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ORIGINAL ARTICLE

Bacterial-biota dynamics of eight bryophyte species from different ecosystems



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Abstract Despite the importance of bryophyte-associated microorganisms in various ecological aspects including their crucial roles in the soil-enrichment of organic mass and N₂ fixation, nonetheless, little is known about the microbial diversity of the bryophyte phyllospheres (epi-/endophytes). To get insights into bacterial community structures and their dynamics on the bryophyte habitats in different ecosystems and their potential biological roles, we utilized the 16S rRNA gene PCR-DGGE and subsequent phylogenetic analyses to investigate the bacterial community of eight bryophyte species collected from three distinct ecosystems from western Japan. Forty-two bacterial species belonging to *γ-proteobacteria* and *Firmicutes* with 71.4% and 28.6%, respectively, were identified among 90 DGGE gel band population. These DGGE-bands were assigned to 13 different genera with obvious predomination the genus *Clostridium* with 21.4% from the total bacterial community. These analyses provide new insights into bryophyte-associated bacteria and their relations to the ecosystems.

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1. Introduction

Bryophytes, a group of lower non-vascular plants that is composed of *Musci* (mosses), *Hepaticae* (liverworts), and *Anthrocrotae* (hornworts), have been taxonomically placed

between the algae and the *pteridophytes*, as first terrestrial plants (Kenrick and Crane, 1997; Edwards et al., 1995). This group entails more than 10,000 species and inhabits a diversity of ecosystems ranging from fresh water sponges in the tropics to the caribou dung patches of the arctic tundra region (Pharo and Zartman, 2007). Bryophytes have been suggested as excellent candidates for appraising the ecological and evolutionary impacts of the habitat fragmentation due to their global ubiquity, fast-growing nature, substrate specificity, and dominant haploid gametophytes (Pharo and Zartman, 2007). They have also been adopted and employed as model organisms and harnessed for different kinds of biotechnological applications (Oliver et al., 2000; Decker et al., 2003). And due to their ability for fast growth, water maintenance and drought tolerance, the utilization of bryophytes as green-roof is growing astoundingly growth (Tani et al., 2011). Previous

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reports have also suggested possible usages for bryophytes as bio-monitors, and as an important factor to document the atmospheric chemistry as well as their importance in the biogeochemical processes (Turetsky, 2003; Cornelissen et al., 2007), and their impact in the ecosystems at large scales (Rocheftort, 2000; Nilsson and Wardle, 2005; Crowley and Bedford, 2011).

These unique properties have turned eyes toward the bryophyte group in order to better exploit it in different aspects. Ecologically, bryophytes colonize unique and microbial-favorable niches, which are supposed to be densely occupied with a huge diversity of microorganisms, but hitherto little is known about the nature and bio-functional diversity of these microbial communities compared with respect to their diverse importance at different aspects (Crowley and Bedford, 2011). This makes the study of bryophyte-associated microorganisms and the understanding of their roles as co-exists with the environment especially interesting.

Despite the efforts that have been paid to investigate the microbial structures of different eco-systems including aqueous, terrestrial, soil animal and plant systems, there is still shortage and little attention regarding this group of important climatic terrestrial plants (Hornschuh et al., 2002). Apart from this, some genera such as *Sphagnum* have been studied thoroughly to understand their microbial community structure and associated biological importance (Hornschuh et al., 2002; Bragina et al., 2013), however, the knowledge on the prevalence and diversity of the bryophyte epi-/endophyte microorganisms remain scarce. Microbial strains such as *Burkholderia*, *Serratia*, *Hafnia*, *Pantoea*, *Methanobacteria* and *Methylobacteria* were found abundantly as endophytes, epiphytes or both in some mosses (Bragina et al., 2013; Opelt and Berg, 2004). Interestingly, some of these microorganisms have been demonstrated to possess the ability in producing plant-growth regulators (PGRs), which is an important characteristic for bio-fertilizing applications and might somehow explain their roles in the moss growth (Hornschuh et al., 2002; Tani et al., 2011). The ability of these moss-associated bacteria in PGR production and their possible interactions with plant tissues during growth are of high interest (Opelt and Berg, 2004). In contrast, other reports have shown that many of these isolated moss-associated bacteria have been demonstrated to possess different antagonistic properties, such as *Pseudomonas putida*, *Xanthomonas* sp., *Serratia* sp., and *Bacillus* sp. (Opelt et al., 2007).

The interaction between the diazotrophic and non-diazotrophic bacterial groups in non-leguminous plants and their roles in such interaction have been previously suggested and named ANFICO, the anaerobic nitrogen-fixing consortium (Minamisawa et al., 2004). Interestingly, in our preliminary screening of bacteria associated with mosses collected from different regions in Japan, we noticed similar predominance of diazotrophic and non-diazotrophic microorganisms that drives us for further investigation on the moss associated microorganisms.

In this study, we screened the microbial diversity of eight bryophyte species using the culture-independent approach of PCR-DGGE based on the divergent regions of the 16S rRNA gene (Muyzer et al., 1993). The relationship between the ecosystem and bryophyte species in association with bacterial community structure fluctuations was discussed and further questions were opened for future researches. Nevertheless, this

report should be treated as a preliminary study and further investigation including additional factors such as seasonal changes, more diverse ecosystems and associated mosses is required for a comprehensive and critical evaluation.

2. Materials and methods

2.1. Sampling sites and strategy

To evaluate the bacterial community structure of the bryophytes, gametophytes of nine bryophytes (representative of eight distinct species) were collected during the spring season of 2009 from the Kurashiki city, which is located in western part of Japan with Latitude: N34.58° 35' and Longitude: E133.77° 46'. Three different sites were purposely targeted for sampling; (a) Kurashiki Ivy Square museum, a region that receives hundreds of visitors weekly (designated as highly populated soil), (b) Tsurugatayama Hill (virgin wet rocks), in

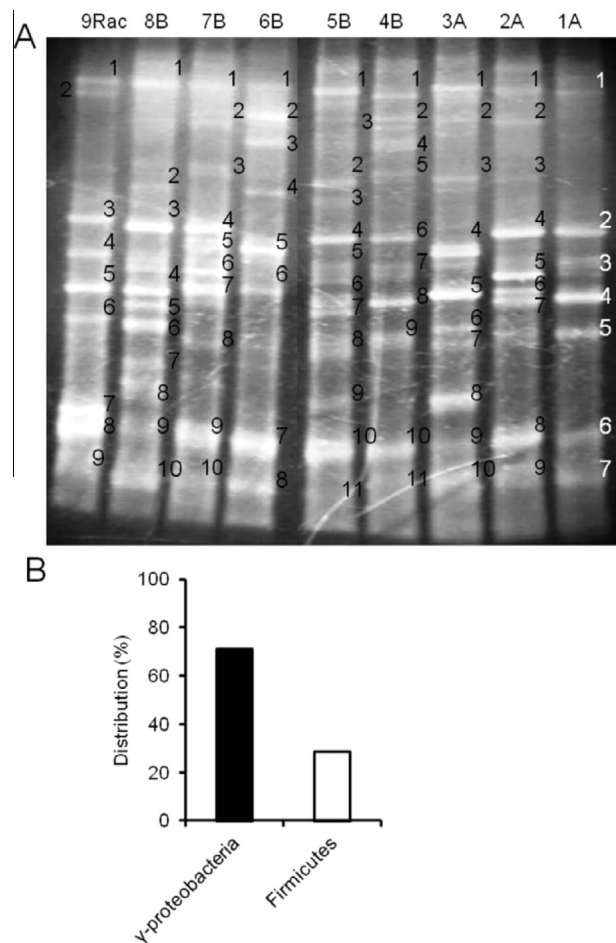


Figure 1 PCR-DGGE gel of bryophytes associated bacteria and the taxonomy distribution. (A) 16S rRNA gene PCR-DGGE characterization of nine bryophyte associated bacteria of three different ecosystems. Letters A, B and Rac symbols indicated the samples of highly populated-soil (intact), virgin-rocks and *Racomitrium* moss isolated from managed soil (green roof), respectively. (B) The taxonomical distribution percentage of the bacterial community isolated from nine bryophytes of different ecosystems.

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