Isolation and Screening of Beneficial Endophytic Bacteria from Rice Grown under Coastal Saline Soils

S. Parvathapriya, R. ThamizhVendan, R. Subhashini, S. Thiruvudai Nambi and R. Renuka

Department of Agricultural Microbiology, Department of Plant Pathology, Department of Biotechnology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai, India

*Corresponding author

Int. J. Curr. Microbiol. App. Sci (2021) 10(01): 2672-2679

Original Research Article

https://doi.org/10.20546/ijcmas.2021.1001.310

A B S T R A C T

Salinity causes disturbance in symbiotic performance of plant and increases susceptibility of plants to soil-borne pathogens. Endophytic bacteria which are associated with their host plants have a beneficial effect for many different plant species and are determinant of cross-tolerance to biotic and abiotic stresses in plants. To maintain the growth and development of crops in saline condition, plant growth promoting rhizobacteria (PGPR) were isolated, and detected their plant growth promoting (PGP) potential under salt stress was investigated. In this study, fifteen endophytic bacteria were isolated from aerial parts of the rice which were grown in different regions of coastal soils. It was concluded that isolates Ri 4 and Ri 12 exhibited higher IAA production, isolates Ri 4 and Ri 5 showed better solubilization in phosphorous, Ri 3 and Ri 4 showed better solubilization of potassium. The result of this study can be used for further investigation in enhancing crop production and maintaining soil health in coastal saline soil environment.

Key words: Beneficial Endophytic Bacteria, Coastal Saline Soils

Introduction

Rice (Oryza sativa) is one of the major staple food crops consumed globally and its production is highly affected by salinity. In India, nearly 9.38 million ha area is occupied by salt-affected soils out of which 5.5 million ha are saline (including coastal regions). Better management practices are needed to improve the productivity and quality of such low productive salty soils. Under the saline conditions the growth and development of rice are reduced because of the salinity-induces changes in metabolism and acidification of apoplast which affects the turgor pressure of cell (Munns and Tester, 2008). In plants, the effect of salinity leading to inhibition of germination, difficulties in crop area establishment, leaf area development, decrease in dry matter production, delay in seed set and also even sterility can occur (Khatun and Flowers., 1995).

Endophytes are the group of microorganisms that colonize the internal tissues of plants
either by symbiotically or in a mutualistic relationship (Dudeja et al., 2012). However some of the endophytic bacteria exert several beneficial effect on host plants, such as stimulation of plant growth, nitrogen fixation, secretion of plant growth regulators (eg: IAA, phosphate solubilization activity) and induction of resistance to plant pathogens (Hung et al., 2004). Osmotic adjustment, stomatal regulation, modification of root morphology, enhance uptake of minerals and alteration of nitrogen accumulation and metabolism are some of the other effects of endophytes infection on the host plants (Zinniel et al., 2008, Stoltzfus et al., 1997). Various researchers reported that bacteria isolated from saline environment are more likely to withstand salt stress. The present investigation was carried out to isolate and screen the beneficial endophytic bacteria from rice (Oryza sativa) which are cultivated in coastal saline soil regions of Tamil Nadu.

Materials and Methods

Sample collection

The samples (rice crop) were collected from different coastal regions of Tamil Nadu (Cuddalore, Puducherry, Karaikal, Nagapattinam, Ramanathapuram). The samples were asymptomatic and healthy. The sterile plastic bags were used to collect all the samples and transported to the laboratory aseptically. The pre-treatment/ surface sterilization and isolation of endophytic bacteria from the collected samples were carried out according to the method described by Sun et al., (2006) with some modification.

Surface sterilization

Fresh leaf sample were washed in running tap water and surface disinfection were carried out in stepwise washing in 70% ethanol for 5min, 2% sodium hypochlorite solution for 1min, 70% ethanol for 30sec and followed by two rinses of sterile water and samples were dried Araujo et al., (2002). To conform whether surface sterilization was done properly, the sterilized leaf samples were imprinted in LB media or the aliquot of sterile water used in final rinse were inoculated into sterile LB broth to examine growth for overnight at 37ºC, if no growth were observed then the disinfection process is successful.

Isolation of endophytic bacteria

After pretreatment, the samples were cut into small pieces and crushed in sterile water using pestle and mortar. About 1ml of crushed leaf sample was serially diluted up to 10⁻⁶ dilution and 0.1ml of aliquot from 10⁻⁵ and 10⁻⁶ were spread on sterile petri-plate containing LB media using sterilized glass rod. All the plates were incubated at 37ºC for 5 days and observations were recorded regularly in order to recover maximum amount of colonies. After incubation, morphologically different isolates were streaked on petri-plate containing LB media and incubated it for 3-5 days at 37ºC.

Morphological and biochemical characterization of endophytic bacterial isolates

Classical gram staining method was used to determined cell morphology (Bathlomew, 1962). Biochemical test for citrate utilization, starch hydrolysis, indole test, methyl red and voges-proskauer were carried out according to the procedure described by Cappuccino and Sherman (2002).

Screening for indole acetic acid producing activity

Indole acetic acid production (Glickmann and Dessaux 1995) was examined colorimetrically using Salkowski’sreagent(1 ml of 0.5M FeCl₃
in 49 ml of 35% perchloric acid). To measure IAA qualitatively, isolated strains were aseptically cultured in respective broth containing 100µg L-tryptophan per ml and incubated at 37°C for 7 days.

After incubation period, the grown isolates were centrifuged at 10,000 rpm for 20 minutes. 1ml of supernatant were mixed with two ml of Salkowski’s reagent and one drop of ortho-phosphoric acid and incubated at room temperature for 25-30 min. Development of pink colour showed IAA production. For quantitative analysis, the absorbance of developed pink colour was measured at 530nm and IAA concentrations were calculated by using IAA standard curve.

**Quantitative determination of phosphate solubilization activity**

Bacterial isolates were screened for their potential to solubilize insoluble calcium phosphate on Pikovskaya agar medium as described by Pikovskaya (1948). Fifteen endophytic bacterial strains were further evaluated quantitatively (Murphy and Riley, 1962), for their P-solubilizing ability in Pikovskaya’s liquid medium in which 0.5% of tri-calcium were added as substrate. The strains were inoculated into 10ml of respective broth and incubated at 30°C for 7 days. Un-inoculated liquid broth was taken as control. After incubation period, the samples were centrifuged at 10,000 rpm for 10 min to remove insoluble potassium. Five ml of culture filtrate was added with 25ml of 1N Ammonium acetate and the mixture samples were kept in mechanical shaker for 10 min. After incubation the samples were filtered through Whatman No.1 filter paper and the collected filtrate were fed into flame photometer to determine the K content. The amount of potassium released were calculated using standard curve in which KCl used as standard. Un-inoculated broth was used as control.

**Statistical analysis**

The experiments were conducted in a completely randomized block design. The mean of three replicates were used to present the results. Standard deviation used to estimate the sample variability. Analysis of variance on the data at CD(0.05%)

**Results and Discussion**

Endophytic bacterial isolates were isolated from rice crop that are grown in coastal saline regions of Tamil Nadu (Cuddalore, Karaikal, Nagapattinam, Puducherry, Ramanathapuram) by using LB media. Some of the endophytic strains have been isolated from aerial tissues of four agronomic crops and from prairie plant species and also from other parts of the plants such as roots.
et al., 2010), stems and seeds (Magnani et al., 2010), petioles, tuber tissues and flowers (Reiter and Sessitsch, 2004) can also be used in isolation of endophytes.

Fifteen isolates were selected for further investigation based on their growth and morphology was shown in Table 1. Gayathri et al., 2010 isolated 36 bacterial strains based on different morphology from mangrove and salt marsh plants. In morphological characterization the endophytic bacterial isolates exhibit the diverse colony shape, colour, margins, elevation including round, circular to irregular colonies with regular and wavy margins.

Out of 15 isolates 6 were pigmented and 9 were non pigmented. Regarding the cell shape and gram staining, 7 gram negative bacilli, 1 gram negative cocci, 4 gram positive bacilli and 3 gram positive cocci were observed. Zinniel et al., (2002) reported an equal presence of both gram positive and negative bacteria in their study about endophytic bacteria isolation. Biochemical tests were carried out in which Ri 3, Ri 4, Ri 6, Ri 7, Ri 10, Ri 11 and Ri 12 were positive in citrate utilization test, they produced blue colour in Simmon's citrate agar (Table 2).

Phosphate solubilization potential of the isolates was evaluated and the results indicated a significant variation among the isolates to solubilize different quantities of PO₄ and release of P into the broth.

Maximum P release was noticed in Ri 5 (0.747±0.003 mg l⁻¹) followed by Ri 4 (0.590±0.004 mg l⁻¹). Minimum was reported with the isolate Ri 15 (0.034±0.004 mg l⁻¹).

**Table 1** Morphological characterization of endophytic bacteria isolated from aerial parts of rice crop grown in coastal saline soils

| ISOLATES | GRAM REACTION | SHAPE   | COLONY MORPHOLOGY          |
|----------|---------------|---------|---------------------------|
|          |               | Form    | Elevation  | Margin | Colony                          |
| Ri1      | gram negative | Bacilli  | Circular   | Raised | Entire Translucent, slimy       |
| Ri2      | gram positive | Bacilli  | Circular   | Raised | Entire Opaque                   |
| Ri3      | gram negative | Bacilli  | Circular   | Flat   | Entire Colourless               |
| Ri4      | gram positive | Cocci    | Irregular  | Raised | Wavy Dark brown                |
| Ri5      | gram negative | Bacilli  | Irregular  | Raised | Wavy Light yellow              |
| Ri6      | gram positive | Cocci    | Circular   | Raised | Entire Rough yellow            |
| Ri7      | gram negative | Cocci    | Irregular  | Umbonate | Entire Opaque                |
| Ri8      | gram negative | Bacilli  | Round     | Flat   | Entire Transparent,slimy,glistening |
| Ri9      | gram positive | Bacilli  | Regular   | Convex | Entire Translucent             |
| Ri10     | gram positive | Cocci    | Round     | Convex | Entire Yellow                  |
| Ri11     | gram positive | Bacilli  | Round     | Raised | Entire Opaque                  |
| Ri12     | gram negative | Bacilli  | Round     | Flat   | Entire Opaque                  |
| Ri13     | gram positive | Bacilli  | Irregular  | Undulate | Wavy Colourless              |
| Ri14     | gram positive | Bacilli  | Circular  | Flat   | Entire Orange                  |
| Ri15     | gram negative | Bacilli  | Circular  | Raised | Entire Light brown             |
### Table 2 Results of biochemical tests for selected endophytic bacteria from rice

| ISOLATES | MR | VP | CATALASE | SIMMON CITRATE UTILIZATION | STARCH HYDROLYSIS | INDOLE |
|----------|----|----|----------|----------------------------|------------------|--------|
| Control  | -  | -  | -        | -                          | -                | -      |
| Ri1      | +  | +  | +        | -                          | -                | -      |
| Ri2      | -  | +  | +        | +                          | +                | +      |
| Ri3      | +  | -  | -        | +                          | -                | +      |
| Ri4      | +  | -  | +        | -                          | +                | ++     |
| Ri5      | -  | +  | -        | +                          | -                | ++     |
| Ri6      | +  | -  | -        | +                          | -                | -      |
| Ri7      | +  | -  | +        | +                          | +                | +      |
| Ri8      | -  | +  | +        | -                          | -                | -      |
| Ri9      | +  | -  | +        | -                          | +                | -      |
| Ri10     | +  | -  | +        | -                          | +                | -      |
| Ri11     | +  | +  | -        | -                          | +                | ++     |
| Ri12     | +  | +  | +        | -                          | +                | -      |
| Ri13     | -  | -  | -        | +                          | -                | -      |
| Ri14     | -  | -  | -        | -                          | -                | -      |
| Ri15     | +  | -  | -        | +                          | -                | ++     |

++ highly positive, + positive, - negative

### Table 3 IAA production, P solubilization and K solubilization activity of endophytic bacterial isolates

| Endophytic bacterial isolates | Phosphorous solubilization (P release mg l⁻¹) | Potassium solubilization (K release mg l⁻¹) | Indole Acetic Acid (µg ml⁻¹) |
|------------------------------|---------------------------------------------|---------------------------------------------|----------------------------|
| Control                      | 0.013±0.002⁹                           | 12.14±0.093⁸                               | 0.177±0.001¹⁰            |
| Ri1                          | 0.312±0.004⁸                           | 14.18±0.220⁷                               | 0.922±0.008⁶             |
| Ri2                          | 0.402±0.001¹                           | 15.56±0.252⁶                               | 1.110±0.009⁴             |
| Ri3                          | 0.590±0.004³                           | 20.14±0.407¹                               | 1.112±0.002³             |
| Ri4                          | 0.747±0.003⁴                           | 19.45±0.165⁴                               | 1.382±0.009¹             |
| Ri5                          | 0.489±0.005⁶                           | 18.56±0.212⁴                               | 1.229±0.003¹             |
| Ri6                          | 0.236±0.003⁷                           | 14.77±0.257⁶                               | 0.541±0.006¹             |
| Ri7                          | 0.520±0.006⁵                           | 19.74±0.125⁴                               | 1.362±0.016⁴             |
| Ri8                          | 0.215±0.003⁶                           | 13.19±0.218⁴                               | 0.726±0.011⁸             |
| Ri9                          | 0.155±0.004⁷                           | 15.15±0.128⁶                               | 0.654±0.007⁸             |
| Ri10                         | 0.448±0.004⁸                           | 12.89±0.016⁷                               | 1.091±0.003⁹             |
| Ri11                         | 0.114±0.004⁵                           | 15.70±0.220⁷                               | 1.247±0.008⁸             |
| Ri12                         | 0.247±0.001¹                           | 16.05±0.352⁴                               | 1.355±0.027⁵             |
| Ri13                         | 0.401±0.002¹                           | 16.88±0.172⁵                               | 0.937±0.013⁸             |
| Ri14                         | 0.034±0.001¹                           | 13.57±0.247⁷                               | 0.564±0.004¹             |
| Ri15                         | 0.28±0.005⁸                            | 14.94±0.127⁷                               | 0.219±0.001¹             |
| Grand Mean                   | 0.324                                  | 15.791                                    | 1.001                    |
| SE.D                         | 0.006                                  | 0.384                                     | 0.019                    |
| CD(0.05)                     | 0.013                                  | 0.782                                     | 0.039                    |
The results were given in Table 3. Endophytic bacteria residing within plant tissues have been reported to promote the plant growth either directly or indirectly through the production of phytohormones and the improvement of nutritional status (Pandey et al., 2008). Huang et al., (2005) reported that most of the phosphate solubilizing endophytic bacteria belonging to Bacillus, Pseudomonas, Klebsiella and Acinetobacter were isolated from maize and rape plants. Thamizh Vendan et al., (2010) studied the endophytes of Gingseng plants and the endophytic isolates belonging to Bacillus cereus and Bacillus megaterium showed notable P-solubilization activity by detecting extracellular solubilization of precipitated tri-calcium phosphate with glucose as sole carbon. Phosphate solubilization by Bacillus sp from salt stressed environment had been observed (Sun et al., 2006) earlier. The amount of potash solubilized was assessed by using Flame photometer. Among the fifteen isolates, significantly higher release of K was observed in Ri3 (20.14 ± 0.407 mg l\(^{-1}\)) followed by Ri4 (19.45 ± 0.165 mg l\(^{-1}\)). Minimum was reported in Ri15 (12.89 ± 0.016 mg l\(^{-1}\)) and the results were tabulated in Table 3. Padma and Sukumar, (2015) reported that a considerably higher concentration of potassium solubilizing bacteria (KSB) are commonly found in the rhizosphere in comparison with non-rhizosphere soil. KSB are usually present in all soils and have been isolated from rhizosphere soil, non-rhizosphere soil, paddy soil (Bakhshandeh et al., 2017) and saline soil (Bhattacharya et al., 2016). IAA production was observed in 15 isolates of endophytic bacteria which were grown in LB broth supplemented with 0.1% tryptophan and the results were presented in Table 3. Most of the isolates exhibited significant variation in IAA production. In this study the isolate Ri 4 produced higher quantity of IAA (1.382±0.009 µg ml\(^{-1}\)) which was significantly higher than other isolates. The minimum production of IAA was recorded in Ri 6 which produced about 0.541±0.006µg ml\(^{-1}\). Long et al.,(2008) reported the production of IAA by the endophytic isolates of Solanumnigrum. Endophytes have also been shown to promote plant growth by producing IAA (Mendes et al., 2007), increases root size and distribution, resulting in greater absorption of nutrient from the soil (Li et al., 2008).

In this study, fifteen endophytic bacteria were isolated from aerial parts of the rice which were grown in different regions of coastal saline soils. It was concluded that isolate Ri 4 and Ri 12 exhibited higher IAA production, isolate Ri 4 and Ri 5 showed better solubilization in phosphorous, Ri 3 and Ri 4 showed better solubilization of potassium. These endophytic bacteria have the potential for phosphate and potassium solubilization and sufficient amount of IAA production. In future studies, these isolates could possibly be utilized for bio-remediation of salt affected soils for agricultural crop production.

References

Aleksandrov, V. G., R. N. Blagodyr, and I. P. Ilev. "Liberation of phosphoric acid from apatite by silicate bacteria." Mikrobiol Z 29, no. 11 (1967): 1-1.

Araújo, Welington L., JoelmaMarcon, Walter Maccheroni, Jan Dirk van Elsas, Jim WL van Vuurde, and Joao LúcioAzevedo. "Diversity of endophytic bacterial populations and their interaction with Xylellafastidiosa in citrus plants." Applied and environmental microbiology 68, no. 10 (2002): 4906-4914.

Bakhshandeh, Esmail, Hemmatollah Pirdashti, and Khadijeh Shahsavarpour Lendeh. "Phosphate and potassium-solubilizing bacteria effect on the growth of rice." Ecological
Bartholomew, James W. "Variables influencing results, and the precise definition of steps in Gram staining as a means of standardizing the results obtained." *Stain Technology* 37, no. 3 (1962): 139-155.

Bhattacharya, Sourish, Pooja Bachani, Deepti Jain, Shailesh Kumar Patidar, and Sandhya Mishra. "Extraction of potassium from K-feldspar through potassium solubilization in the halophilic*Acinetobacter soli* (MTCC 5918) isolated from the experimental salt farm." *International Journal of Mineral Processing* 152 (2016): 53-71.

Cappuccino, J. G., and N. Sherman."Techniques for isolation of pure cultures. Cultural Characteristics of Microorganisms." *Microbiology A Laboratory Manual, Pearson Education* 6 (2002): 13-23.

Dudeja, S. S., Rupa Giri, Ranjana Saini, Pooja Suneja-Madan, and Erika Kothe."Interaction of endophytic microbes with legumes." *Journal of Basic Microbiology* 52, no. 3 (2012): 248-260.

Gayathri, S., D. Saravanan, M. Radhakrishnan, R. Balagurunathan, and K. Kathiresan. "Bioproducting potential of fast growing endophytic bacteria from leaves of mangrove and salt-marsh plant species." (2010).

Glickmann, Eric, and Yves Dessaux."A critical examination of the specificity of the salkowski reagent for indolic compounds produced by phytopathogenic bacteria." *Applied and environmental microbiology* 61, no. 2 (1995): 793-796.

Hung, Pham Quang, and K. Annapurna."Isolation and characterization of endophytic bacteria in soybean (Glycine sp.)." *Omonrice* 12 (2004): 92-101.
(1962): 31-36. 
Padma, S. D., and J. Sukumar. "Response of mulberry to inoculation of potash mobilizing bacterial isolate and other bio-inoculants." Global J. Bio. Sci. Biotechnol 4 (2015): 50-53.

Pandey, Anita, Namrata Das, Bhavesh Kumar, K. Rini, and Pankaj Trivedi. "Phosphate solubilization by Penicillium spp. isolated from soil samples of Indian Himalayan region." World Journal of Microbiology and Biotechnology 24, no. 1 (2008): 97-102.

Pikovskaya, R. I. "Mobilization of phosphorus in soil in connection with vital activity of some microbial species." Mikrobiologiya 17 (1948): 362-370.

Sessitsch, Angela, Birgit Reiter, and Gabriele Berg. "Endophytic bacterial communities of field-grown potato plants and their plant-growth-promoting and antagonistic abilities." Canadian journal of microbiology 50, no. 4 (2004): 239-249.

Stanford, George, and Leah English. "Use of the flame photometer in rapid soil tests for K and Ca." Agronomy Journal 41, no. 9 (1949): 446-447.

Stoltzfus, J. R., R. M. P. P. So, P. P. Malarvithi, J. K. Ladha, and F. J. De Bruijn. "Isolation of endophytic bacteria from rice and assessment of their potential for supplying rice with biologically fixed nitrogen." Plant and Soil 194, no. 1-2 (1997): 25-36.

Sun, Lijun, Zhaoxin Lu, Xiaomei Bie, Fengxia Lu, and Shengyuan Yang. "Isolation and characterization of a co-producer of fengycins and surfactins, endophytic Bacillus amyloliquefaciens ES-2, from Scutellaria baicalensis Georgi." World Journal of Microbiology and Biotechnology 22, no. 12 (2006): 1259-1266.

Vendan, Regupathy Thamizh, Young Joon Yu, Sun Hee Lee, and Young Ha Rhee. "Diversity of endophytic bacteria in ginseng and their potential for plant growth promotion." The Journal of Microbiology 48, no. 5 (2010): 559-565.

Zinniel, Denise K., Pat Lambrecht, N. Beth Harris, Zhengyu Feng, Daniel Kuczmański, Phyllis Higley, Carol A. Ishimaru, Alahari Arunakumari, Raúl G. Barletta, and Anne K. Vidaver. "Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants." Applied and environmental microbiology 68, no. 5 (2002): 2198-2208.

How to cite this article:
Parvathapriya, S., R. Thamizh Vendan, R. Subhashini, S. Thiruvudai Nambi and Renuka R. 2021. Isolation and Screening of Beneficial Endophytic Bacteria from Rice Grown under Coastal Saline Soils. Int.J.Curr.Microbiol.App.Sci. 10(01): 2672-2679.
doi: https://doi.org/10.20546/ijcmas.2021.1001.310