Journal of Young Pharmacists 5 (2013) 95–97

Contents lists available at SciVerse ScienceDirect

Journal of Young Pharmacists

journal homepage: www.elsevier.com/locate/jyp

Short communication

Diversity of endophytic bacteria in Malaysian plants as revealed by 16S rRNA encoding gene sequence based method of bacterial identification☆

Chye Ying Loh [a], Yin Yin Tan [a], Rahim Rohani [b], Jean-Frédéric F. Weber [b], Subhash Janardhan Bhore [a,*]

[a] Department of Biotechnology, Faculty of Applied Sciences, AIMST University, Bedong 08100, Kedah, Malaysia
[b] Atta-ur-Rahman Research Institute of Natural Products for Drug Discovery (RiND) and Faculty of Pharmacy, MARA University of Technology (UiTM), Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia

ARTICLE INFO

Article history:
Received 18 May 2013
Accepted 2 July 2013
Available online 30 July 2013

Keywords:
Dicots
Endophytes
Monocots
Natural compounds
Bioprospecting

ABSTRACT

Bacterial endophytes do have several potential applications in pharmacy, medicine and agricultural biotech industry. The main objective of this study was to understand types of bacterial endophytes associated with dicotyledonous (dicot) and monocotyledonous (monocot) plant species. Isolation of the endophytic bacteria was performed using surface-sterilized various tissue samples, and identification of the endophytic bacterial isolates (EBIs) was completed using 16S rRNA encoding gene sequence similarity based method. In total, 996 EBIs were isolated and identified from 1055 samples of 31 monocot and 65 dicot plant species from Peninsular Malaysia. The 996 EBIs represented 71 different types of bacterial species. Twelve (12) out of 71 species are reported as endophytes for the first time. We conclude that diverse types of bacterial endophytes are associated with dicot and monocot plants, and could be useful in pharmacy, medicine and agricultural biotechnology for various potential applications.

1. Introduction

Symbiotic association with microbes allows plants (including medicinal plants) to survive in adverse living conditions. The success of plant-microbe interactions at the molecular level[1] determines the ability of soil bacteria to reside inside plants as benign endophytes.[2] Bacterial endophytes have several potential applications in pharmacy, drug discovery[3,4] as well as in agriculture that include alteration of plant growth and development,[5] increasing shelf-life of agricultural commodities,[6,7] enhancing productivity of crop plants[8] and phytoremediation.[9,10]

This study was conducted to have a collection of bacterial endophytes from tropical plant species. The long-term aim of this study is to explore endophytic bacteria for various economically important bioactive compounds such as novel antibiotics, molluscicidal agents, and plant growth regulators. The main objective of this first phase of the study was to understand types of endophytic bacteria associated with dicot and monocot plant species. The diversity of the bacterial endophytes in monocot and dicot plants species from Peninsular Malaysia is reported in this paper.

2. Materials and methods

Healthy tissue samples (such as leaves, stems, petioles, fruits etc.) from 1055 plants belonging to 31 monocot and 65 dicot plant species were collected from road sides, gardens and plantations from all over Peninsular Malaysia. The culturable endophytic bacterial strains were isolated from the surface-sterilized tissues as discussed somewhere else.[6,11] In brief, the collected leaves, stems, petioles, fruits etc. samples were washed under plenty of running tap water, and surface-sterilization of tissue samples was carried out carefully to avoid the contamination.[6]

To isolate endophytic bacteria, plant tissue samples were inoculated aseptically in the Petri plates containing Luria–Bertani (LB) agar medium. Petri plates were incubated (in the dark) up to 18–20 h at 37 °C (±3 °C) in an incubator.

The cultivation of isolated EBIs, amplification of their 16S rRNA encoding gene fragments, 16S rRNA gene fragments sequencing and identification of isolates were carried out using routinely used techniques with minor modifications.[6]

☆ Presentation at a meeting: Nil

* Corresponding author: Tel.: +60 4 429 8176; fax: +60 4 429 8109.
E-mail addresses: subhashbhore@gmail.com, subhash@aimst.edu.my (S.J. Bhore).

0975-1483/$ – see front matter
http://dx.doi.org/10.1016/j.jyp.2013.07.001

Table 1
Types (varieties) of endophytic bacteria associated with monocot and dicot plant species in Peninsular Malaysia.

No.	Bacterial endophyte	M[a]	D[a]
1	*Acinetobacter baumannii*	0	+
2	*Acinetobacter baylyi*	+	+
3	*Acinetobacter calcoaceticus*	0	+
4	*Acinetobacter radioresistens*	+	0
5	*Acinetobacter soli*	0	+
6	*Acinetobacter* sp.	0	+
7	*Aeromonas jandaei*	0	+
8	*Bacillus acidiceler*	0	+
9	*Bacillus altitudinis*	+	+
10	*Bacillus amyloliquefaciens*	+	+
11	*Bacillus anthracis*	+	+
12	*Bacillus aquimaris*	+	0
13	*Bacillus badius*	0	+
14	*Bacillus cecembensis*	+	0
15	*Bacillus cereus*	+	+
16	*Bacillus drentensis*	+	0
17	*Bacillus endophyticus*	0	+
18	*Bacillus flexus*	+	+
19	*Bacillus fusiformis*	+	0
20	*Bacillus koreensis*	+	+
21	*Bacillus licheniformis*	+	+
22	*Bacillus marisflavi*	+	+
23	*Bacillus megaterium*	+	+
24	*Bacillus mycoides*	+	+
25	*Bacillus pumilus*	+	+
26	*Bacillus* sp.	+	0
27	*Bacillus subtilis*	+	+
28	*Bacillus tequilensis*	+	0
29	*Bacillus thuringiensis*	+	+
30	*Bacillus vallismortis*	+	0
31	*Bacillus vireti*	0	+
32	*Brevibacterium linens*	+	0
33	*Burkholderia cepacia*	+	0
34	*Cloacibacterium normanense*	0	+
35	*Cronobacter sakazakii*	+	+
36	*Enterobacter cancerogenus*	0	+
37	*Enterobacter cloacae*	+	+
38	*Enterobacter cowanii*	+	+
39	*Enterobacter hormaechei*	+	+
40	*Enterobacter ludwigii*	+	+
41	*Enterobacter oryzae*	+	0
42	*Enterobacter pulveris*	+	0
43	*Enterobacter* sp.	+	0
44	*Enterococcus casseliflavus*	0	+
45	*Erwinia soli*	+	0
46	*Erwinia* sp.	+	0
47	*Escherichia coli*	+	+
48	*Escherichia hermannii*	0	+
49	*Exiguobacterium acetylicum*	0	+
50	*Jeotgalicoccus psychrophilus*	+	0
51	*Klebsiella oxytoca*	+	+
52	*Klebsiella pneumoniae*	+	0
53	*Lysinibacillus fusiformis*	+	0
54	*Lysinibacillus sphaericus*	+	+
55	*Micrococcus yunnanensis*	+	0
56	*Paenibacillus illinoisensis*	+	+
57	*Paenibacillus xylanilyticus*	+	0
58	*Pantoea agglomerans*	+	+
59	*Pantoea ananatis*	+	+
60	*Pantoea dispersa*	+	+
61	*Pantoea eucrina*	+	0
62	*Pantoea stewartii*	+	+
63	*Pasteurella pneumotropica*	+	0
64	*Pectobacterium cypripedii*	+	0
65	*Pseudomonas oleovorans*	0	+
66	*Pseudomonas putida*	+	0
67	*Sporosarcina aquimarina*	0	+
68	*Staphylococcus pasteuri*	+	0
69	*Stenotrophomonas maltophilia*	0	+
70	*Terribacillus saccharophilus*	0	+
71	*Vibrio parahaemolyticus*	+	0

[a] Note: *M*, monocotyledonous plants; *D*, dicotyledonous plants; +, present; 0, not found among isolates.

3. Results and discussion

As a part of a broad study to explore potential applications of bacterial endophytes, 996 culturable endophytic strains were isolated from the surface-sterilized tissues of 1055 plant samples. Out of the 996 strains, 977 were identified to species level where their 16S rRNA encoding gene sequences showed at least 96% similarity with closest hits from the GenBank/DDBJ/EMBL database. Annotated, 16S rRNA encoding gene fragment's nucleotide sequences of all 996 EBIs have been deposited in the GenBank/DDBJ/EMBL DNA database under accession numbers: HQ844622–HQ844643, HQ694233–HQ694466, HQ694155–HQ694231, HQ693995–HQ694150, HQ683764–HQ684020, HQ670424–HQ670630, HQ650771–HQ650786, HQ638090–HQ638091, HQ638086–HQ638088, HQ637458, HQ634273–HQ634288, and HQ418463–HQ418467.

The isolates represented 71 bacterial species (Table 1), corresponding to 27 genera belonging to 6 classes, namely Actinobacteria, Bacilli, Betaproteobacteria, Cocci, Flavobacteria and Gammaproteobacteria (see Supplementary data). The host plants of EBIs, host plant's location on Peninsular Malaysia, identity of the isolates, and all other details are depicted in the Supplementary data.

By far, the most abundant isolates belong to the *Bacillus* genus, with 24 species and 824 isolates (83% of all isolates). Five (5) *Bacillus* species account for 82% of all isolates of this genus or 68% of all isolates: *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Bacillus pumilus*, and *Bacillus anthracis*. On the other hand, 11 genera are represented by single isolates. As far as geographic distribution is concerned, it can be noted that the prevalence of *Bacillus* isolates is lower in the southernmost state, Johor, (48%) than in the other states (75–99%). No clear pattern was observed for the botanical distribution of these isolates. Yet, a few plant species yielded *Bacillus* isolates only; these include notably *Ananas comosus* L. (pineapple), *Artocarpus heterophyllus* Lam. (jackfruit), *Calamus rotang* L. (rotan), *Nelumbo nucifera* Gaertn. (lotus), *Nephelium lappaceum* L. (rambutan) and *Tamarindus indica* L. (tamarind).

Out of the 71 identified species, twelve (12) are reported as endophytes for the first time. These species include *Acinetobacter soli, Aeromonas jandaei, Bacillus cecembensis, Bacillus koreensis, Bacillus marisflavi, Brevibacterium linens, Cloacibacterium normanense, Enterobacter pulveris, Erwinia soli, Jeotgalicoccus psychrophilus, Pantoea eucrina*, and *Vibrio parahaemolyticus*. To explore potential applications of these bacterial endophytes, we intend to make all these isolates available to the interested scientists for the further research.

4. Conclusion

In a nutshell, 996 strains of bacterial endophytes were isolated, which depicts their wide range of diversity in monocot and dicot plant species. We are aware that the diversity of endophytic bacteria reported here is somewhat restricted to the limited areas in which collections were done, and even greater microbial diversity is expected from plants in undisturbed areas (primary forests). However limited, we also strongly believe in the uniqueness of this collection of EBIs and its usefulness in pharmacy, medicine and agricultural biotechnology. Further research is required to explore the potential applications of these isolates.

Acknowledgement

We are grateful to Dr. G. Strobel for his comments on earlier draft of the manuscript. This project was funded by the Ministry of Agriculture and Agro-Based Industry (MoA), Malaysia (Grant Code

Number: 05-02-16-SF1001) and MARA University of Technology (UiTM), Puncak Alam Campus, Selangor, Malaysia. LCY and TYY contributed equally to this work. The authors declare no competing financial interests.

Sources of support

Authors are grateful to the Ministry of Agriculture and Agro-Based Industry (MoA), Malaysia (Grant Code Number: 05-02-16-SF1001), and MARA University of Technology (UiTM), Puncak Alam Campus, Selangor, Malaysia for the financial support.

Conflicts of interest

All authors have none to declare.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jyp.2013.07.001.

References

1. Spaink HP. Plant-microbe interactions: a receptor in symbiotic dialogue. *Nature*. 2002;417:910–911.
2. Bulgarelli D, Rott M, Schlaeppi K, et al. Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature*. 2012;488:91–95.
3. Strobel G, Daisy B. Bioprospecting for microbial endophytes and their natural products. *Microbiol Mol Biol Rev*. 2003;67:491–502.
4. Guo B, Wang Y, Sun X, Tang K. Bioactive natural products from endophytes: a review. *Prikl Biokhim Mikrobiol*. 2008;44:153–158.
5. Hardoim PR, van Overbeek LS, Elsas JD. Properties of bacterial endophytes and their proposed role in plant growth. *Trends Microbiol*. 2008;16:463–471.
6. Bhore SJ, Ravichantar N, Loh CY. Screening of endophytic bacteria isolated from leaves of Sambung Nyawa [*Gynura procumbens* (Lour.) Merr.] for cytokinin-like compounds. *Bioinformation*. 2010;5:191–197.
7. Ali S, Charles TC, Glick BR. Delay of flower senescence by bacterial endophytes expressing ACC deaminase. *J Appl Microbiol*. 2012;113:1139–1144.
8. Mei C, Flinn BS. The use of beneficial microbial endophytes for plant biomass and stress tolerance improvement. *Recent Pat Biotechnol*. 2010;4:81–95.
9. Weyens N, van der Lelie D, Taghavi S, Vangronsveld J. Phytoremediation: plant-endophyte partnerships take the challenge. *Curr Opin Biotechnol*. 2009;20:248–254.
10. Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN. Bacterial endophytes: recent developments and applications. *FEMS Microbiol Lett*. 2008;278:1–9.
11. Fisher PJ, Petrini O, Lappin Scott HM. The distribution of some fungal and bacterial endophytes in maize (*Zea mays* L.). *New Phytol*. 1992;122:299–305.