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Plant growth promoting endophytic *Pseudomonas aeruginosa* RD10 isolated from water hyacinth for phenol degradation

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ABSTRACT

A total of twelve endophytic bacteria were isolated from the root of *E. crassipes*. All these bacterial strains were analyzed for phenol degradation potential. Twelve endophytic bacteria were subjected to polyphenol resistance analysis and all showed phenol tolerance up to 300 ppm. Among the twelve bacterial strains, the strain RD10 was resistant up to 800 ppm, and reduced growth were observed at higher concentrations. The plant growth-promoting properties of endophytic bacterial strains were analyzed. Among the isolated bacterial strain, only five strains produced ACC deaminase. IAA production was detected in seven bacterial isolates, while siderophore production was determined from only five bacterial strains. Among the isolated endophytes, the strain RD10 exhibited ACC deaminase, IAA, and siderophore activity in submerged fermentation. ACC deaminase production of 125.2 ± 4.8 µm/ml was shown by strain RD10 and IAA production was 89.7 ± 5.1 µg/ml. Moreover, maximum siderophore production was observed (53.9 ± 4.9% siderophore units) in strain RD3. Catechol 2,3-dioxygenase activity of the bacterial strains was analyzed. Catechol 2,3 dioxygenase activity ranges from 0.03 ± 0.01 U/mg to 35.2 ± 1.1 U/mg. Phenol degradation by the isolated endophytes was studied and the initial phenol concentration was 500 ppm. The strain RD1 degraded 1.2 ± 0.2% phenol and more than 50% phenol degradation was observed in strains RD3, RD4, RD6, RD7, RD9, RD10, and RD11, respectively. The endophytic bacterial strain was Gram-negative, rod-shaped and motile. It was non-spore-forming bacteria, capsulated and, showed a positive reaction to oxidase and catalase. In MacConkey agar medium, strain RD10 forms smooth and flat colonies, and the diameter of the colonies ranged from 2 to 3 mm. The colonies have an alligator skin-like appearance on top view and have regular margins. The strain RD10 was streaked on blood agar medium and the strain RD10 produced strong pigmentation on blood agar. Antibiotic sensitivity of strain RD10 against various antibiotics in terms of zone of inhibition was analyzed. The endophytic strain RD10 was sensitive to all selected antibiotics. Based on biochemical characters, pigment analysis and, molecular characterization, the strain was confirmed as *P. aeruginosa* RD10. In this study, *P. aeruginosa* RD10 was cultured at 400, 500 and, 600 mg/l phenol concentration for 96 h. Optimum growth was achieved at 500 mg/l concentration and an inhibitory effect was observed at 600 mg/l phenol concentration. The results confirmed that the selected strain can withstand and grow well at 500 mg/l phenol concentration. The strain RD10 was cultured at various incubation temperatures with 500 mg/l phenol concentration. Optimization of environmental factors can lead to maximum degradation of phenol. The results revealed that phenol degradation was influenced by temperature. The highest phenol degradation was achieved at 30 °C in Erlenmeyer flask culture and decreased at high temperatures. *P. aeruginosa* RD10 could degrade phenol at pH 5.5 (10.2 ± 0.5% removal),

at pH 6.0, 15.4 ± 1.1% of the phenol was degraded after incubation for 72 h at 30 °C. Phenol removal (%) declined to 70.4 ± 2.9% at higher initial pH value (9.0).

Key words : *Endophyte, Bacteria, Phenol resistant, Biodegradation, Eco-friendly*

Introduction

Water hyacinth is one of the rapidly-growing macrophytes and is an invasive plant species in various countries and native to South America. It has a lot of potentials to produce large amounts of biomass and has rapid vegetative propagation (Sierra-Carmona *et al.,* 2022). The role of water hyacinth-associated microbes on organic contaminants, pesticides, heavy metals, nutrient uptake and dissolved oxygen content was reported (Luo *et al.,* 2015). Root microbiome assembly is a dynamic and highly complex that can be significantly affected by various factors, including, geographical, growth conditions and environmental aspects such as pH, temperature and nature of the sediment, and morphological, genetic, developmental and physiological conditions of the macrophytes (Edwards *et al.,* 2015). A microbiome study revealed synergistic functional properties between bacteria and fungi, mainly when exposed to adverse climatic conditions (Luo *et al.,* 2015). Some of the bacteria occurring in water hyacinth roots involved in the biogeochemical cycle, and nitrogen fixation, are essential not only for water hyacinth growth but also for various functional properties of ecosystem processes (Liengaard *et al.,* 2012).

Plant growth promoting bacteria improve plant growth and these associated bacteria are used for the treatment of effluent and soil. Endophytic bacteria improved phytoremediation due to the improved metabolic activities which improved the degradation of various organic pollutants (Wang *et al.,* 2019). Root-associated bacteria such as *Enterobacter* strains, *Bacillus* and *Pseudomonas* can improve phytodegradation because of the production of growth promoters and enzymes (Afzal *et al.,* 2014). Ullah *et al.* (2015) reported plant growth promoting bacteria such as *Beijerinckia*, *Klebsiella*, *Gluconacetobacter*, *Enterobacter*, *Bacillus*, *Serratia*, *Flavobacterium*, *Burkholderia*, and *Pseudomonas*. Bacteria such as *Bacillus safensis* and *Bacillus cereus* are involved in phytoremediation (Wu *et al.,* 2019). Endophytes could improve the growth of plants and the main objective of this paper is to isolate endophytic bacteria for the degradation of phenolic compounds from the water.

Materials and Methods

Isolation of endophytic bacteria from the *E. crassipes* plant

In this study, *E. crassipes* plant was collected from the coir retting contaminated region in Kerala (India). The collected water hyacinth was transferred to a sterile container aseptically and transported to the laboratory. The endophytic bacterial strains were initially isolated from the root of water hyacinth after surface sterilization as described previously (Afzal *et al.,* 2014). Root was washed for 2 min with sterile double distilled water and surface sterilized with ethanol (70%) for 5 min, followed by sodium hypochlorite treatment (1%) for 60 seconds and finally rinsed with sterile double distilled water three times. The surface sterility was verified using Nutrient Agar medium (Hi-media, Mumbai, India). The Nutrient Agar plates were incubated for 24 h at 37 ± 1 ºC and no visible growth was observed, which indicated sterility. Then the surface sterile root (0.2 g) was homogenized using 2 ml physiological saline (0.9% NaCl). About 0.1 ml of sample was spread onto Nutrient Agar plates containing 25% coir retting effluent (filter sterilized). The plates were incubated for 24 – 48 h at 37 ± 1 ºC and the isolated bacteria were subcultured using the agar medium and the morphologically different bacterial strains were maintained. Based on colony morphology and rapid proliferation, a total of 19 bacteria were selected.

Initial screening of phenol degradation by endophytes

The isolated 12 endophytes were cultured in nutrient broth medium (Hi-media, Mumbai, India) and incubated for 18 h at 37 ± 1 ºC in an orbital shaker for 150 rpm. After 24 h incubation, cells were centrifuged at 10,000 rpm and the cells were resuspended in physiological saline. The cell density was adjusted

to approximately 1 × 10^7 CFU/ml. A total of two-millilitre bacterial suspensions of all 12 bacterial suspensions was inoculated in sterile retting effluent (250 ml). The colour and polyphenol content of retting effluent was observed.

Screening of polyphenol resistance bacteria

The screened bacteria were subjected to polyphenol resistance analysis. An overnight culture of the selected isolates was cultured in peptone water and was further inoculated into the increasing concentration of phenol (100 – 1000 ppm) in nutrient agar medium. The nutrient agar plates were incubated for 24 – 48 h and the growth was determined. The maximum tolerant limit was registered and the prominent isolate was further used for characterization studies.

Analysis of growth-promoters from the bacteria

The amount of IAA production by the selected bacterial isolates was determined by using a UV Visible spectrophotometer. Briefly, the sample was incubated with perchloric acid and iron trichloride as described earlier by Bric *et al.* (1991) with minor modifications. The bacterial strain was inoculated in Erlenmeyer flask containing 50 ml nutrient broth medium with 0.2% tryptophan. It was incubated for two days at optimum temperature (28 ± 2 °C) at 125 rpm in a rotary shaker. It was centrifuged at 6000 rpm for 8 min and the cell-free supernatant was used for analysis. The sample was incubated with a substrate for 20 min and the colour was detected. IAA was prepared at various concentrations (10 – 100 µg/ml) and the standard curve was prepared. The ACC deaminase production was carried out using a UV-Visible spectrophotometer at 540 nm against the reagent blank. α-ketobutyrate was prepared at various concentrations (1 to 10 µM) and a calibration curve was plotted. One ACC deaminase unit was expressed as the amount of sample required to liberate nmol of α-ketobutyrate per mg/h (Honma and Shimomura, 1978). The total protein content of the culture was estimated as described previously (Bradford, 1976) using bovine serum albumin (BSA) as a standard. The amount of siderophore production was evaluated using Chrome Azurol sulphonate assay. Briefly, 0.5 ml of Chrome Azurol sulphonate solution was mixed with 0.5 ml of culture supernatant and incubated for 10 min. Then the optical density of the sample was measured at 630 nm. The amount of siderophore production was assayed and the percentage siderophore production was determined. The result was expressed as siderophore units (%) using the following formula.

$$\%SU = \frac{A_r - A_s}{A_r} \times 100$$

SU- siderophore units
Ar – Absorbance of the reference
As – Absorbance of the sample

Catechol 2,3-dioxygenase activity of endophtic bacteria was analyzed. The cell-free extract (50 ìL) was treated with phosphate buffer (pH 7.5, 900 ìL) and 50 ìL of catechol (50 mM) in a total volume of 1 mL. It was incubated at 37 ºC for 10 min. The formation of 2-hydroxymuconic semialdehyde was determined at 375 nm using a UV-Visible spectrophotometer. Control reaction was performed without any crude sample for each enzyme assay. One unit of enzyme activity was defined as the amount of enzyme required to generate 1 ìmol of product/min under standard assay conditions and the final result was expressed as U/mg protein.

Phenol degrading ability of bacterial strains

The endophytic bacterial strains were subjected to the degradation of phenol. The selected strains were cultured in a minimal medium at 500 ppm phenol. The culture was centrifuged after 24 h and phenol degrading potential was analyzed. Briefly, 0.2 ml culture supernatant was mixed with sodium carbonate (0.5 M) (3 ml) and mixed. It was treated with Folin-Ciocalteaeu reagent and the absorbance was read at 725 nm. The amount of residual phenol content was determined and the percentage of phenol degradation was calculated. Catechol was used as the standard.

Characterization of phenol degrading bacteria

The potent strain was identified up to the genus level by biochemical characters and morphological properties as described in Bergey's Manual of Determinative Bacteriology. The selected bacterial strain, RD10 was cultured in Luria Bertani broth medium (Hi-media, Mumbai, India) and incubated for 18 h at 37 ºC. The cell pellet was centrifuged (10000 rpm, 10 min) and DNA was extracted by solvent extraction method using chloroform and isoamyl alcohol. The extracted DNA was purified using DNA purification kit as described by the manufacturer (Merck, Germany). The purity of the DNA was tested spec-

trophotometry method using a Nanodrop Machine. The purified DNA was used as a template DNA in PCR. The total PCR mixture volume was 25 µl comprising, DNA polymerase, and forward (5′-AGAGTTTGATCMTGG-3′) and reverse (5′-ACCTTGTTACGACTT3′) primers. PCR was performed using a Thermocycler machine (Applied Biosystems, USA) and the purity was analyzed using 1.5% agarose gel electrophoresis. The purified fragment was sequenced and the sequences were compared using NCBI blast analysis. The 16S rDNA sequence of strain RD10 isolated from the root of water hyacinth was deposited in GenBank and accession number (OP271307) was assigned.

Screening of bacteria using MacConkey Agar plates

Mac Conkey agar plate was used for the differentiation of genus *Pseudomonas* and non- fastidious gram-negative rods. The isolated strain was streaked on Mac- Conkey agar plates (peptone-17 g; protease peptone – 3, lactose monohydrate – 10 g, bile salts – 1.5 g, sodium chloride – 5 g, neutral red – 0.03 g, crystal violet – 0.001 g, agar – 13.5 g, distilled water - 1 L) and incubated for 24 h at 37 ºC and the result was observed.

Determination of haemolytic assay

The bacterial strain was cultured on the blood agar medium (Hi-media, Mumbai, India). The plates were incubated for 48 h at 37 °C. Sheep blood (7 ml) was added with 100 ml blood agar medium. After 48 h incubation, haemolytic activity was analyzed. The final result was classified as, α-hemolysis, β-hemolysis and γ-hemolysis.

Antibiotic sensitivity analysis

An antibiotic sensitivity test was carried out using commercial antibiotic discs (Hi-media, Mumbai, India). The Mueller Hinton Agar plates were incubated for 24 h and the antibiotic sensitivity of strain RD10 was analyzed. Based on the zone of inhibition around the bacterial strain, bacteria were classified as sensitive, intermediate or resistant (Wu *et al.*, 2020).

Growth kinetics of phenol degrading bacteria

The bacterial strain was cultured in a minimal medium containing phenol (400 – 600 mg/l) and incubated for 96 h at 37 ºC. The broth culture was withdrawn every 12 h and the absorbance was read at 600 nm.

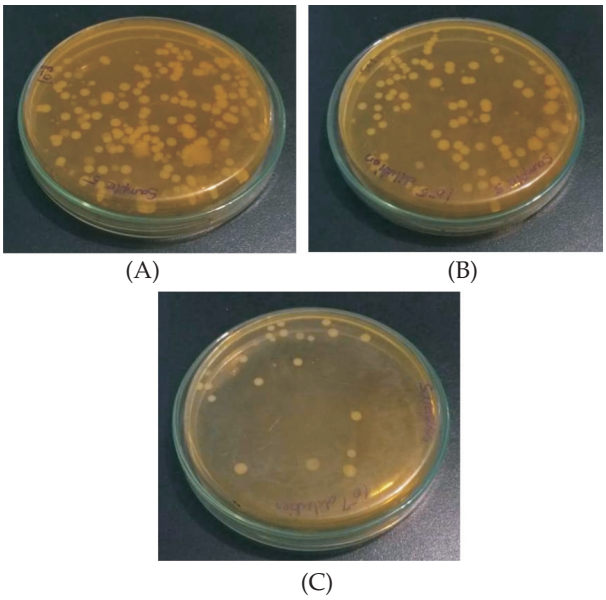
Optimization of environmental factors on phenol degradation

The effect of incubation temperature and pH on phenol degradation was analyzed. Effect of temperature (25, 27, 29, 31, and 33 ºC) and pH (5.5, 6.0, 6.5, 7.0, 7.5, and 8.0) on phenol degradation was analyzed.

Results

Isolation of endophytic bacteria from *E. crassipes*

A total of twelve endophytic bacteria were isolated from the root of *E. crassipes*. All these bacterial strains were analyzed for phenol degradation potential. The colour and phenol content of retting effluent was observed after treatment. Among the twelve bacteria, seven isolates showed prominent polyphenol degrading activity in initial observation. The growth of isolated endophytes in a nutrient broth medium was described in Fig. 1.



(A) (B)

(C)

Fig. 1. Growth of endophytic strains at 10^{-3} (A), 10^{-5} (B) and 10^{-7} (C) dilution on nutrient agar medium screened from *E. crassipes* root.

Endophytic bacterial and phenol resistance

Twelve endophytic bacteria were subjected to polyphenol resistance analysis and all showed phenol tolerance up to 300 ppm. Among the twelve bacterial strains, five were resistant up to 700 ppm. The strain RD10 was resistant up to 800 ppm and re-

duced growths were observed at higher concentrations. The phenol resistance pattern of the selected bacterial strain was described in Table 1.

Table 1. Maximum tolerance limit of phenol by the endophytic bacteria isolated from *E. crassipes* root.

Bacteria	Phenol tolerance (ppm)
RD1	300
RD2	500
RD3	700
RD4	700
RD5	500
RD6	700
RD7	500
RD8	500
RD9	700
RD10	800
RD11	700
RD12	500

Plant growth-promoting activities of bacteria isolated from the root

The plant growth-promoting properties of twelve endophytic bacterial strains were described in Table 2. Among the isolated bacterial strain, only five strains produced ACC deaminase. IAA production was detected in seven bacterial isolates, while siderophore production was determined from only five bacterial strains. Among the isolated endophytes, the strain RD10 exhibited ACC deaminase, IAA and siderophore activity in submerged fermentation. ACC deaminase production of 125.2 ± 4.8 µM/ml was shown by strain RD10 and IAA production was 89.7 ± 5.1 µg/ml. Moreover, maximum siderophore production was observed (53.9 ± 4.9% siderophore units) in strain RD3 (Table 2).

Catechol 2,3-dioxygenase activity

Catechol 2,3-dioxygenase activity of the bacterial strains was analyzed. Catechol 2,3 dioxygenase activity ranges from 0.03 ± 0.01 U/mg to 35.2 ± 1.1 U/mg. All selected endophytes showed Catechol 2,3 dioxygenase activity and the result was described in Table 3.

Table 3. Catechol 2,3-dioxygenase activity in endophtic bacteria isolated from *E. crassipes* root

Bacteria	Catechol 2,3 dioxygenase (U/mg protein)
RD1	8.5 ± 0.21
RD2	0.03 ± 0.01
RD3	17.4 ± 1.9
RD4	0.41 ± 0.5
RD5	18.5 ± 3.1
RD6	19.2 ± 1.9
RD7	5.3 ± 0.5
RD8	10.2 ± 1.2
RD9	20.4 ± 2.1
RD10	35.2 ± 1.1
RD11	13 ± 0.2
RD12	17.3 ± 2.8

Phenol degradation by endophytes

Phenol degradation by the isolated endophytes was studied and the initial phenol concentration was 500 ppm. The strain RD1 degraded 1.2 ± 0.2% phenol and more than 50% phenol degradation was observed in strains RD3, RD4, RD6, RD7, RD9, RD10, and RD11, respectively. Among the strains, RD10 degraded 93.1 ± 0 phenol from the water and the

Table 2. Endophytic bacteria isolated from *E. crassipes* root and plant growth-promoting activities.

Bacteria	ACC deaminase (µM)	IAA production (µg/ml)	Siderophore units (%)
RD1	–	10.3 ± 2.1	-
RD2	–	0.52 ± 0.03	-
RD3	-	1.5 ± 0.4	53.9 ± 4.9
RD4	–	29.4 ± 1.5	-
RD5	38.2 ± 1.1	20.4 ± 2.6	-
RD6	29.4 ± 2.7	-	-
RD7	–	-	14.8 ± 2.1
RD8	10.1 ± 1.1	-	-
RD9	17.6 ± 2.5	-	-
RD10	125.2 ± 4.8	89.7 ± 5.1	27.6 ± 1.6
RD11	–	59.2 ± 1.2	30.2 ± 2.2
RD12	–	-	40.4 ± 3.3

Table 4. Phenol degradation by the endophytes isolated from *E. crassipes* root.

Bacteria	Phenol degradation (%)
RD1	1.2 ± 0.2
RD2	14.3 ± 1.5
RD3	50.2 ± 5.2
RD4	67.2 ± 3.3
RD5	14.2 ± 3.2
RD6	69.4 ± 8.2
RD7	57.9 ± 0.7
RD8	9.4 ± 0.1
RD9	90.2 ± 5.2
RD10	93.1 ± 0
RD11	90 ± 2.2
RD12	17.3 ± 2.8

result was described in Table 4.

Analysis of morphological, biochemical and molecular characters

Biochemical characterization of strain RD10

The endophytic bacterial strain was Gram-negative, rod-shaped and motile. It was non-spore-forming bacteria, capsulated and showed a positive reaction to oxidase and catalase. Moreover, it showed a negative reaction to the MR-VP test. In addition, the selected strain was positive for the citrate test, urease test, and nitrate reduction test. It hydrolyzed casein and produced lipase. Green pigmentation was observed on nutrient agar plates and was negative for H_2S production. The biochemical characteristics of strain RD10 was described in Table 5.

Growth characteristics of strain RD10 on selective media

In MacConkey agar medium, strain RD10 forms smooth and flat colonies and the diameter of the colonies ranged from 2 to 3 mm. The colonies have an alligator skin-like appearance on top view and have regular margins. MacConkey agar medium was differentiated strain RD10 based on lactose fermentation. The colonies were non-lactose fermenting types (Fig. 2A). The strain RD10 was streaked on blood agar medium and the strain RD10 produced strong pigmentation on blood agar. It showed a grape-like odour because of the presence of 2-aminoacetophenone (Fig. 2B). The strain RD10 was streaked on a nutrient agar medium and incubated for 24 h. A greenish-blue pigmentation was observed (Fig. 2C).

Table 5. Biochemical test and identification of endophytic strain RD10

Biochemical test	Result
Gram-staining	Negative
Shape	Rod
Motility	Motile
Spore	Non-sporing
Capsule	Non-Capsulated
Oxidase test	Positive
Catalase test	Positive
MR	Negative
VP	Negative
Citrate test	Positive
Indole test	Negative
Urease test	Positive
Hydrogen sulphide	Negative
Nitrate-reduction	Positive
Casein-hydrolysis	Positive
Pigment production	positive
Hemolysis	Negative
Glucose-fermentation	Negative
Lactose-fermentation	Negative
Lipase-production	Positive

Antibiotic sensitivity

Antibiotic sensitivity of strain RD10 against various antibiotics in terms of zone of inhibition was analyzed. The endophytic strain RD10 was sensitive to

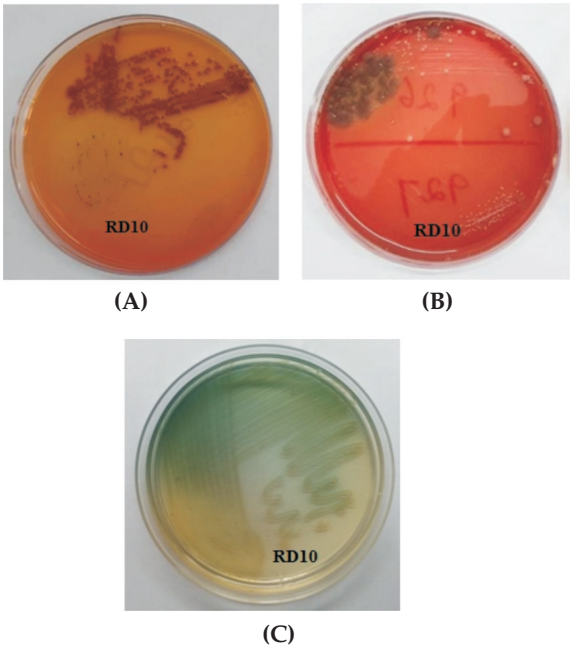


Fig. 2. Growth characteristics of endophytic strain RD10 on MacConkey agar medium (A), blood agar medium (B) and nutrient agar medium (C).

all selected antibiotics. These include amikacin, cefepime, ciprofloxacin, ofloxacin, nitrofurantoin, gentamycin, imipenem, cefotaxime, cephalothin, meropenem, norfloxacin, ceftazidime, levofloxacin, chloramphenicol and cefixime (Fig. 3).

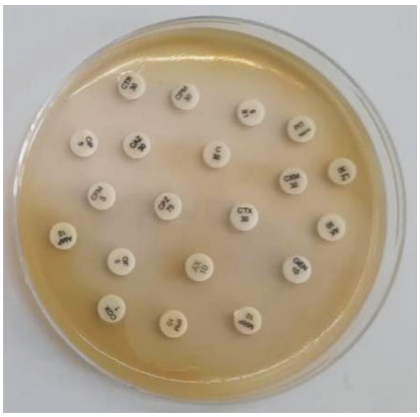


Fig. 3. Antibiotic resistance pattern of strain RD10 isolated from the root.

16S rDNA gene sequencing

BLAST analysis was performed to determine the closely related genera from the NCBI database and the result revealed that the strain RD10 was closely related to *Pseudomonas aeruginosa* and named *P. aeruginosa* RD10. Based on biochemical characters, pigment analysis and molecular characterization, the strain was confirmed as *P. aeruginosa* RD10. BLAST analysis revealed that the strain RD10 was very closely related to *P. aeruginosa* with 99% sequence similarity.

Growth kinetics of *P. aeruginosa* RD10 at various concentrations of phenol

The initial phenol concentration is one of the important factors that influenced bacterial growth. In this study, *P. aeruginosa* RD10 was cultured at 400, 500 and 600 mg/l phenol concentration for 96 h. Optimum growth was achieved at 500 mg/l concentration and an inhibitory effect was observed at 600 mg/l phenol concentration. Fig. 4 shows the growth profile of phenol-resistant *P. aeruginosa* RD10 cultured at various concentrations of phenol. The results confirmed that the selected strain can with-

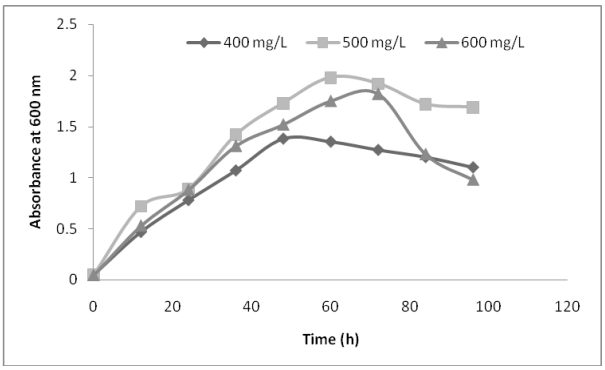
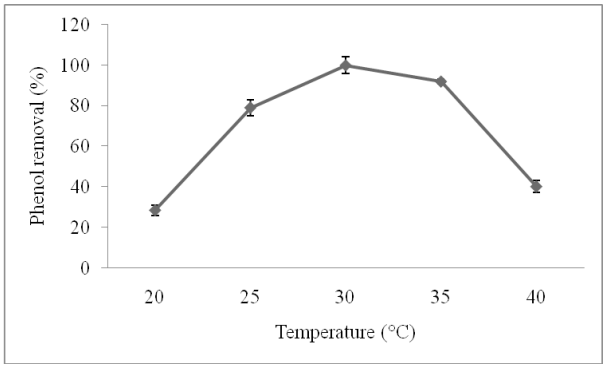
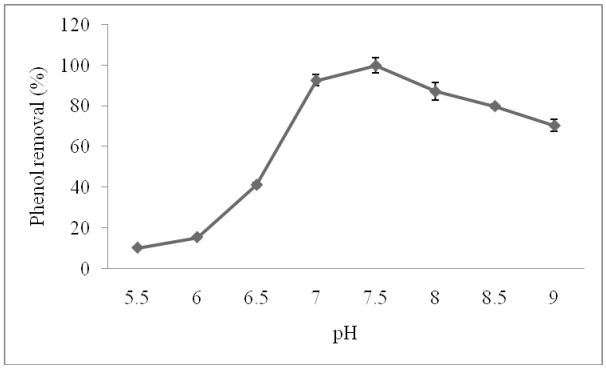


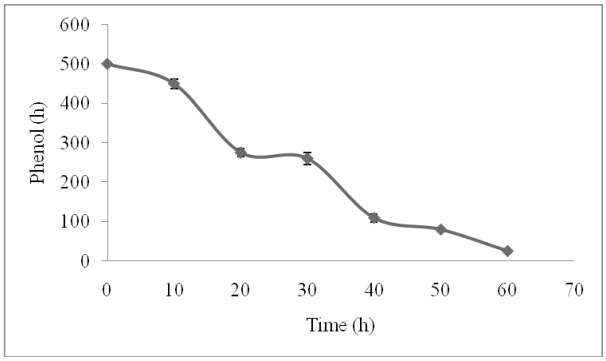
Fig. 4. Growth kinetics of *P. aeruginosa* RD10 at various concentrations of phenol



(A)



(B)



(C)

Fig. 5. Effect of initial temperature (A), pH (B), and incubation time (C) on phenol degradation after 72 h incubation temperature at 30 °C and the initial phenol concentration was 500 mg/l.

stand and grow well at 500 mg/l phenol concentration.

Optimization of phenol degradation

The strain RD10 was cultured at various incubation temperatures with 500 mg/l phenol concentration. The environmental parameter has a potential effect on phenol degradation by bacteria. Optimization of environmental factors can lead to maximum degradation of phenol. The results revealed that phenol degradation was influenced by temperature. The highest phenol degradation was achieved at 30 °C in Erlenmeyer flask culture and decreased at high temperatures (Fig. 5A). The effect of pH on phenol degradation (500 mg/l initial concentration) was analyzed in this study. Figure 5B revealed that the optimum pH for the selected strain RD10 was 7.5. *P. aeruginosa* RD10 could degrade phenol at pH 5.5 (10.2 ± 0.5% removal), at pH 6.0, 15.4 ± 1.1% of the phenol was degraded after incubation for 72 h at 30 °C. When pH was at 7.5, maximum phenol degradation was achieved and was considered as 100%. Phenol removal (%) declined to 70.4 ± 2.9% at higher initial pH value (9.0). The strain RD10 was cultured in optimized condition (pH 7.5, 500 mg/l phenol concentration, and 30 °C) for 10 – 60 h (Fig 5C).

Discussion

The isolated bacterial strains showed phenol tolerance ability when bacteria were exposed to various concentrations. The endophytic strains showed a tolerance range from 300 to 800 mg/l phenol. The plant growth-promoting properties of twelve endophytic bacterial strains were determined in this study. The selected 12 bacteria showed ACC deaminase, IAA and siderophore activity. Among the isolated bacterial strain, IAA production exhibited in seven bacteria, while siderophore production was determined from five bacterial strains. Based on phenol tolerance ability, and plant growth promoting properties, the candidate strain isolated from the root of *E. crassipes* root was identified as *P. aeruginosa* RD10.

Recently, endophytic bacterial strains from the genus *Pseudomonas* were reported from the plant endosphere. Most of the previous studies have observed similar findings that the endophytic bacteria colonize the interior part of a plant, whereas, rhizospheric bacteria proliferate and acclimatize the rhizosphere (Ijaz *et al.,* 2016). Recent studies revealed bacterial species of endophytic and rhizospheric origin which can be used in combination with macrophytes for better removal of organic pollutants, including phenol (Liu *et al.,* 2016).

Bacteria isolated from *E. crassipes* showed plant growth promoter activity. Endophytic bacteria remain an important source that could positively improve the capacity of plants in phytoremediation. In this study, plant growth promoting *P. aeruginosa* RD10 was inoculated in phenol-containing culture medium and observed decreased phenol load in the medium and this result was similar to previous findings (Demoling and Bååth, 2008). Among the isolated endophytes, the strain RD10 exhibited improved ACC deaminase, IAA and siderophore production in submerged fermentation. It has been previously reported that ACC deaminase-producing bacteria improved plant growth and stimulated phytoremediation (Girard *et al.,* 2021).

The isolated endophytes showed enhanced production of IAA and siderophore. Similarly, endophytic bacteria isolated from the plant showed enhanced production of IAA and siderophores and improved plant growth. Phytohormones including, gibberellins (GAs) and indole-3-acetic acid can produced by various endophytic bacteria, including *Pseudomonas* sp. and *Bacillus* sp. (Chen *et al.,* 2010). Endophytic bacteria such as *Paracoccus*, *Panniobacter, Planococcus,* and *Rhizobium* reduced BOD, COD, and colour from the wastewater (Sierra-Carmona *et al.,* 2022). Endophytic bacterial strain, *Bacillus* sp. decreased BOD level in wastewater. Inoculated *Bacillus cereus* reduced 66%, 61% BOD and COD from the wastewater. *Bacillus* sp. (SBER3) isolated from the roots produced siderophores and HCN and improved biodegradation ability (Bisht *et al.,* 2014).

In this study, the optimized culture medium removed >90% phenol from the medium by *P. aeruginosa* RD10. Chakraborty *et al.* (2010) isolated bacteria from the wastewater and achieved 76.69% phenol removal at neutral pH and 30 °C. Endophytes improved shoot and the root length of plants and helped to tolerate stress conditions and to improve phytoremediation of types of phenolic compounds (Ho *et al.,* 2012). Iqbal *et al.* (2018) reported complete degradation of phenol by endophytic bacterial isolates from wastewater. Bacteria such as *Pseudomonas* sp. LCRH90, *Bacillus cereus* LORH97 and *Acinetobacter lwofii* ACRH76 were isolated from the floating macrophyte *T. domingensis* and removed phenol from the wastewater effectively (Saleem et

al., 2018). In this study, *P. aeruginosa* RD10 attained maximum growth at 500 mg/l phenol concentration than 400 mg/l in the culture medium. Bacteria use phenol as a sole source of carbon; hence the growth rate was higher at increased phenol concentration in the culture (Mahiudddin *et al.,* 2012). The increased growth in the culture medium containing phenol revealed the consumption of phenol as a sole source of carbon by the strain RD10.

The isolated plant endophyte showed effective phenol removal efficiency. Afzal *et al.* (2014) characterized endophytic bacteria and reported the efficiency to reduce phenol from the wastewater. Plants are unable to remove phenol-containing compounds completely from the wastewater. Phenol degrading bacteria were coded with catechol 2, 3-dioxygenase and this enzyme contributed to the transformation of phenols and reduced phenol concentration from the wastewater (Wang *et al.,* 2007). Wang *et al.* (2011) reported plant growth promoting *Pseudomonas aeruginosa* isolated from the contaminated environment. It produced plant growth-promoting hormones including IAA and siderophores. In this study, the selected strain degraded >90% phenol from the water. The efficiency of phenol degradation varied based on pH value, temperature, initial phenol concentration and degradation time. In optimized condition, *P. aeruginosa* RD10 removed 100% phenol from the medium. Bacteria transformed phenol and plants use organic acids and improved pH value. The endophytic bacteria isolated from the root of macrophyte can effectively utilize phenol from the water as a carbon source and be the potential to remove phenol from the coir retting wastewater.

Conclusion

The endophytic strain RD10 isolated from *E.crassipes* showed phenol resistance at various concentrations. The isolated strain showed plant growth-promoting properties including, ACC deaminase, IAA production, and catechol 2,3-dioxygenase activity. The strain RD1 degraded phenol considerably and was characterized as *P. aeruginosa* RD10. Optimization of environmental factors can lead to maximum degradation of phenol. The results revealed that phenol degradation was influenced by temperature. The highest phenol degradation was achieved at 30 °C in Erlenmeyer flask culture and decreased at high temperatures. The endophytic *P. aeruginosa* has the potential to remove phenol from the coir retting wastewater.

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