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# ENDOPHYTIC BACTERIA AS MITIGATORS OF BIOTIC AND ABIOTIC STRESS TOLERANCE IN *SALVADORA* SPECIES.

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Endophytic bacteria are capable of augmenting plant growth, both under normal conditions as well as stressful conditions. This is accomplished by fulfillment of a number of routine procedures that aid in plant growth and metabolism, both under normal physiological conditions as well as anarchical conditions when plant survival is jeopardized. Endophytic bacteria act as modulators of plant growth both in direct as well as indirect manner by production of phytohormones that regulate plant growth, facilitation of nutrient uptake by plants in the form of enhanced nitrogen fixation, iron uptake and phosphate solubilization, abiotic stress tolerance against salinity drought and heavy metal stress as well as biotic stress tolerance by killing of pests and phytopathogens that jeopardize plant survival. The current study is an attempt to isolate the population of bacterial endophytes inhabiting soil around Salvadora plants in Jaipur region following their biochemical and morphological characterization coupled with sequencing and phylogenetic analysis to aid in bacterial classification. Furthermore, the results showcased in the study demonstrate unparalleled ability of endophytic bacteria to aid in both biotic as well as abiotic tolerance against both gram positive and gram-negative bacteria and salinity stress. In this context, the current study further strengthens the assertion of other existing studies unveiling the plant growth promoting attributes of endophytic bacteria and highlights their ameliorative role towards achievement of the goal of sustainable crop development and agriculture.

**Keywords:** Abiotic stress tolerance, Biotic stress tolerance, Endophytic bacteria, Plant growth promotion, *Salvadora* species.

## Introduction

Plants, the primary producers on earth, that form the main driving force for maintenance of ecological balance and perfect homeostasis on earth are aided by a population of endophytes. Plant endophytes can be best defined as microbes with the ability to colonize different plant tissues such as root, stem, leaf, flowers, and seeds[1]. However, unlike the conventional microbial strains, which act as phytopathogens and wreak havoc on the plants; these endophytic micro-organisms contribute to effective growth and development of plants and contribute to plant fitness by aiding in biotic and abiotic stress tolerance. There are several ways in which endophytic bacteria aid in promoting plant growth such as:

- Production of phytohormones and plant growth stimulators that fuel the plant metabolism and development such as auxins, gibberellins, cytokinins and ethylene.

- Facilitating availability of nutrients to plants by nitrogen fixation, siderophore production to augment iron uptake and solubilization of phosphate.
- Providing resistance to abiotic stress in the form of salinity and drought tolerance.
- Aiding in plant survival by bioremediation of heavy metals and hydrocarbon contaminants.
- Aiding in plant survival by antibiosis to neutralize plant pathogens by production of lytic enzymes, allelochemicals and induced systemic resistance[2].

Considering all these beneficial traits, the endophytic bacteria have rightly been coined to act as PGPRs (plant growth promotion regulators). The diversity of bacterial endophytes present in plants is controlled by a number of factors, that include plant specific factors (plant species, growth stage, geographical location) as well as environment specific factors (climate, drought, salinity, season, type of soil, pathogens).

Based on all these factors, the current study has been carefully drafted to focus on the endophytic bacteria residing in Salvadora plants growing in Jaipur region. For this, the bacterial endophytes residing in Salvadora plants were isolated followed by their morphological and biochemical characterization using a array of tests to gain deeper insights into their morphology as well as well biochemical behavior. This was followed by sequencing of isolated bacterial strains followed by phylogenetic analysis to aid in their classification and nomenclature. Furthermore, the results shown in the later half of the study demonstrate the ability of endophytic bacteria to produce secondary metabolites, showcase strong antioxidant potential and potent antimicrobial activity against both gram positive and gram-negative bacteria. Furthermore, the isolated bacterial endophytes were found capable of aiding in plant survival under hypersaline conditions by demonstrating unparalleled growth in presence of salinity stress.

## Material and Methods

*Ioslation and characterization of endophytic bacteria:*

Sampling of the *Salvadoraoleoides* was done from Jaipur, Rajasthan. Roots were taken for the experiment. Collected healthy roots of *Salvadora* were surface sterilized by immersion in 90% (v/v) ethanol for one minute followed by 1% (v/v) NaOCl for 10 min and then washed six times with sterile distilled water. Sterilized roots were pleated on nutrient agar (NA) medium and incubated for 24-48 h at 30°C. The colonies appeared under the roots were picked up and diluted by dilution series and subsequently streaked on Triphenyltetrazolium chloride(TZC) agar plates. Colonies showing similar characteristics were confirmed by plating on various semi selective media, and by other biochemical and molecular studies. Obtained sequences were submitted at National Centre for Biotechnology Information(NCBI) to obtain accession number.

*Determination of secondary metabolites from the isolated endophytic bacteria:*

The determination of secondary metabolites from the isolated endophyte was done. The isolated endophytic bacteria were inoculated in nutrient broth (autoclaved) and incubated at 30°C for 7 days. Then the culture was centrifuged at 10,000 rpm for 15 minutes for biomass removal. To this biomass, equal volumes of ethyl acetate and chloroform was added followed by continuous shaking.

The organic solvent layer was collected in a conical flask and the solvent was evaporated. The extract was transferred to a 5 ml sterile vial and left to dry at room temperature.

*Antioxidant potential of the endophytic extract:*

For determination of DPPH radical scavenging potential of the extracted samples 1,1-diphenyl 2-picryl-hydrazil (DPPH) method was applied. The mixing of 100 µl aliquot form samples was done in 3.9 ml taken from 0.1 mM DPPH (methanolic) solution. Then blend was subjected to vortex and left for incubation in the dark for 30 min. Its OD was calculated at 515 nm while methanol was used as blank.

The radical scavenging activity was determined by the ratio

$$= \left(\frac{Ab_{control} - Ab_{sample}}{Ab_{control}}\right) \times 100$$

Where $Ab_{control}$ is presenting the absorbance of the DPPH solution and absorbance of the DPPH solution with sample is denoted by $Ab_{sample}$.

Linear plot of concentration versus % inhibition was plotted and by this $IC_{50}$ values were determined. The antioxidant potential of each extract was showed in form of $IC_{50}$ (stated as the quantity of concentration necessary to prevent DPPH radical development by 50%), find out with the help of inhibition curve.

*Antibacterial screening:*

Extracts obtained from the isolated endophytic bacteria were screened for antibacterial potential against *Escherichia coli* and *Staphylococcus aureus* at different concentrations by well diffusion assay. Inhibition zone and Activity index were calculated in reference to standard antibiotic drugs.

*Effect of salt concentration on the growth of the isolated endophytic bacteria:*

The isolated endophytic bacteria was inoculated in different culture tubes having nutrient broth with varied salt concentrations (0%, 0.5%, 1%, 1.5%, 2%, and 2.5% NaCl) and incubated at 30°C for 72 hours. Then absorbance was taken at 660 nm by using UV visible spectrophotometer.

## Results and Discussion

*Isolation of different endophytic bacteria associated with Salvadora sp.:*

The current study shows results pertaining to morphological and biochemical characterization of different endophytic bacterial strains associated with *Salvadora* sp. For this study, nearly 25 unknown endophytic bacterial were collected from root of *Salvadora* from Jaipur, Rajasthan and designated as JR (JR 1 to JR 25). Pictogram in Figure 1 shows isolated bacteria from roots of *Salvadora* species.

*Morphological and biochemical analysis of bacterial strains associated with Salvadora sp.:*

The isolated endophytic bacteria were characterized to identify their morphological and biochemical characteristics in terms of 15 different attributes such as Gram staining, Catalase test, Amylase test, MR test, Lipase test, Indole test, VP test, Gelatinase test, Urease test, Citrate utilization test, motility, Protease test, H2S production, Phosphate solubilization test and siderophore test. Each of these tests was performed with a motive to gain deeper insights into the characteristic traits of every unknown bacterial strain to enable its characterization and classification as per the microbiological standards.

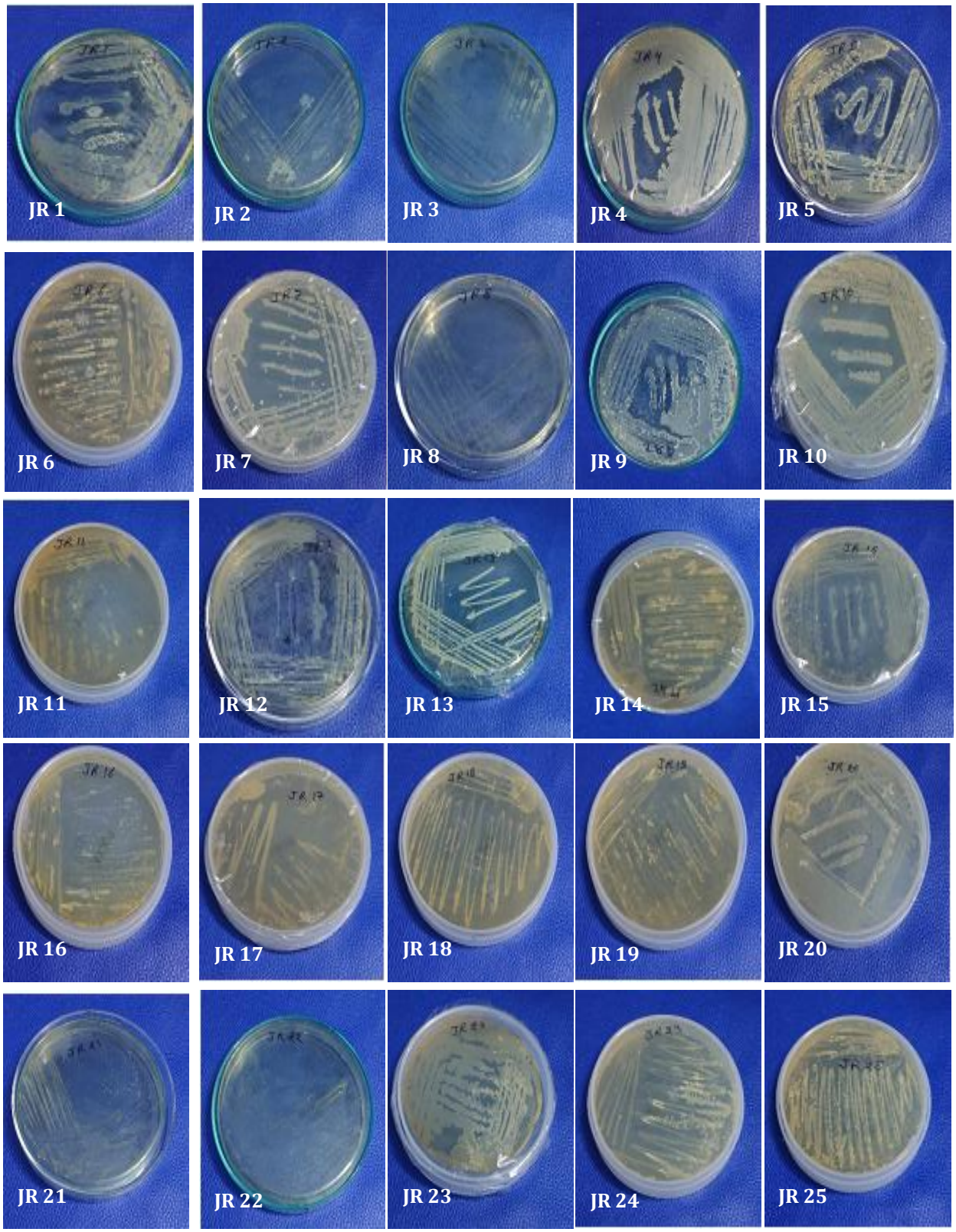


**Figure 1: Endophytic bacteria isolated from roots of *Salvadora sp*. collected from Jaipur, Rajasthan.**

**Table 1: Morphological and biochemical characterizations of bacterial isolates from Roots of *Salvadora* sp. collected from Jaipur.**

| Strain code | Gram staining | Shape | Catalase | Amyalse | MR | Lipase | Indole | VP | Gelatinase | Urease | Citrate | Motility | Protease | H2S | Phosphate solublization | Siderophore test |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **JR1** | + | Rod | + | + | - | + | - | + | - | + | + | + | + | - | - | - |
| **JR2** | - | cocci | + | - | - | + | - | + | - | + | + | + | + | - | + | - |
| **JR3** | + | Rod | - | - | - | + | - | + | - | + | + | + | + | - | + | - |
| **JR4** | + | Rod | + | + | - | + | + | + | - | + | + | + | + | - | + | - |
| **JR5** | - | Cocci | - | + | - | + | - | - | - | + | + | + | + | - | + | - |
| **JR6** | - | cocci | - | + | - | - | - | + | - | + | + | + | + | - | - | - |
| **JR7** | + | Rod | + | + | - | + | + | + | - | + | + | + | + | - | + | - |
| **JR8** | - | cocci | + | - | - | + | - | + | - | + | + | - | + | - | + | - |
| **JR9** | + | Rod | + | + | - | + | + | + | + | + | + | + | + | - | + | + |
| **JR10** | + | Rod | + | + | - | + | + | + | + | + | + | + | + | - | + | + |
| **JR11** | - | cocci | + | + | - | + | - | + | - | + | - | - | + | - | + | - |
| **JR12** | + | Rod | + | - | + | + | + | - | - | + | - | + | + | - | - | - |
| **JR13** | + | Rod | + | - | + | + | + | - | - | + | - | + | + | - | - | - |
| **JR14** | + | Rod | + | + | - | - | - | + | - | + | + | + | + | - | + | - |
| **JR15** | + | Rod | + | + | - | + | + | + | - | + | + | + | + | - | + | - |
| **JR16** | - | Cocci | + | + | + | + | - | + | - | + | - | + | + | - | + | - |
| **JR17** | + | Rod | + | - | - | + | + | + | - | + | + | + | + | - | + | - |
| **JR18** | + | cocci | + | + | - | + | + | + | - | + | + | - | + | - | + | - |
| **JR19** | + | Rod | + | + | - | + | - | + | - | + | - | + | + | - | - | - |
| **JR20** | + | Rod | - | + | - | + |  | + | - | + | + | - | + | - | + | - |
| **JR21** | - | Rod | + | - | - | - | + | - | + | + | - | + | + | + | - | + |
| **JR22** | - | Cocci | - | + | - | + | - | + | - | + | + | + | + | - | - | - |
| **JR23** | + | Rod | + | + | - | + | + | + | - | + | + | + | + | - | + | - |
| **JR24** | - | Rod | + | - | - | - | + | - | + | + | - | + | + | + | - | + |
| **JR25** | + | Rod | - | + | - | + | - | + | - | + | + | + | + | - | - | - |

**Table 2: Quantitative determination of secondary metabolites in the isolated bacterial strains.**

| S. no. | Sequence code | Name of bacterial isolate | Flavonoids (mg/gdw) | Alkaloids (mg/gdw) | Sterols (mg/gdw) |
|---|---|---|---|---|---|
| **1** | JR7 | *Bacillus cereus* | 10.26 | 13.11 | 7.11 |
| **2** | JR9 | *Bacillus thuringiensis* | 9.11 | 5.38 | 21.84 |
| **3** | JR13 | *Bacillus nitratireducens* | 4.93 | 17.65 | 17.39 |
| **4** | JR18 | *Staphylococcus* sp. | 7.48 | 7.83 | 11.88 |
| **5** | JR24 | *Proteus mirabilis* | 10.57 | 4.27 | 24.16 |

The results in Table 1 present an outline of different characteristics of the endophytic bacteria collected from root of *Salvadora* plants.

*Molecular identification of the isolated bacterial strains:*

On the basis of biochemical tests, bacteria showing plant growth promoting activity (positive Indole test), 5 isolates were selected and identified by molecular sequencing followed by submission of the sequences to NCBI to obtain accession number of each.

Out of the 25 bacterial sequences from Jaipur root (JR1 to JR25), the 5 bacterial strains that were subjected to sequencing included JR7, JR9, JR13, JR18 and JR24. The brief summary of sequencing results is as follows:

- JR7 bearing the accession number OR512174, was identified as *Bacillus cereus*, with a DNA length of 1505 bp, belonging to the phyla firmicutes. The phylogenetic analysis of JR7 revealed its 99-100% sequence similarity with different bacterial strains including *Bacillus sp.*, *Bacillus cereus*, *Bacillus tropicus* and *Bacillus thuringenesis.*
- JR9 bearing the accession number OR512175, was identified as the *Bacillus thuringenesis*, with a DNA length of 1468 bp, belonging to the phyla firmicutes. The phylogenetic analysis of JR9 revealed its 99-100% sequence similarity with different bacterial strains *including Bacillus cereus*, *Bacillus thuringenesis* and *Bacillus anthracis*.
- JR13 bearing the accession number OR512176, was identified as the *Bacillus nitratireducens* with a DNA length of 1470 bp, belonging to the phyla firmicutes. The phylogenetic analysis of JR13 revealed its 99-100% sequence similarity with different bacterial strains including *Bacillus nitratireducens, Bacillus cereus, Bacillus anthracis, Bacillus paramycoides* and *Bacillus wiedmanni*.
- JR18 bearing the accession number OR512177, was identified as the *Staphylococcus* spp., with a DNA length of 1516 bp, belonging to the phyla firmicutes. The phylogenetic analysis of JR18 revealed its 100% sequence similarity with *Staphylococcus aureus.*
- JR24 bearing the accession number OR512178, was identified as the *Proteus mirabilis*, with a DNA length of 1443 bp, belonging to the phyla enterobacteria. The phylogenetic analysis of JR24 revealed its 100% sequence similarity with *Proteus mirabilis*.

*Phytochemical analysis of isolated endophytic bacterial strains:*

All the five identified bacterial strains were undergone for their phytochemical analysis for presence of secondary metabolites, both qualitatively as well as quantitatively. The results of the qualitative analysis showed presence of all the three secondary metabolites (alkaloids, flavonoids and steroids) in all the 5 bacterial strains. The quantitation of each of the secondary metabolites showed presence of variable amount of alkaloids, flavonoids and steroids in all the endophytic bacteria (Table2, Figure 2). Brief summary of the results is as follows:

The trend for alkaloids was:
*Bacillus nitratireducens* (JR13) > *Bacillus cereus* (JR7) > *Staphylococcus* sp. (JR18) > *Bacillus thuringiensis* (JR9) > *Proteus mirabilis* (JR24)

The trend for flavanoids was:
*Proteus mirabilis* (JR24) > *Bacillus cereus* (JR7) > *Bacillus thuringiensis* (JR9) > *Staphylococcus* sp. (JR18) > *Bacillus nitratireducens* (JR13)

The trend for steroids was:
*Proteus mirabilis* (JR24) > *Bacillus thuringiensis* (JR9) > *Bacillus nitratireducens* (JR13) > *Staphylococcus* sp. (JR18) >*Bacillus cereus* (JR7)

*Antioxidant potential of the isolated bacterial strains:*
The presence of secondary metabolites has also been reported to contribute to antioxidant activity of bacteria. Considering this, the antioxidant potential of different bacterial strains was assessed using DPPH assay at different doses (20, 40, 60, 80 and 100 mg/L). The results indicate potent antioxidant activity of all the bacterial strains in a dose dependent manner.
The trend in DPPH activity was as follows:
*Staphylococcus* sp. (JR18) > *Proteus mirabilis* (JR24) > *Bacillus nitratireducens* (JR13) > *Bacillus cereus* (JR7) > *Bacillus thuringiensis* (JR9) (Table 3, Figure 3).

**Table 3: DPPH Free radical scavenging potential of the isolated endophytic bacteria.**

| S. No. | Code of isolates | Name of bacterial strain | Antioxidant potential at different concentrations (mg/L) | | | | | $IC_{50}$value (mg/L) |
|---|---|---|---|---|---|---|---|---|
| | | | 20 | 40 | 60 | 80 | 100 | |
| 1 | JR7 | *Bacillus cereus* | 9.22 | 11.28 | 14.72 | 18.53 | 22.65 | 263.50 |
| 2 | JR9 | *Bacillus thuringiensis* | 6.53 | 8.22 | 11.38 | 13.42 | 16.73 | 362.69 |
| 3 | JR13 | *Bacillus nitratireducens* | 15.27 | 18.53 | 20.44 | 24.17 | 27.64 | 249.53 |
| 4 | JR18 | *Staphylococcus* sp. | 28.47 | 33.07 | 35.70 | 39.53 | 43.26 | 137.66 |
| 5 | JR24 | *proteus mirabilis* | 15.78 | 19.13 | 22.54 | 25.73 | 29.11 | 225.62 |

**Table 4: Antibacterial potential of the isolated endophytic bacteria.**

| S. no. | Sequence code | Name of endophytic bacteria | Antibacterial activity at different concentrations | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 50 mg/L | | 100 mg/L | | 150 mg/L | | 200 mg/L | |
| | | | IZ | AI | IZ | AI | IZ | AI | IZ | AI |
| ***E. coli*** | | | | | | | | | | |
| **1** | JR13 | *Bacillus nitratireducens* | 13 | 0.361111 | 18 | 0.486486 | 19 | 0.5 | 21 | 0.525 |
| **2** | JR18 | *Staphylococcus* sp. | 11 | 0.305556 | 14 | 0.378378 | 15 | 0.394737 | 18 | 0.45 |
| **3** | JR24 | *Proteus mirabilis* | 16 | 0.444444 | 18 | 0.486486 | 19 | 0.5 | 24 | 0.6 |
| ***S. aureus*** | | | | | | | | | | |
| **1** | JR13 | *Bacillus nitratireducens* | 15 | 0.441176 | 17 | 0.485714 | 19 | 0.5 | 21 | 0.538462 |
| **2** | JR18 | *Staphylococcus* sp. | 9 | 0.264706 | 10 | 0.285714 | 16 | 0.421053 | 18 | 0.461538 |
| **3** | JR24 | *Proteus mirabilis* | 12 | 0.352941 | 14 | 0.4 | 15 | 0.394737 | 19 | 0.487179 |

*Antimicrobial activity of the isolated endophytic bacteria:*
Out of 5 identified isolated endophytic bacteria, total 3 isolates were selected for evaluating their antimicrobial potential against *E. coli* (Gram negative) and *S. aureus* (Gram positive bacteria). Each extract of endophytic bacteria was screened at different concentrations (50-200 mg/L) and Inhibition zone (IZ) was measured in mm (milimeter). Activity was also compared with standard antibiotic streptomycin at different concentrations and Activity index (AI) values were calculated (Table 4).

The results indicate variable antimicrobial activity of the isolated endophytic bacteria, both against *E. coli* as well as *S. aureus.*

The trend in antimicrobial activity of endophytic bacteria against *E. coli* was as follows (Figure 5):

50 mg/L: *Proteus mirabilis* (JR24) > *Bacillus nitratireducen* (JR13) > *Staphylococcus* sp. (JR18)

100 mg/L: *Bacillus nitratireducens* (JR13) > *proteus mirabilis* (JR24) > *Staphylococcus* sp. (JR18)

150 mg/L: *Bacillus nitratireducens* (JR13) > *proteus mirabilis* (JR24) > *Staphylococcus* sp. (JR18)

200 mg/L: *Proteus mirabilis* (JR24) > *Bacillus nitratireducens* (JR13) > *Staphylococcus* sp. (JR18)

The trend in antimicrobial activity of endophytic bacteria against *S. aureus* was as follows (Figure 6):

50 mg/L: *Bacillus nitratireducens* (JR13) > *proteus mirabilis* (JR24) > *Staphylococcus* sp. (JR18)

100 mg/L: *Bacillus nitratireducens*(JR13) > *proteus mirabilis* (JR24) > *Staphylococcus* sp. (JR18)

150 mg/L: *Bacillus nitratireducens* (JR13) > *Staphylococcus* sp. (JR18) > *Proteus mirabilis* (JR24)

200 mg/L: *Bacillus nitratireducens* (JR13) > *proteus mirabilis* (JR24) > *Staphylococcus* sp. (JR18)

*Effect of salt concentrations on the growth of endophytic bacteria:*
The isolated and identified nine endophytic bacteria were inoculated in different culture tubes having nutrient broth with varied salt concentrations (0%, 0.5%, 1%, 1.5%, 2%, and 2.5% NaCl) and incubated at 30°C for 72 hours. Then absorbance was taken at 660 nm by using UV visible spectrophotometer. The trend in salt tolerance ability of bacterial endophytes was as follows (Table 5, Figure 4):

0% conc.: *Proteus mirabilis* (JR24) > *Bacillus nitratireducens* (JR13) > *Staphylococcus* sp. (JR18)

0.5% conc: *Proteus mirabilis* (JR24) > *Bacillus nitratireducens* (JR13) > *Staphylococcus* sp. (JR18)

1% conc: *Staphylococcus* sp. (JR18) > *Proteus mirabilis* (JR24) > *Bacillus nitratireducens* (JR13)

1.5% conc: *Proteus mirabilis* (JR24) > *Staphylococcus* sp. (JR18) > *Bacillus nitratireducens* (JR13)

2% conc: *Proteus mirabilis* (JR24) > *Bacillus nitratireducens* (JR13) > *Staphylococcus* sp. (JR18)

2.5% conc: *Proteus mirabilis* (JR24) > *Bacillus nitratireducens* (JR13) > *Staphylococcus* sp. (JR18)

The results indicate variable values of resistance against salinity stress by different bacterial strains at all the tested concentrations.

Plants, which form the basis of sustenance of all the lifeforms on earth owing to their attribute to supply much needed oxygen to the living beings, are themselves at the mercy of a diverse plethora of endophytic organisms. These endophytes not only aid in plant growth under normal conditions but also assume the role of comrade in arms under the perilous conditions when survival of plant is jeopardized. Therefore, it would be appropriate to quote here, that each and every plant that has ever existed on earth owes its survival to the bacterial endophytes that aid in effective maintenance of plant homeostasis. Endophytic bacteria can be defined as the microbes isolated from surface-sterilized plant tissues, which aid in survival of host plants in a number of direct and indirect ways, such as:

- Production of key phytohormones that are crucial for plant growth such as auxins, gibberlins and ethylene[4].
- Production of phytostimulators and other plant growth regulators
- Facilitating growth of plants by iron uptake, phosphate solubilization and nitrogen fixation[5].
- Safegurading the plant from being attacked by plant pathogens[6].
- Abiotic and biotic stress tolerance[7].

Considering the crucial role of endophytic bacteria in plant growth and development, the current study is an attempt to isolate and characterize the bacterial endophytes inhabiting *Salvadora* spp. from the soil in Jaipur. This was followed by a series of biochemical and morphological tests coupled with sequencing and phylogenetic analysis to gain deeper insights into classification of the isolated bacterial strains and their biochemical attributes. Furthermore, the study showed production of several secondary metabolites by these endophytic bacteria, which were further found to aid in antioxidant activity as well as antimicrobial activity of the isolated endophytic bacteria. In addition to this, the study also showed ability of endophytic bacteria to provide abiotic stress tolerance to plants in the form of salinity stress.

The study began with isolation of 25 different endophytic bacteria, which were characterized into different classes on the basis of their biochemical and morphological analysis. The biochemical analysis of the isolated endophytic bacteria was performed on the basis of 15 different tests, namely, Gram staining, Catalase test, Amylase test, MR test, Lipase test, Indole test, VP test, Gelatinase test, Urease test, Citrate utilization test, motility, Protease test, $H_2S$ production, Phosphate solubilization test and siderophore test.

**Table 5: Growth of the isolated endophytic bacteria at different concentrations.**

| S. No. | Code of bacteria | Name of bacteria | Absorbance at different Salt concentration (%) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 0 | 0.5 | 1 | 1.5 | 2.0 | 2.5 |
| 1 | JR13 | *Bacillus nitratireducens* | 0.192 | 0.316 | 0.432 | 0.318 | 0.266 | 0.187 |
| 2 | JR18 | *Staphylococcus* sp. | 0.165 | 0.286 | 0.463 | 0.334 | 0.217 | 0.115 |
| 3 | JR24 | *proteus mirabilis* | 0.213 | 0.378 | 0.436 | 0.529 | 0.413 | 0.257 |

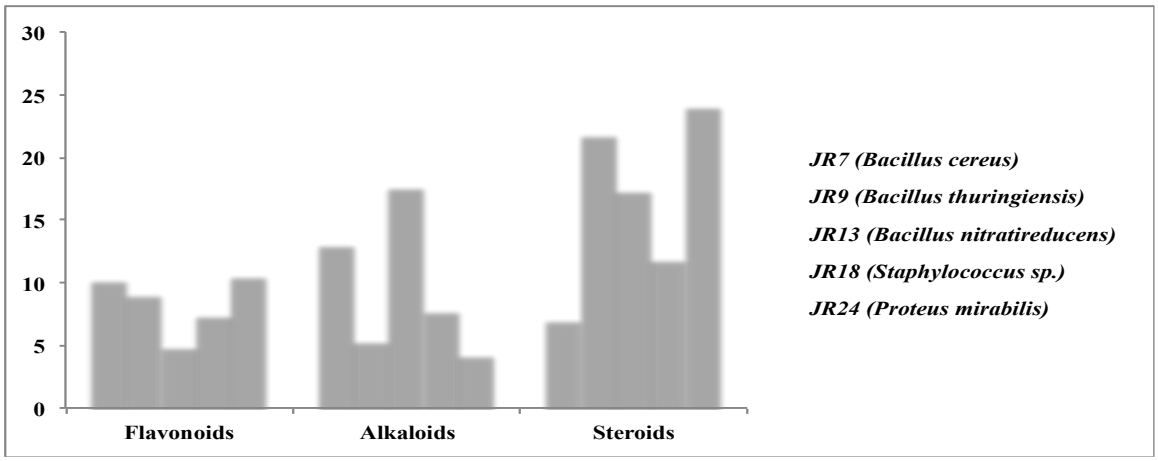


**Figure 2: Quantitative determination of secondary metabolites in the isolated bacterial strains.**

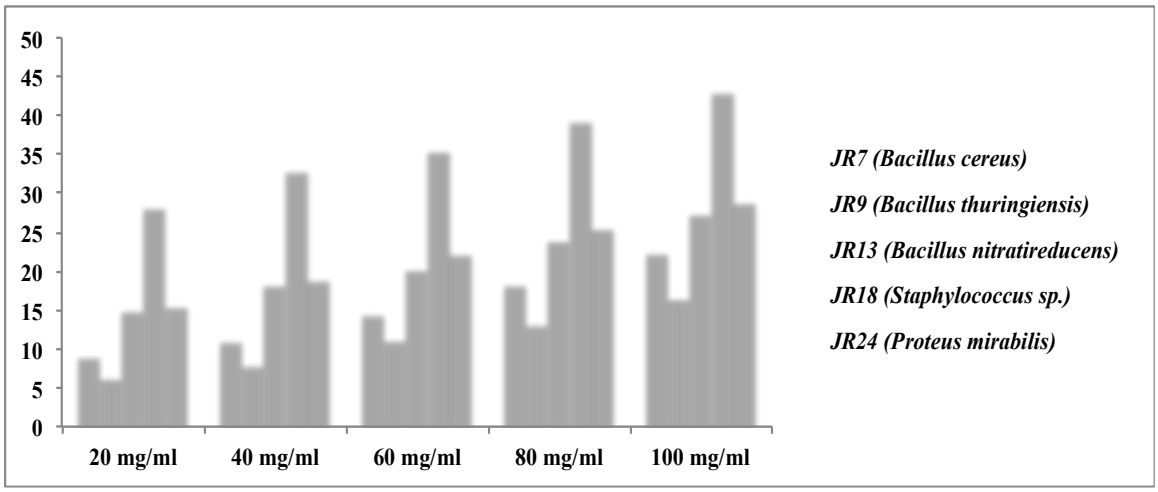


**Figure 3:DPPH Free radical scavanging potential of the isolated endophytic bacteria.**

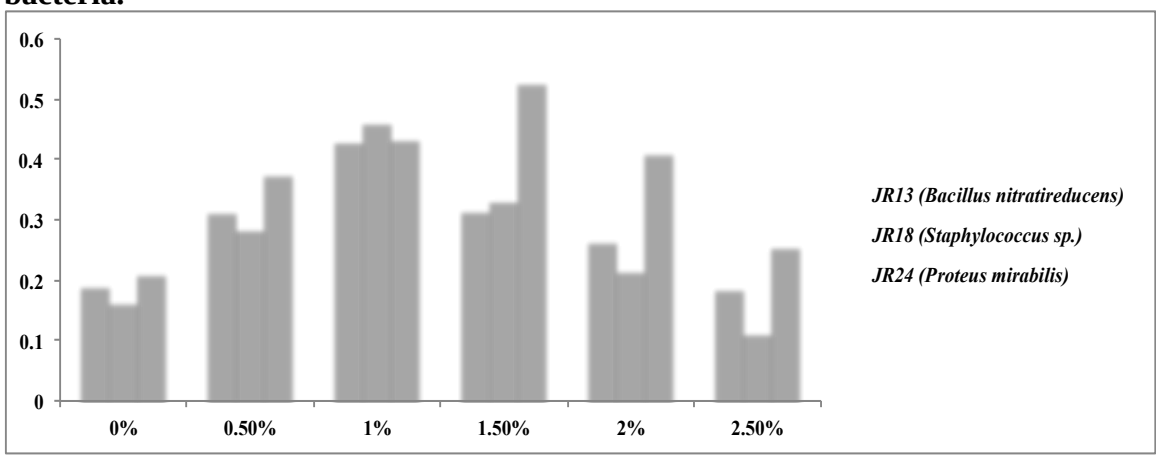


**Figure 4: Growth of the isolated endophytic bacteria at different concentrations.**

The results of these tests suggested that most of the isolated bacteria were gram-positive rods showing positive catalase and amylase activity. Most of these bacterial strains were VP positive with positive test for urease, citrate and protease. Furthermore, nearly 60% of the isolated bacterial strains were found to be phosphate solubilizing in nature and around 50% of the isolated bacterial strains were found to be indole positive. Both phosphate solubilization and indole production by bacteria are considered one of the most important plant growth promoting trait for endophytic bacteria owing to the stimulatory role of bacterial IAA in augmenting plant growth and development[8], fortification of plant immune system[9], increase in bacterial colonization efficiency of plants by rhizospheric and phyllospheric bacteria[10,] enhanced absorption of water and nutrients by plant roots[11], thus leading to an overall increase in plant growth, development and fitness. Therefore, considering the above hypothesis, 5 bacterial strains were selected on the basis of their indole production efficiency and subjected for sequencing for their molecular identification.

Thereafter, the sequencing results identified the isolated endophytic bacteria to be *Bacillus cereus, Bacillus thuringiensis, Bacillus nitratireducens, Staphylococcus* sp. and *Proteus mirabilis.* The isolated endophytic bacteria were further analyzed for their ability to produce secondary metabolites. All the five strains of isolated endophytic bacteria were found to produce flavonoids, alkaloids and steroids, all of which are believed to contribute to antioxidant activity of the endophytic bacteria, as indicated by results of DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The results shown in this study are in complete correlation with other studies, where too, researchers have demonstrated presence of alkaloids, steroids and flavonoids in bacterial endophytes[12-14]. Alkaloids have been reported for their anti-fungal, anti-viral and antibacterial activity, therefore, production of alkaloids by endophytic bacteria may be beneficial for host plant in augmenting plant immunity and boosting plant defense system against attack by nefarious pathogens. Similarly, antimicrobial activity of terpenoids and steroids has also been reported in a number of previous studies against disease due toplant pathogens[15-16]. Considering this, production of these three secondary metabolites (alkaloids, steroids and flavonoids) form a great combination as plant growth promoting trait for endophytic bacteria owing to their crucial role in fortifying plant immune system and safeguarding the plant from different microbial assailants. Antioxidant activity of bacterial endophytes such as *Staphylococcus* sp., *Bacillus pumilus, Bacillus safensis, Proteus mirabilis, Bacillus nitratireducens, Microbacterium testaceum* and *Bacillus thuringiensis* has previously been reported in a number of studies[17-18].

Thereafter, the researchers progressed to evaluate the antimicrobial efficacy of the endophytic bacteria against both *E. coli* (Gram negative) and *S. aureus* (Gram positive bacteria). The results indicate highest antimicrobial activity against *S. aureus* by *Bacillus australimaris, Bacillus safensis* and *Bacillus nitratireducens* at all the tested concentrations, whereas, lowest antimicrobial activity was observed in case of *Bacillus thuringiensis* and *Staphylococcus* sp.. Similar results showing antimicrobial activity of endophytic bacteria against *S. aureus*

have been demonstrated in previous studies[19-20]. The remarkable antimicrobial activity of all the tested endophytic bacteria maybe attributed to the presence of several bioactive secondary metabolites such as alkaloids, steroids and flavonoid; all of which have been reported to be anti-microbial in nature. In addition to this, the results further show remarkable abiotic stress tolerance ability of the endophytic bacteria by showcasing their ability to grow in presence of increasing concentrations of salt, which may be beneficial for the host plant in the long run since these endophytic bacteria shall continue to flourish in the plants even under conditions of extreme salinity, when plants are exposed to hypersaline soils. Also, these endophytic bacteria shall continue to support plant growth and render their plant growth promoting benefits even under hypersaline conditions, thus augmenting plant fitness and overall development.

## Conclusion

The study highlights the plant growth promoting attributes of the isolated endophytic bacterial strains, evident from their ability to produce IAA, produce secondary metabolites, exhibit potent antioxidant activity, showcase unparalleled antimicrobial activity and the ability to tolerate salinity stress. All these attributes are suggestive of the pivotal role of endophytic bacteria in plant growth and development while also providing both biotic as well as abiotic stress tolerance. However, the only drawback of this study is that the plant growth promoting traits of endophytic bacteria have not been reproduced at the translational level. Nonetheless, this study has prepared a solid groundwork for future studies where these isolated endophytic bacteria can be transfused into the plant system and their plant growth promoting attributes can be visualized at the translational level.

In this whole scenario of usage of endophytic bacteria for improving plant productivity and growth is; usage of these bacterial strains would eliminate the need of synthetic fertilizers as well as pesticides to increase plant productivity and protect them from attack by pathogens. Minimizing the utility of chemical pesticides and fertilizers would be a great step towards restoring the disturbed ecological balance and ameliorate the deteriorating environmental condition.

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