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Isolation and Identification of Endophytic Bacteria from Day Flower of *Commellina benghalensis*

Archana S Injal[1]*, Tejaswini C Mendke[2], Tara N Matnale[3], Somnath Kshirsagar[4]

[1]Department of Genetics, Walchand College of Art and Science, Solapur, India
[2]Department of Nanotechnology, Walchand College of Art and Science, Solapur, India
[3]Department of Biotechnology, Walchand College of Art and Science, Solapur, India
[4]Department of Zoology, K.N. Bhise College of arts and science, Solapur, India

Research Article

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***For Correspondence**

Archana S Injal, Department of Genetics, Walchand college Of Art and Science, Solapur, India.

E-mail: injalarchana29@gmail.com

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ABSTRACT

Objective: Endophytes are nothing but microflora of plant. Each plant has its own endophytic flora.*C. benghalensis* is weed. Day flower is unique feature of *Commelina benghalensis*. Which is relatively difficult to control by herbicide? So, objective of this work is to isolate and identify endophytic bacteria from dayflower of *Commelina benghalensis* and finding role of entophyte in herbicide resistivity.

Methods: The plant of *C. benghalensis* was collected from nearby Solapur area and re-cultivated in college botanical garden. Dayflowers were collected from underground roots of *C. benghalensis*. Separated dayflowers were brought in laboratory and wash thoroughly by tap water. Surface sterilization was carried out by 70% alcohol, Tween 20, Sodium hypochloride and distilled water. Each dayflower was cut into two pieces from its central axis. These two cut pieces were separately inoculated on nutrient agar medium (cut portion facing toward medium) and incubate for 24 hours at 37 °C.

Result: After 24 hours of incubation, white bacterial growth was observed on nutrient agar medium exactly near surrounding area of cut portion of dayflower. Colonies were isolated and used for further biochemical tests.The Biochemical tests were performed on Vitek 2 biomérieux vitek 2 systems by using the identification software PIBWIN Version 2.0.0. On the basis of analysis of this system, it is finalized that the isolated bacterial strain was Micrococcusspp.

Conclusion: This resistivity to herbicides might be due to presence of *micrococcus spp*. in its dayflower. Because, Micrococcus actively participate in catabolic reactions by utilizing a wide range of unusual substrate like herbicides, pyridine, oil and chlorinated biphenyls.

INTRODUCTION

Bacteria and fungi which live inside plant cells without any harm to plant are called endophytes[1]. Most plant species accommodate one, or more than one, kind of endophyte. Endophytesdevelop some associations with the host plant [2-6]. These associations can be commensal or mutualistic but, sometime few bacteria and fungi live as parasite inside the plant cells [7]. More work was done on endophytic fungi than bacterial endophytes [8,9]. In this study, we have isolated endophytic bacteria from day flower of *Commelinabenghalensis*. The *Commellinabenghalensis* is a weed plantbelongs to family *commelinaceae* [10].These weeds are normally found in crop field during monsoon season (June to September).

Commellinabenghalensis commonly known as the Benghal dayflower, trophical spiderwort, wandering jew, kanshira in Bengali language while in Hindi it is called as Kana and in Sanskrit: kanchata, kosapuspi, marishajalaja. Day flower is a unique character of *commelinance* family. This day flower is self -pollinating. It is a native to tropical Asia and Africa.

The study suggested that, there is a great diversity in reproductive systems of dayflower of Commellina [11]. It produces

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three types of flowers one male flower, chasmogamous and twohermaphroditeschasmogamous andcleistogamous. All three types develop on aerial branches. From this hermaphrodite chasmogamous flowers areon subaerial branches and cleistogamous flowers grow on underground branches. Also some plants may produce female flowers on aerial branches. The dayflowers have three ovules per ovary [11].

As per the review of literature maximum work was done on different plant parts of *C. benghalensis* expect dayflower and a bit of work was carried out on endophytes of this plant. So effort was made to isolated endophytic bacteria from day flower of *Commelina benghalensis* other than different plant portion.

MATERIALS AND METHODS

Collection of plant

The plant of *C. benghalensis* was collected from nearby Solapur areaand re-cultivated in college botanical garden **(Figures 1 and 2).**

Figure 1. *Commelina benghalensis* plant.

Figure 2. *Commelina benghalensis* plant.

Isolation of Endophytic bacteria

For isolation of endophytes, dayflowers were collected from underground roots of *C.benghalensis* **(Figure 3)**. Separated dayflowers were brought in laboratory andwash thoroughly by tap water. Surface sterilization was carried out by 70% alcohol, Tween 20, Sodium hypochloride and distilled water. Each dayflower was cut into two pieces from its central axis. These two cut pieces were separately inoculated on nutrient agar medium (cut portion facing toward medium) and incubate for 24 hours at 37°C.

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Figure 3. Dayflowers of *commelina benghalensis*.

Biochemical characterization of Endophyte

The isolated endophytic bacterial colonies were identified on the basis of morphological, cultural and biochemical characteristics. The Biochemical tests were performed on Vitek 2 biomérieuxvitek 2 systems using the identification software PIBWIN Version 2.0.0 [12].

RESULTS

Isolation of Entophytic bacteria

In the present study, After 24 hours of incubation, white bacterial growth was observed on nutrient agar medium exactly near surrounding area of cut portion of dayflower. Four quadrant streaking method was performed to isolate single colony of respective bacteria. Well isolated colony was streak and maintain on separate nutrient agar slant. These isolated colonies were used for further biochemical tests **(Figure 4).**

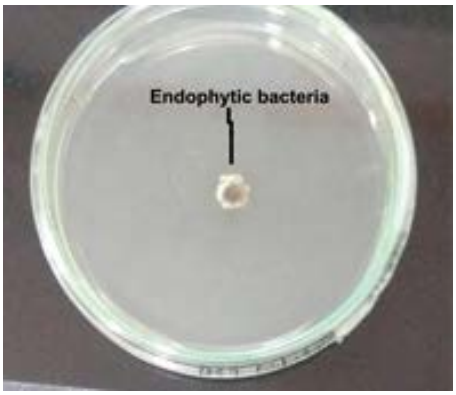

Figure 4. Isolation of endophytic bacteria of Dayflower of *commelina benghalensis*.

Biochemical test

The isolated endophyticbacteria were gram positive with white coloured colony morphology **(Figure 5).** These strain showed positive results for biochemical tests such as Catalase, Arginine, Hihydrolase 1, Beta galactosidase, Alpha glucosidase, Lascinarmylamidase, L-prolinearmylamidase, Alanine, Arylamidase, Tyrosine armylamidase, Urease, D-glucose, D-ribose, N-acetyl - D-glucosamine, D-maltose, Bactracin resistance, D-mannitol, D-mannose, Methyl -B-D-glucopyranoside, Saccharose \ Sucrose, D-trehalose and optochin resistance. The Biochemical tests were performed on Vitek 2 biomérieuxvitek 2 systems by using the identification software PIBWIN Version 2.0.0. On the basis of analysis of this system, it is finalized that the isolated bacterial strain was *Micrococcusspp*. The result of biochemical tests **(Table 1).**

Table 1. Biochemical tests for identification of isolated entophytic strains from Dayflower of *Commellina benghalensis*.

Biochemical Tests	Result
White Creame Colony	-
Yellow Orange colony	-
Pink Red colony	-
Catalase	+
Oxidase	-
Coagulase	-
Amygdalin	-

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Phosphatidylinositol Phospholipase C	-
Xylose	-
Arginine Hihydrolase 1	+
Beta Galactosidase	+
Alpha Galactosidase	+
Cyclocextrin	-
L Aspartate Arylamidase	-
Beta Galactopyranosidase	-
Alpha Mannasidase	-
Phosphatase	-
Leucine Arylamidase	+
L-proline Arylamidase	+
Beta Galacuronidase	-
Alpha- Galactosidase	+
L-Pyrrolidonyl- Aeylamidase	+
Beta- Glucuronidase	-
Alanine Arylamidase	+
Tyrosine Arylamidase	+
D-sorbitol	-
Urease	+
Polymyxin B Resistance	-
D- Galactose	+
D-Ribose	+
L-Lactate Alkatiniczation	-
Lactose	-
N-Acetyl-D-Glucosamine	+
D-Maltose	+
Bacteacin Resistance	+
Novobiocin Resistance	-
Growth In 6.5% NaCl	-
D- Mannose	+
D-Mannose	+
Methyl I-B-D-Glucopyrqanoside	+
pullutan	-
D-Raffinose	-
O/129 Resistance (Comp. Vibrio.)	-
Salicin	-
Saccharose/Sucrose	+
D-Trechalose	+
Arginine Dihydrolase-2	-
Optochin Resistance	+
Identification	Micrococcus Spp.

Figure 5: Isolation of single colony by four quadrant streaking method.

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DISCUSSION

The planet having about 300,000 species of plants, from these, majority of plants contain endophytes [13]. In nature, an endophyte-free plant is a rare exception [14]. Endophytes help plant to cope up with infections developed by pathogen and various environmental stress [15].

The most frequently found genera of bacterial endophytes are *Pseudomonas, Micrococcus, Bacillus, Stenotrophomonas, Burkholde-ria, Pantoea, Microbacterium*, [16-22]. Genus micrococcus have been detected an endophytes in several other plants including the *Aloe vera* [23], *zea* [6], cotton [24], rice seed [5], the roots of *Polysporaaxillaris* [25] and *Aquilariasinensis* [26].

The result of this study indicates that the isolated strain of endophyte shows distinct features of different species of genus micrococcus, that it should be described as novel species in day flower of *C. benghalensis*. This Micrococcus spp. are likely involved in biodegradation of different environmental pollutant to and decrease their toxicity [27-30]. Some Micrococcus isolates also can produce different useful products, such as long-chain aliphatic hydrocarbons for lubricating oils.

CONCLUSION

Medicinal plants are source of great economic value all over the World. On literature available it can be said that *C. benghalensis* plant, show unique characteristic of presence of day flower whose entophytic screening were studied. According to this study an endophytic bacteria of dayflower of said plant is nothing but *Micrococcus spp*.

C. benghalensis is relatively difficult to control by herbicide. This resistivity to herbicides might be due to presence of *micrococcusspp.* In its dayflower. Because, Micrococcus actively participate in catabolic reactions by utilizing a wide range of unusual substrate like herbicides, pyridine, oil and chlorinated biphenyls.

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REFERENCES

1. Wilson D. Endophyte: The evolution of a term, and clarification of its use and definition. Oikos. 1995; 73:274–276.
2. Adams PD, et al. Seed-borne bacterialendophytcs in different cotton cultivars. Phytopathology. 1996.86: 597.
3. James EK, et al. Infection and colonization of rice seedlings by the plant growth-promoting bacterium Herbaspirillumseropedicae Z67. Mol Plant Microbe Interact. 2002; 15: 894–906.
4. Sessitsch A, et al. Cultivation independent population analysis of bacterial endophytes in three potato varieties based on eubacterial and Actinomycetes-specific PCRof 16S rRNA genes. FEMS Microbiol Ecol 2002; 39: 23–32.
5. Kaga H, et al. Rice seeds as sources of endophytic bacteria. Microbes Environ. 2009; 24: 154–162.
6. Johnston MD, et al. Conservation and diversity of seed associated endophytes in Zea across boundaries of evolution, ethnography and ecology. PLoS ONE. 2011; 6; e20396.
7. Rodriguez R, et al. More than 400 million years of evolution and some plants still can't make it on their own: plant stress tolerance via fungal symbiosis. J Exp Bot. 2008; 59: 1109–1114.
8. Verma S, et al. Piriformosporaindica,gen. sp. nov., a new root Z-colonizing fungus. J Mycologia. 1998; 90: 896–903.
9. Waller F, et al. Theendophytic fungus Piriformosporaindica reprograms barley tosalt-stress tolerance, disease resistance, and higher yield. Proc Natl Acad Sci USA. 2005; 102: 13386–13391.
10. Kharade A, et al. Photochemical investigation in commelinabenghalensis and cyanotiscristata, Res J pharm App sci. 2013; 3: 46-48.
11. Kaul V, et al. Reproductive effort and sex allocation strategy in Commelinabenghalensis L, a common monsoon weed. Bot J Linn Soc. 2002; 140: 403–413.
12. Karad D. Vitek 2 biomérieuxvitek 2 system using the identification software PIBWIN Version 2.0.0. 2018.
13. Smith SA, et al. Bioactive endophytes warrant intensified exploration and conservation. PLoS One. 2008; 3: e3052.
14. Partida-Martínez LP, et al. The microbe-free plant: fact or artifact? Front Plant Sci. 2011; 2: 100.
15. Timmusk S, et al. Bacterial distribution in the rhizosphere of wild barley under contrasting micro climates. PLoS One. 2011; 6: e17968.

e-ISSN: 2320-3528
p-ISSN: 2347-2286

16. Hallmann J, et al. Bacterialendophytes in agricultural crops. Can J Microbiol. 1997; 43: 895–914.

17. SturzAV Nowak J. Endophytic communities of rhizobacteria and thestrategies required to create yield enhancing associations with crops. Appl Soil Ecol. 2000; 15: 183–190.

18. Sun Y, et al. The presence of a1-aminocyclopropane-1-carboxylate (ACC) deaminase deletion mutationalters the physiology of the endophytic plant growth-promoting bacterium Burkholderiaphytofirmans PsJN. FEMS Microbiol. Lett. 2009; 296: 131–136.

19. Rosenblueth M, et al. Bacterial endophytes and theirinteractions with hosts. Molec. Plant Microbe Interact. 2006; 19: 827–837.

20. Marquez-Santacruz HA, et al. Diversity of bacterial endophytesinroots of Mexican husk tomato plants (Physalisixocarpa) and their detection intherhizosphere. Gen Mol Res. 2010; 9: 2372–2380.

21. Shi Y, et al. Illumina-based analysis ofendophytic bacterial diversity and space-time dynamics in sugar beet on thenorth slope of Tianshan mountain. Appl Microbiol Biotechnol. 2014; 98: 6375–6385.

22. Romero FM, et al. The communities of tomato (Solanumlycopersicum L) leaf endophytic bacteria, analyzed by 16S-ribosomalRNA gene pyrosequencing. FEMS Microbiol Lett. 2014; 351: 187–194.

23. Om Prakash, et al. Description of Micrococcus aloeveraesp. nov., anendo phyticactino bacterium isolated from Aloe vera, Int J Syst Evol Microbiol. 2014; 64: 3427–3433.

24. Mcinroy JA, et al. Survey of indigenous bacterial entophytes from cotton and sweet corn. Plant Soil. 1995; 173: 337–342.

25. Zhao GZ, et al. Micrococcus yunnanensis sp. nov, a novelactinobacterium isolated from surface-sterilized Polysporaaxillarisroots. Int J SystEvol Microbiol. 2009; 59: 2383–2387.

26. Chen HH, et al. Micrococcus endophyticus sp. nov., isolatedfrom surface-sterilized Aquilariasinensis roots. Int J Syst Evol Microbiol. 2009; 59: 1070–1075.

27. Doddamani H, et al. Biodegradation of carbaryl by a Micrococcus species. Curr Microbiol. 2001; 43: 69–73.

28. Sims GK, et al. Riboflavin Production during Growth of Micrococcus luteuson Pyridine. Appl Environ Microbiol. 1992; 58: 3423–3425.

29. Wilson AK. Commelinaceae - a review of the distribution, biology and control of the important weeds belonging to this family. Tropical Pest Management. 1981; 27: 405-418.

30. Huang W, et al. Importance of Gram-positive naphthalene-degrading bacteria in oil-contaminated tropical marine sediments. Lett Appl Microbiol. 2003; 36: 251–257.