

RESEARCH ARTICLE

Screening of stress tolerance endophytic bacteria isolated from six stress bearing plants

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ABSTRACT

In the present study, salt tolerance and heavy metal tolerance, bacterial endophytes were isolated from six plants collected from Himachal Pradesh and Punjab states of India. Fifty isolates were obtained. The isolates were checked for their phosphate solubilization, nitrogen fixation, Urease, and Indole producing activity. Isolates were subjected to different NaCl concentrations ranging from 2% to 16% and heavy metal concentrations (lead and cadmium) 2-6%. Among 50 isolates, two isolates DL3R2 and A1S1S1 showed positive responses to phosphate solubilization, nitrogen fixation, Urease, and Indole producing activity. On comparing their ODs with control, it was found that the increase in the concentration of salt is a decrease in growth of the bacterial culture. Only two isolates R1L2 and A2L2L2 had growth at 12, 14 and 16% NaCl concentration. However, isolates had a sharp decrease in growth with the increase in heavy metal stress. Results indicate that three isolates DL2R2, R1L2, and A1S1S1 had growth at 6% lead concentration (0.229 ± 0.015 , 0.224 ± 0.01 , 0.425 ± 0.024), and isolates R1L2, DL3R2, and DP1L1L1 showed growth at 6% cadmium concentration. The ODs recorded at 600nm were 0.34 ± 0.013 , 0.637 ± 0.023 , 0.633 ± 0 . This study suggests that such endophytes have a scope to be used in the future to promote plant growth under salt and heavy metal stress thus promising an alternative method to make plants tolerable to different environmental stress conditions.

Keywords: Abiotic stress, endophytes, heavy metal, salt stress

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INTRODUCTION

Salinity and accumulation of heavy metal in the soil have resulted in the loss of productivity of soil (Ali et al., 2013). Salinity leads to osmotic and ionic imbalance in the plants, which ultimately affects the turgor pressure, cell division and cell elongation. It also affects the photosynthetic rate and transpiration rate (Vaishnav et al., 2019).

The presence of cadmium and lead in the soil is a serious threat to agricultural land. It may result in the contamination of ground water and also affect the food chain (Afzal et al., 2017). Its toxicity disturbs the photosynthesis activity of plants and may cause chlorosis resulting in plant death. Cadmium has a negative effect on seed germination, early seedling growth, and plant biomass. Photosynthesis, relative water content, transpiration rate, stomatal conductance, and electrolyte leakage all

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change as a result (Genchi et al., 2020). Microorganisms that live in the host tissue and do not possess any threat to plants are known as endophytes. They are known to have the potential to survive in harsh conditions such as salt and heavy metal stress (Gupta et al., 2020). Endophytic bacteria play an important role in plant growth promotion under normal and stress. It improves their nutrient intake ability as well as make them tolerable to cope up with the changing environmental condition. They produce many hormones which make competent with the stress. *Bacillus*, *Pantoea*, *Microbacterium*, *Curtobacterium*, *Cupriavidus*, *Siphonobacter*, and *Pseudomonas* are some of the Zinc and cadmium-tolerant endophytic bacteria isolated from *Murdannia spectabilis* (Rattanapolsan et al., 2020). The present research focuses on the isolation and screening of salt and heavy metal tolerance endophytic bacteria from six plants. The isolates obtained could be exploited to promote plant growth under stress.

MATERIALS AND METHODS

Isolation of endophytes

Host plants

Lantana camara, *Phoenix dactylifera*, *Hemerocallis fulva*, *Salvia Rosmarinus*, *Commiphora wightii*, and *Abutilon indicum* were collected from Himachal Pradesh and Punjab. Roots stem and leaves of the plant were washed with running or distilled water to remove dirt or soil attached to the samples. Surface sterilization is done with ethanol (70%) and sodium hypochlorite (3%). They were again rinsed with distilled water (Khalil et al., 2020). Roots, stems and leaves were crushed in sucrose solution with mortar and pestle and were added to nutrient broth and were incubated at 37°C for 24 hours. Colonies of the bacteria were sub cultured on nutrient agar plates and purification was done.

Screening of Isolates for Indole acetic acid

The development of indole acetic acid was confirmed on all 50 isolates. The culture was inoculated and incubated for 24-48 hours in tryptophan broth. Salkowski's reagent was used to determine the IAA produced. After incubation, the broth was centrifuged, and the supernatant was preserved. The supernatant was mixed with Salkowski reagent and kept at room temperature for 30 minutes. IAA productions were characterized by the solution's red to pink colour (Fouda et al., 2021).

Phosphate solubilization test

All the isolates were inoculated on Pikovskayas Agar plates and incubated at 28 °C for 48-120 hrs. The appearance of a clear zone indicates a positive result (Kaur and Kaur 2021).

Urease test

Christensen's Urea Agar containing urea was prepared and dispensed in test tubes. The culture was streaked and incubated at 37°C for 48hr to 7 days. The formation of pink colour indicates that the reaction is positive (Hall, 2013).

Nitrogen fixation test

Cultures were inoculated into Jensen's media (an N-free broth media) and were incubated at 37°C for 24-48hrs. Growth indicates the nitrogen-fixing capacity of bacteria (Bag et al., 2017).

Screening for salt tolerance

The isolates obtained were screened for their growth in culture media containing different NaCl concentration ranging from 2-16%. Tolerance to different NaCl concentration was assessed. The ODs of selected isolates at 600nm were taken and compared (Abedinzadeh et al., 2019). Triplicates were taken for each isolate.

Screening for heavy metal tolerance

The isolates were screened for two heavy metal named lead and cadmium tolerance. The isolates were subjected to different lead and cadmium concentration (2-6%). Isolates were grown on NA plates containing heavy metals and were incubated at 30±5 for 24-48hrs. For the assessment of growth each isolate was grown in NB medium containing heavy metal and ODs were recorded (Abidina et al., 2020; Zhao et al., 2017).

RESULTS

Fifty endophytic bacteria were isolated from six plants. All the isolates were screened for IAA, Phosphate solubilization, Urease and Nitrogen fixation (Table 1).

Table 1: Endophytes with their PGP activity

| Isolates | Indole | Phosphate solubilization | Nitrogen-fixing bacteria | Urease | Isolates | Indole | Phosphate solubilization | Nitrogen-fixing bacteria | Urease |
|----------|--------|--------------------------|--------------------------|--------|----------|--------|--------------------------|--------------------------|--------|
| G1R1 | - | - | + | + | L1S1 | - | - | - | - |
| G1R2 | - | - | - | - | L1S2 | - | - | - | - |
| G1S1 | - | - | - | - | L2L1 | - | - | + | - |
| G1S2 | - | - | - | - | L2L2 | - | - | + | + |
| G2R1 | + | - | - | - | L3L1 | - | - | - | - |
| G2R2 | - | - | - | + | L3L2 | - | - | - | - |
| G2S1 | - | - | - | - | L3S3S1 | - | - | - | - |
| G3R1 | - | - | + | + | A1S1S | - | + | + | + |
| G3R2 | - | - | + | + | A1S1S2 | - | - | + | + |
| G3S1 | - | - | - | - | A2S2S1 | - | - | - | - |
| G3S2 | - | - | - | - | A2S2S2 | - | - | - | - |
| DL1R1 | - | - | - | - | A2S2S3 | - | - | - | - |
| DL1R2 | - | - | - | + | A2L2L1 | - | - | - | - |
| DL2R1 | + | - | - | + | A2L2L2 | - | - | - | - |
| DL2R2 | - | - | + | + | DP1L1L1 | - | - | - | - |
| DL3R1 | - | - | + | + | DP1L1L2 | - | - | - | - |

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| | | | | | | | | | |
|--------------|---|---|---|---|----------------|---|---|---|---|
| DL3R2 | + | + | + | + | DP1S1S1 | - | - | - | - |
| DL3L2 | | | + | + | DP1S1S2 | - | - | - | - |
| DL3L1 | + | - | + | + | DP2S2S1 | - | - | - | - |
| R1L1 | - | - | + | + | DP2S2S2 | - | - | - | - |
| R1L2 | - | - | + | + | DP2L2L1 | + | - | - | - |
| R2L1 | - | - | + | + | DP3L3L1 | - | - | - | - |
| R3L1 | - | - | - | - | DP3L3L2 | - | - | - | - |
| L1L1 | - | - | - | - | DP3S3S1 | - | - | - | - |
| L1L2 | + | - | - | - | DP3S3S2 | - | - | - | - |

The isolates were further screened for salt and heavy metal stress tolerance. The isolates were grown on different NaCl concentration (2-16%) and heavy metal lead and cadmium concentration (2-6%). The ODs of the isolates in triplicates were taken at 600nm and compared with the control (Table 2 and 2a).

Table 2: The ODs of isolates at different NaCl concentrations (2-16%).

| Isolates | NaCl concentration | | | | | | | | |
|----------|--------------------|-------------|--------------|--------------|-------------|-------------|-----|-----|-----|
| | Control | 2% | 4% | 6% | 8% | 10% | 12% | 14% | 16% |
| G1R1 | 1.731±0.168 | 1.081±0.005 | 0.854±0.0431 | - | - | - | - | - | - |
| G1R2 | 1.443±0.107 | 0.988±0.028 | 0.979±0.006 | - | - | - | - | - | - |
| G1S1 | 1.422±0.085 | 1.306±0.018 | - | - | - | - | - | - | - |
| G1S2 | 1.373±0.054 | 1.296±0.022 | 0.916±0.020 | - | - | - | - | - | - |
| G2S1 | 1.550±0.094 | 1.048±0.008 | 0.849±0.017 | - | - | - | - | - | - |
| G2R2 | 1.286±0.074 | 0.938±0.012 | 0.831±0.009 | - | - | - | - | - | - |
| G3R1 | 1.557±0.091 | 1.264±0.026 | 0.920± 0.024 | 0.333±0.0120 | 0.322±0.018 | 0.234±0.009 | - | - | - |
| G3R2 | 1.552±0.109 | 1.087±0.006 | 0.944 ±0.074 | - | - | - | - | - | - |
| G3S1 | 1.595±0.037 | 1.527±0.136 | - | - | - | - | - | - | - |
| DL1R1 | 1.606±0.077 | 1.486±0.293 | 1.094±0.062 | - | - | - | - | - | - |
| DL1R2 | 1.230±0.121 | 1.193±0.113 | | - | - | - | - | - | - |
| DL2R1 | 1.902±0.055 | 1.884±0.009 | 0.869±0.011 | - | - | - | - | - | - |
| DL2R2 | 1.443±0.162 | 1.348 ±0.14 | 0.958±0.003 | 0.247± 0.012 | - | - | - | - | - |
| DL3R1 | 1.624±0.090 | 1.094±0.010 | 0.715±0.011 | 0.217± 0.006 | - | - | - | - | - |
| DL3R2 | 1.649±0.174 | 1.475±0.006 | 1.211±0.051 | 0.522± 0.062 | 0.463±0.014 | - | - | - | - |
| DL3L1 | 1.47±0.167 | 1.321±0.074 | 0.790±0.006 | - | - | - | - | - | - |
| DL3L2 | 1.42±0.014 | 1.299±0.044 | 1.068±0.185 | 0.373± 0.123 | 0.22± 0.008 | - | - | - | - |

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|---------|--------------|-------------|--------------|--------------|-------------|-------------|-------------|-------------|-------------|
| R1L1 | 1.180±0.020 | 1.171±0.013 | 0.915±0.022 | - | - | - | - | - | - |
| R1L2 | 1.167±0.019 | 1.102±0.019 | 0.768±0.585 | 0.280±0.013 | 0.27±0.005 | 0.22±0.008 | 0.219±0.17 | 0.196±0.015 | 0.176±0.062 |
| R2L1 | 1.632±0.162 | 1.320±0.011 | 0.765±0.004 | 0.215±0.007 | - | - | - | - | - |
| R3L1 | 1.725±0.053 | 1.408±0.074 | - | - | - | - | - | - | - |
| L1L1 | 1.795±0.134 | 1.525±0.052 | 1.313±0.006 | - | - | - | - | - | - |
| L1L2 | 1.510±0.179 | 1.418±0.077 | 1.002±0.017 | 0.239± 0.018 | 0.211±0.010 | 0.208±0.006 | - | - | - |
| L1S1 | 1.1203±0.038 | 0.980±0.051 | 0.937±0.005 | - | - | - | - | - | - |
| L1S2 | 1.1566±0.039 | 1.076±0.005 | 0.929±0.052 | - | - | - | - | - | - |
| L2L1 | 1.453±0.171 | 1.179±0.055 | 0.987±0.007 | - | - | - | - | - | - |
| L2L2 | 1.579±0.050 | 1.333±0.061 | 1.437±0.079 | - | - | - | - | - | - |
| L3L1 | 1.271±0.107 | 0.926±0.025 | - | - | - | - | - | - | - |
| L3L2 | 1.966±0.021 | 1.717±0.007 | 1.536±0.020 | - | - | - | - | - | - |
| L3S3S1 | 1.531±0.125 | 1.254±0.081 | - | - | - | - | - | - | - |
| A1S1S | 1.046±0.168 | 0.878±0.013 | 0.798±0.007 | - | - | - | - | - | - |
| A1S1S2 | 1.516±0.080 | 1.399±0.055 | 0.747±0.005 | 0.231± 0.011 | - | - | - | - | - |
| A2S2S1 | 1.26±0.581 | 0.883±0.007 | - | - | - | - | - | - | - |
| A2S2S2 | 1.1993±0.073 | 0.865±0.027 | 0.659±0.041 | - | - | - | - | - | - |
| A2S2S3 | 1.312±0.120 | 1.007 ±0.01 | 0.722±0.01 | 0.252±0.035 | 0.227±0.006 | 0.216±0.005 | - | - | - |
| A2L1L1 | 0.925±0.009 | 0.801±0.068 | - | - | - | - | - | - | - |
| A2L2L2 | 1.260±0.023 | 0.865±0.029 | 0.77 ± 0.017 | 0.348±0.035 | 0.319±0.004 | 0.250±0.008 | 0.226±0.005 | 0.191±0.028 | 0.141±0.051 |
| DP1L1L1 | 1.471±0.063 | 1.363±0.492 | - | - | - | - | - | - | - |
| DP1L1L2 | 1.619±0.070 | 0.643±0.548 | 0.539±0.007 | 0.279±0.009 | 0.253±0.057 | 0.236±0.006 | - | - | - |
| DP1S1S1 | 1.516±0.284 | 1.088±0.009 | - | - | - | - | - | - | - |
| DP2S2S1 | 1.151±0.075 | 1.064±0.027 | - | - | - | - | - | - | - |
| DP2S2S2 | 1.792±0.064 | 1.604±0.009 | - | - | - | - | - | - | - |
| DP2L2L2 | 1.1753±0.184 | 0.980±0.005 | - | - | - | - | - | - | - |
| DP3L3L1 | 1.422±0.084 | 1.281±0.058 | - | - | - | - | - | - | - |
| DP3L3L2 | 1.128±0.020 | 1.092±0.004 | - | - | - | - | - | - | - |
| DP3S3S1 | 1.646±0.028 | 1.418±0.074 | - | - | - | - | - | - | - |
| DP3S3S2 | 1.315±0.089 | 1.285±0.006 | - | - | - | - | - | - | - |

Table. 2 (b) ANOVA results for the effect of salt concentration on the growth of screened isolate

| | Isolates | |
|---------------------|----------|-------|
| | R1I2 | DL3R2 |
| P value | <0.05 | <0.05 |
| Significance | Yes | Yes |

There is a decrease in the growth of isolates with the increase in salt concentration. Among 50 isolates, only 6 isolates showed growth at 10% salt concentration, whereas at 12,14 and 16% salt concentration, only two isolates showed growth. A decrease in the optical density of isolate A2L2L2 and R1L2 at different salt concentrations were compared to control. Highest ODs were recorded at 2% and 4%, whereas the least were recorded at 14% and 16% with $p < 0.05$

Table 3(a): ODs of isolates at different cadmium concentrations at 600nm absorbance

| Isolates | Cadmium concentration (%) | | | |
|----------------|---------------------------|--------------|-------------|-------------|
| | Control | 2% | 4% | 6% |
| G1R1 | 1.731±0.168 | 0.344±0.039 | 0.344±0.049 | - |
| G2R2 | 1.286±0.074 | 1.27±0.073 | 1.24±0.141 | - |
| G2S1 | 1.550±0.094 | 1.028±0.014 | - | - |
| R1L2 | 1.167±0.019 | 0.941±0.082 | 0.623±0.033 | 0.34±0.013 |
| DL3L1 | 1.47±0.167 | 1.113±0.012 | 1.11±0.088 | - |
| DL3R2 | 1.649±0.174 | 1.436±0.048 | 1.37±0.010 | 0.637±0.023 |
| L1L1 | 1.795±0.134 | 0.681±0.007 | - | - |
| DP1L1L1 | 1.471±0.063 | 1.1413±0.006 | 1.105±0.565 | 0.633±0.009 |
| DP2L2L2 | 1.1753±0.184 | 0.585±0.019 | - | - |
| A1S1S | 1.046±0.168 | 0.747±0.009 | - | - |
| A2S2S3 | 1.312±0.120 | 1.087±0.010 | 0.780±0.049 | - |

Table 3(b): ANOVA results for the effect of cadmium concentration on the growth of screened isolates

| | Isolates | |
|---------------------|----------|-------|
| | R1I2 | DL3R2 |
| P value | <0.05 | <0.05 |
| Significance | Yes | Yes |

The growth of the isolates varies at different concentrations. Among 50 isolates, 11 showed growth at different concentrations (1-6%). G1R1, G2R2, R1L2, DL3R2, L1L1 were able to grow at 4% concentration. DL3R2, R1L2, and DP1L1L1 are the isolates showing growth at 6% cadmium concentration (Table 3 a and b) . However, comparing their growth with control, we found that their growth decreases with increased cadmium concentration.

The growth of the isolates was found to decrease at every concentration. R1L2, A1S1S, and DP2L2L2 are the isolates that can stand with 6% lead concentration. 13 isolates grew at 2% lead concentration, 6 at 4%, and 3 at 6% concentration (Table 4 a and b).

Table 4 (a) ODs of isolates at a different lead concentration at 600nm absorbance

| Isolates | Lead concentration (%) | | | |
|----------|------------------------|-------------|-------------|-------------|
| | control | 2% | 4% | 6% |
| G2S1 | 1.550±0.094 | 1.623±0.027 | 0.662±0.021 | - |
| G3R1 | 1.557±0.091 | 1.274±0.043 | - | - |
| G3R2 | 1.552±0.109 | 0.474±0.008 | - | - |
| DL1R1 | 1.606±0.077 | 1.362±0.017 | - | - |
| DL2R1 | 1.902±0.055 | 1.123±0.010 | 0.665±0.003 | - |
| DL2R2 | 1.443±0.162 | 0.783±0.761 | 0.345±0.005 | 0.229±0.015 |
| DL3R1 | 1.624±0.090 | 0.233±0.030 | - | - |
| DL3R2 | 1.649±0.174 | 0.973±0.019 | - | - |
| R1L2 | 1.167±0.019 | 1.256±0.216 | 0.383±0.004 | 0.224±0.01 |
| R2L1 | 1.632±0.162 | 1.321±0.012 | 0.285±0.057 | - |
| A1S1S | 1.046±0.168 | 1.037±0.044 | 0.855±0.048 | 0.425±0.024 |
| DP2L2L2 | 1.175±0.184 | 1.065±0.012 | - | - |

Table 4(b) ANOVA results for the effect of lead concentration on the growth of screened isolates

| P value | Isolates | | |
|--------------|----------|-------|---------|
| | R1I2 | DI3R2 | DP1L1L1 |
| P value | <0.05 | <0.05 | <0.05 |
| Significance | Yes | Yes | Yes |

DISCUSSION

Endophytes are known to promote tolerance of plants against many stresses such as salt, heavy metal, temperature etc. Studies has shown that endophytic bacteria procured from six plants showed rise in sugar and GSH content in rice plants under salt stress. It has also reduced the ABA quantity and have also produced many organic compounds (Khan et al., 2020).

In Present study 50 endophytic bacteria were isolated from six plants and were screened for IAA, Ureaase, Phosphate solubilization and Nitrogen fixation. They were further screened for salt and heavy metal tolerance. Isolates were subjected to different NaCl concentration in the range 2-16%. Among 50 isolates three isolates R1L2 and A2L2L2 showed tolerance to 12%, 14% and 16% salt concentration but there is a sharp decrease in growth with the increase in salt concentration. Studies showed that on inoculating the endophytic bacteria there is a decrease in IAA production with the increase in NaCl concentration (100–400 mM). however, there is an increase in growth of soybean plants under salt stress. (Khan et al., 2019).

It was observed that the with the increase in the heavy metal concentration there is a substantial decrease in growth of the bacterial isolate. A study has shown that on inoculating the three endophytic bacteria separately and together with *B. mutica* at four different concentration of cadmium i.e. 100, 200, 400, and 1000 mg, plant physiological parameters, biomass production, bacterial colonization, and Cd accumulation were measured. The use of endophytic bacteria in combination was more effective. The maximum activity of consortium was seen at 100 mg Cd kg⁻¹ of soil (Ahsan et al., 2019). *B. cereus* demonstrated high potential of Pb uptake (4.540.38 mg/g) and removal (8.360.70 percent) with no substantial difference ($p>0.05$) from the other strains, with the exception of *P. psychrophila* (1.360.23 mg/g of Pb uptake, and 2.600.444 percent removal) (Yongpisanphop et al., 2020).

This study shows that heavy metal application was found to have major effects on the plants. Plant growth indices under Zn²⁺ and Cd²⁺ 20 treatments had shown significant differences ($P<0.05$) as compared to plants in the control treatment. Plants respond positively on the Zn²⁺ 21 treatment while the Cd²⁺ 22 treatment had the opposite effect (Afzal et al., 2020).

CONCLUSION

All bacteria isolated from six plants were screened for salt and heavy metal tolerance. Some of them were able to tolerate salt stress up to 16% concentration whereas few showed no growth after 10% salt concentration. Similarly, three isolates showed growth at 6% concentration of lead and cadmium. This research provides us the basis of the utilization of such stress tolerance endophytes for plant cultivation under stress condition as such endophytes helps the plants to tolerance wide concentration of salt and heavy metal stress thus suggesting an alternate method to conserve plants under stress.

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