16S rRNA sequence-based analysis of bacterial communities associated with the selected mosses of Mount Abu (Rajasthan), India

**Abstract**

The phyllosphere of bryophytes offers an excellent habitat to various other microorganisms to flourish. This fascinating association among bryophytes and soil borne microorganisms have significant ecological and physiological roles including N\(_\text{2}\) fixation, soil-enrichment, promotion of protonemal growth and bud formation. However, our understanding regarding the ecological aspects of this association is still little known, and if consider at molecular level it becomes rare. So far, only sporadic works have been done, and for better understanding related to this ignored concern and there is a necessity to address this. Hence, the present study was done to elucidate the structure and biological roles of bacterial diversity coupled with the bryophytes and their dynamics on selected taxa in different ecosystems. Phylogenetic analysis of bacterial community associated with selected mosses was carried out. The work is primarily based on the 16S rRNA sequencing approach. Moss taxa viz., *Anoectangium clarum* Mitt., *Hyphila involuta* (Hook.) A. Jaeger and *Atrichum undulatum* (Hedw.) P. Beauv., were collected from different locations of Mount Abu, Rajasthan. The outcome of the study estimated that the microbial communities associated with these mosses belong mainly to phylum proteobacteria and firmicutes with the dominance of γ-proteobacteria. This genus found frequently associated to the species of different habitat, soil, near water channel and the rocks are *Pseudomonas*, *Aeromonas*, *Routella* and *Halobascillus*. This study provides the groundwork information about the exceptional relationship of these bacteria with mosses and would be helpful for the future research in this direction.

**Keywords:** bacterial community, bryophytes, endophytes, mosses

**Abbreviations:** FISH, fluorescent in situ hybridization; CLSM, confocal laser scanning microscopy; SSCP, single-strand conformation polymorphism

**Introduction**

The plant-microbes association is considered indispensable for the growth and development of plants.\(^1\) Bacteria associated with the plants are mainly endophytes and bare epiphytes that help to improve the plant growth by promoting soil enrichment,\(^2\) degradation of organic pollutants,\(^3-5\) plant hormones and vitamin synthesis, N\(_\text{2}\) fixation, suppression of the development of bacterial and fungal diseases,\(^6-8\) and can also enhance the host capability to adapt under extreme environment.\(^9\) Bacterial associations are present in nearly all the plant species that exists on earth.\(^9\) Despite this prevalent dependence, astonishingly, the majority of research focused only on the higher plants-bacterial association and the structure or diversity of bacteria associated with lower plants such as bryophytes has been neglected.\(^10\) Bryophytes are the primitive and simplest non-vascular plants/first land plants that are composed of mosses, liverworts and hornworts, and are considered to be the ancestors of pteridophytes and all other tracheophytes.\(^11,12\) Bryophytes consists of nearly 25,000 species worldwide\(^13\) distributed in diverse ecological condition ranging from polar and alpine regions to tropic\(^14\) and also played an important role in biota formation along with the settlement of land by plants.\(^15\) Bryophytes have been suggested as excellent candidates for evaluating the ecological and evolutionary impacts of the habitat fragmentation due to their global ubiquity, fast-growing nature, substrate specificity, and dominant haploid gametophytes.\(^14\) They have also been adopted and employed as model organisms and exploited for different kinds of biotechnological applications.\(^16-18\) Bryophytes play imperative roles in nutrient cycling and can act as bio-indicators of air pollutants or heavy metals, making them crucial to the environmental health of many ecosystems.\(^18\)

Nowadays, bryophytes are gaining great attention from several researchers because of its distinctive properties and availability of biotechnological tools to exploit it in different aspects useful for better understanding of evolution and ecology. A number of studies showed that bryophytes-microorganism association is prevalent in the environment,\(^19-21\) but till now the nature and bio-functional diversity of these microbial communities inhabiting in/on the bryophytes remains unknown.\(^20\) This draws the attention of several researchers to study the bryophytes-associated microorganisms and their relationship with bryophytes and its habitat. Currently the detailed understanding of microbial community structure and its associated biological role are available for only few genera of bryophytes.\(^20,22\) The peat moss, *Sphagnum* is the most exploited genus among bryophytes to study the abundance, structure and function of the endo/ectophytic bacteria associated with plant.\(^19,24\) Apart from this, other genera such as *Hygroamblystegium* spp., *Entodon* spp., *Grimmiia* spp.,\(^21\) *Brachythecium* spp., *Haploludium* spp., *Bryum* spp.,\(^20\) *Funaria* spp.,\(^20\) *Tortula*\(^20\) has been studied for microbial association. Several studies provide insights into the correlation between the ecosystem

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or geographical region and abundance of microbial community. Several methods have been used to study the bryophyte-microbial association such as scanning electron/fluorescence microscopy, Fluorescent in situ Hybridization (FISH) and Confocal Laser Scanning Microscopy (CLSM). Single-strand conformation polymorphism (SSCP), 16S rRNA and 16S rDNA sequencing, DGGE-PCR sequencing and Illumina sequencing. Microbial strains such as Burkholderia, Serratia, Hafnia, Pantoea, Methanobacteria and Methylbacteria were found abundantly as endophytes, epiphytes or both, in some mosses. Some of these microorganisms possess unique characteristic property such as N₂ fixation, mineral phosphate solubilization, ACC deaminase activity and PGR (Plant Growth Regulators) production, which make them valuable tools for agricultural biotechnology. In addition, many of isolated mass-associated bacteria have antagonistic property against several phytopathogenic fungi. In this study, the diversity and community structure of three bryophyte species collected from three different locations of Mount Abu were investigated and compared based on culture-independent approach of 16S rRNA sequencing method of Tian. As the study is based on only a few sequences so it provides only the groundwork scenario of microbial community diversity and the relationship between the ecosystem and bryophyte species in association with bacterial community. For comprehensive and significant evaluation of the associated microbial community of the Mount Abu region, diverse ecosystem and associated mosses should be taken into account for upcoming study.

Materials and methods

Sampling sites and surface treatment

To evaluate the bacterial community structure of the bryophytes three mosses, Anoectangium clarum, Hyophila involuta and Atrichum undulatum were collected during the spring season of 2014 from Mount Abu, which is located in the western part of India with longitude and latitude 72.7083°E 24.5925°N respectively. Three sites were selected for sampling; (a) surface of rock, (b) moist soil, (c) near water channels (Table 1). The collected samples were submitted and identified as taxonomically distinct species at Banasthali University Herbarium, Rajasthan and used for further analysis. The samples were washed with running tap water to remove attached soil. Then, the samples were immersed in 70% ethanol for 3 min and followed by washing five times with sterile distilled water.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of Samples</th>
<th>Herbarium number</th>
<th>Source of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anoectangium clarum Mitt. (Pottiaceae)</td>
<td>BURI7860301</td>
<td>Moist Soil</td>
</tr>
<tr>
<td>2</td>
<td>Hyophila involuta (Hook.) Jaeg. (Pottiaceae)</td>
<td>BURI7860318</td>
<td>Near water Channel</td>
</tr>
<tr>
<td>3</td>
<td>Atrichum undulatum (Hedw.) P. Beaux (Polytrichaceae)</td>
<td>BURI786</td>
<td>Shaded rocky ledges</td>
</tr>
</tbody>
</table>

Total DNA extraction and PCR amplification

The genomic DNA of bacterial consortia was extracted from 1gm of surface treated moss using Fast DNA spin kit for soil (MPBIO) according to manufacturer’s instruction. The eluted DNA was used diluted and used for the PCR reaction.

Amplification of bacterial 16S rRNA genes and Phylogenetic analysis

The 16S rRNA gene fragments were amplified with the universal bacterial primer sets 790F (50- AACAGGATTAGATACCCTTG-30) and 1492R (50- TACGGYTACCTGGTACGACT-30). The PCR was performed using a 1.5ul mixture containing 25 ng/µl of DNA 2.5 mM each of dNTPs, 1.5 mM MgCl₂, 10µM of forward and reverse primers and, 0.2 U of Taq DNA polymerase (Genei). The amplifications were performed using a thermal cycler (Primus 96) with subsequent plan, i.e. initial denaturation at 94°C for 5min; 35 cycles of 1 min at 94°C, 1 min at annealing temperature 53°C and 2 min at 72°C; and a final elongation step of 5 min at 72°C. PCR products were separated by the electrophoresis technique using a 1% agarose gel. The six bands of approximately 250 bp in size were excised and purified by the Hiyield Gel/PCR DNA Mini Kit as described by the manufacturer (Figure 1). The sequencing of the purified eluted product was performed by Applied Biosystems (India). Similarity searches in GenBank were performed using BLAST. The DNA sequences were analyzed for the closest neighbours among sequenced 16 rDNA regions of different bands using PAUP version 4 (Figure 2).
Bacterial species identified in all geographical regions belong to three phylogenetical group α-proteobacteria, γ-proteobacteria and fermicutes. The γ-proteobacteria genera such as Pseudomonas and Aeromonas were found associated with bryophyte species of soil habitat (Aneotangium clarum). And also the bryophytes species (Hyphila involuta) collected from water channel were found associated with γ-proteobacteria i.e. Pseudomonas and uncultured α-proteobacteria. In contrast, bryophyte species (Atrichum undulatum) collected from rock habitat contains both γ-proteobacteria and fermicutes genera, Pseudomonas and Halobacillus respectively. The genus Pseudomonas was found associated with bryophyte species of both soil and rock habitat. This result showed that the bacteria community structures are different at only species level irrespective of the habitat of bryophyte species. Bragina et al.,21 reported that the bacterial community structures are highly specific to their masses. However, the study involves only a few sequences due to lack of resources and contamination in samples the only the few microbial species and their distribution in different habitat were studied. So this study is preliminary attempt to understand the microbial community structure of mosses from different habitat of Mount Abu. Further study can be done to study other associated microbial community and its biological role and also its specificity is host specific or habitat specific and the reason for fluctuation of diversity in different habitats.

Conclusion

We report the bacterial community structure of three mosses collected from three different habitats of Mount Abu. The data indicate the presence of two phyla proteobacteria and fermicutes associated with mosses. However, due to inadequate resources and contamination in samples the only the few microbial species and their distribution in different habitat were studied. So this study is preliminary attempt to understand the microbial community structure of mosses from different habitat of Mount Abu. Further study can be done to study other associated microbial community and its biological role and also its specificity is host specific or habitat specific and the reason for fluctuation of diversity in different habitats.

Acknowledgments

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Conflicts of interest

The author declares there is no conflict of interest.

References

3. Morgan JA, Bending GD, White PJ. Biological costs and benefits to plant-


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