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The Diversity and Composition of Cyanobacteria in the Muscovite Mineral – An Endolithic Ecosystem

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ABSTRACT

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Small subunit (16S rRNA) gene-based surveys were employed to investigate the phylogenetic diversity within the unique muscovite ecosystem. This approach unveiled a broader scope of diversity compared to traditional culture-based methods. Notably, cyanobacterial diversity remained remarkably stable at the phylum level, underscoring the ecological resilience of Muscovite. In Muscovite, the phylum Cyanobacteria accounted for 0.5983% of the microbial population. Within this phylum, classes including ML635J-21, Nostocophycidae, Synechococcophycidae, and Oscillatoriothycidae were identified, alongside an unclassified category. Notably, 517 cyanobacterial clones were detected, signifying their presence in the microbial community. Intriguingly, a substantial proportion (0.2616%) of cyanobacterial clones fell into unclassified orders, indicating potential unidentified microbial lineages within this group. Among the identified classes, ML635J-21 exhibited the highest abundance, with 225 clones. However, all classes within Cyanobacteria were underrepresented. The presence of an unclassified category further highlighted the need for comprehensive taxonomic characterization within this environment. These results emphasize the diverse and potentially novel cyanobacterial communities thriving in the Muscovite ecosystem, prompting further exploration to uncover their ecological roles and contributions.

Introduction

Endolithic bacterial communities are microbial ecosystems that thrive within the porous spaces of rocks, minerals, or other hard substrates (Wierchos and Ascaso, 1994; Friedmann, 1982). These communities are found in a wide range of environments, from deserts to polar regions, and even deep-sea environments. Endolithic bacteria

play crucial roles in biogeochemical cycles, weathering of minerals, and can have significant implications for astrobiology and the search for life on other planets. Endolithic bacteria are found in diverse habitats, including hot and cold deserts, arctic and alpine environments, coastal areas, caves, and deep-sea environments. These bacteria are adapted to extreme environmental conditions, such as high salinity, low water availability, extreme

temperatures, and high levels of radiation (Walker and Pace, 2007; Pointing and Belnap, 2012). Endolithic bacteria play crucial roles in biogeochemical cycles, including carbon, nitrogen, and sulfur cycling. They contribute to the weathering and alteration of minerals, which has implications for soil formation and nutrient availability (Northup and Lavoie, 2001; Chan *et al.*, 2012). Studying endolithic communities provides insights into the potential for life on other planets, particularly Mars, where similar environments may exist. The search for endolithic life forms part of the broader field of astrobiology (Cabrol *et al.*, 2007).

Endolithic cyanobacteria are a group of photosynthetic microorganisms that inhabit the interiors of rocks or mineral substrates. They are known for their ability to withstand extreme environmental conditions, such as high temperatures, desiccation, and high levels of radiation. These cyanobacteria play important roles in various ecosystems, including desert environments, where they contribute to carbon and nitrogen cycling (Garcia-Pichel and Pringault, 2001; Friedmann and Ocampo-Friedmann, 1995).

Endolithic cyanobacteria are commonly found in arid and semi-arid environments, including deserts and other rocky habitats. They colonize the porous spaces within rocks, where they are protected from harsh external conditions. (Wynn-Williams and Edwards, 2000).

Endolithic cyanobacteria belong to various taxonomic groups, including Chroococciopsidales, Nostocales, and Oscillatoriales (Pointing and Belnap, 2012). They display a wide range of morphologies and adaptations to their specific environmental niches. Endolithic cyanobacteria have evolved various physiological adaptations to survive in their extreme habitats, including mechanisms for water retention and protection against high radiation levels (Billi, 2000). They are capable of metabolizing inorganic substrates and contribute to primary production in these environments (Azua-Bustos, 2011).

Endolithic cyanobacteria exhibit remarkable diversity in their specialized habitats, with presence documented in a variety of extreme environments, such as deserts, caves, and polar regions. These microorganisms have undergone specific evolutionary adaptations to thrive under harsh conditions characterized by high salinity, limited water availability, extreme temperatures, and elevated levels of radiation. These adaptations enable them to colonize porous spaces within rocks and mineral substrates. In addition to their resilience, endolithic cyanobacteria play pivotal roles in biogeochemical cycles, encompassing processes such as carbon fixation, nitrogen fixation, and mineral weathering, thus contributing to nutrient cycling within rock matrices. Furthermore, the investigation of endolithic communities in extreme environments, notably exemplified by the Atacama Desert, holds implications for astrobiology and the quest for extraterrestrial life, as these environments serve as analogs for extraterrestrial habitats.

This study also unveils the extensive phylogenetic diversity of microorganisms, including cyanobacteria, dwelling within muscovite and soil. These findings underscore the broader scope of diversity when compared to conventional culture-based approaches. Moreover, investigations into soil-based ecosystems highlight the rich diversity of endolithic cyanobacteria, emphasizing their presence and ecological significance in various terrestrial ecosystems. Phylogenetic analysis and taxonomic classification of 16S rRNA gene sequences of cyanobacteria have constituted a central focus in elucidating their diversity and evolutionary relationships within these habitats. The identification of unclassified orders and potentially novel lineages within the Cyanobacteria group within muscovite suggests the existence of previously unrecognized microbial groups, thus emphasizing the necessity for further taxonomic characterization. In summary, these collective findings emphasize the diverse and distinctive nature of endolithic cyanobacteria, their capacity to adapt to extreme environments, and their substantial

contributions to biogeochemical processes. Additionally, the research conducted on muscovite highlights the potential for the discovery of novel microbial lineages within these habitats.

Muscovite, a prevalent mineral encountered in igneous, metamorphic, and sedimentary rocks, belongs to the mica group of minerals, encompassing biotite, phlogopite, and others. Its chemical composition is represented by the formula $KAl_2(AlSi_3O_{10})(OH)_2$, classifying it as a member of the phyllosilicate mineral class.

Typically, muscovite occurs as thin, transparent to translucent sheet-like crystals, exhibiting a range of colors from colorless to pale shades of brown, green, or pink. Muscovite's excellent basal cleavage property allows it to be easily split into thin, flexible sheets, giving it the common name "white mica". This mineral is commonly found in igneous rocks like granites and pegmatites, as well as metamorphic rocks such as schists. Sedimentary rocks formed from the alteration of pre-existing minerals can also contain muscovite.

Muscovite has found various industrial applications, particularly in the electrical, electronic, and construction sectors. Its exceptional dielectric properties make it valuable as an insulator in electrical equipment. Muscovite sheets are used in the manufacturing of windows, lampshades, and decorative items. In some cultures, muscovite is believed to possess metaphysical properties associated with self-reflection, clarity of thought, and insight.

The name "muscovite" itself is derived from "Muscovy," an antiquated term for Russia, where it was historically used as a source of window panes. While muscovite is not considered a high-value economic mineral by itself, it plays a pivotal role in the formation of specific types of pegmatite deposits that can contain economically significant minerals like lithium, beryllium, and gemstones. Thus, muscovite holds geological, industrial, and cultural significance worldwide.

Materials and Methods

In this study, a dataset comprising 197924 16S rRNA clone sequences derived from Muscovite samples was utilized. These sequences were originally obtained from grounded muscovite mineral, sourced from an underground mine located at Sydapuram, Gudur, Division, Nellore district (latitude of 14°10' and 14°19' E longitude of 79°35' and 79°45' W) Andhra Pradesh.

The construction of the clone library and subsequent sequence analysis were previously described in detail by Elshahed *et al.*, (2008). Operational Taxonomic Units (OTUs) were defined at a 97% similarity threshold using the DOTUR software.

The representative sequences for each cyanobacteria OTU, as identified in the original study, were aligned using the Greengenes NAST alignment tool (DeSantis *et al.*, 2006). These aligned sequences, along with closely related 16S rRNA sequences, were incorporated into the Greengenes (Ludwig *et al.*, 2004).

The Pintail online tool (Ashelford *et al.*, 2005) was employed to scrutinize individual sequences within the cyanobacteria dataset. Suspicious sequences, identified by Bellerophon (Huber *et al.*, 2004) or those showing unclear phylogenetic affiliation, were used as queries. The closest cultured relative or a reliably closely-related abundant KFS OTU sequence (n>50) was chosen as the reference sequence.

After removing chimeric sequences, a total of 517 OTUs, were taxonomically classified at the species level. Novel families were defined as groups of at least two clone sequences showing >92–93% sequence similarity with each other but <92–93% sequence similarity to sequences from already-named families. The definition of novel orders followed a similar criterion, using 90% sequence similarity as a general cutoff, although these values varied between each class of cyanobacteria.

Results and Discussion

Small subunit (16S rRNA) gene-based surveys have clearly shown that the scope of phylogenetic diversity in muscovite is much broader than that implied using culture-based approaches. Although having a remarkably stable cyanobacterial phylum level diversity, Muscovite is an extremely diverse ecosystem at the order, family, genus and species levels. The detailed phylogenetic analysis and taxonomic placements of 16S rRNA gene sequences of cyanobacteria has been the main focus of the present diversity studies. In muscovite the phylum cyanobacteria represents 0.5983%. The most abundant class is ML635J-21, Nostocophycidae, Synechococcophycidae, Oscillatoriophycidae and unclassified (Table-1).

The composition and novel or unclassified lineages within different classes of Cyanobacteria in Muscovite presents several significant findings. Cyanobacteria, as a whole, are represented by 517 clones in the microbial community, indicating their presence. Interestingly, the data reveals a notable percentage (0.2616%) of Cyanobacteria clones falling into unclassified orders, implying the existence of unidentified microbial lineages within this group in Muscovite. Although no novel orders are specified, it highlights the potential for undiscovered microbial diversity (Table-2). The phylum cyanobacteria further distinguishes different classes within Cyanobacteria, with ML635J-21, Nostocophycidae, Synechococcophycidae, and Oscillatoriophycide classes each under represented.

These classes show varying clone counts, with ML635J-21 class being the most abundant with 225 clones. Additionally, the unclassified category further underscores the need for taxonomic characterization within Cyanobacteria in this environment. These results emphasize the diversity and potential novelty of Cyanobacteria in Muscovite, inviting further exploration to uncover their ecological roles and contributions. The distribution of orders and families within

Cyanobacteria in Muscovite, yields valuable insights into the composition of this microbial group (Fig-1 and Fig-2). Notably, there are no orders or families classified as abundant, indicating that specific orders or families within Cyanobacteria are not highly prevalent in this ecosystem. However, three orders and three families fall into the intermediate category, suggesting a moderate level of diversity and representation within Cyanobacteria. Similarly, two orders and two families are categorized as rare, emphasizing the lower prevalence of these microbial groups within Cyanobacteria in the Muscovite environment. In total, there are five distinct orders and five families identified, underscoring the diversity and complexity of Cyanobacteria in this unique environment. These classifications set the stage for further investigations into the ecological roles and contributions of these Cyanobacteria orders and families in the Muscovite ecosystem.

The results you provided describe the diversity and composition of Cyanobacteria in the muscovite ecosystem, highlighting both known and potentially novel lineages within this phylum. Here is a discussion of the results along with suggested citations: The study utilized small subunit (16S rRNA) gene-based surveys, revealing a much broader phylogenetic diversity in muscovite compared to culture-based approaches. This observation underscores the importance of molecular techniques in uncovering microbial diversity that may otherwise remain undetected (Elshahed *et al.*, 2008). At the phylum level, Cyanobacteria represent a relatively small proportion (0.5983%) of the microbial community in muscovite. However, this group displays remarkable stability in diversity, indicative of its ecological significance in this ecosystem. Within Cyanobacteria, the study identified several classes, including ML635J-21, Nostocophycidae, Synechococcophycidae, and Oscillatoriophycidae, as well as an unclassified category. These findings shed light on the taxonomic distribution and relative abundance of different classes within Cyanobacteria in muscovite.

Table.1 Composition and abundance of phylum Cyanobacteria in Muscovite and soil sample

Name of the Sample	% abundance of Cyanobacteria	% of each class with in Cyanobacteria					
		ML63 5J-21	Nostocophycideae	Synechococcophycideae	Oscillatorioophycideae	4C0 d-2	Unclassified
Muscovite	0.5983%	0.2604	0.2025	0.1296	0.0046	-	0.0012
Soil	3.8176%	0.0168	2.5731	0.1642	0.0084	0.0105	1.0444

Table.2 Composition and novel and unclassified lineages within the different classes of Cyanobacteria in Muscovite

Lineage	No. of clones	Total No. of orders	No. of novel orders	% of clones in unclassified order	Total No. Of families	No. of novel families	% of clones in unclassified families
Cyanobacteria total	517	05	-	0.2616	05	-	0.4641
ML635J-21	225	01	-	0.2604	01	-	0.2604
Nostocophycideae	175	01	-	-	01	-	0.2025
Synechococcophycideae	112	01	-	-	01	-	-
Oscillatorioophycide	4	01	-	-	01	-	-
Unclassified	1	01	-	0.0012	01	-	0.0012

Fig.1 Distribution of Abundant, Intermediate and rare orders in Cyanobacteria phylum of muscovite

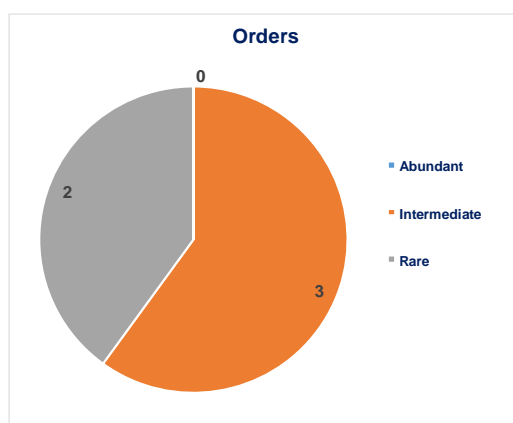
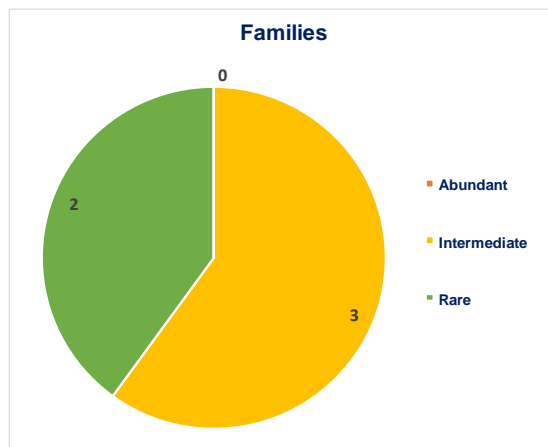


Fig.2 Distribution of Abundant, Intermediate and rare families in Cyanobacteria phylum of muscovite



Remarkably, a substantial portion (0.2616%) of Cyanobacteria clones were classified as unclassified orders, suggesting the potential presence of unidentified and potentially novel microbial lineages within this group. This underscores the need for further taxonomic characterization and exploration of this ecosystem's microbial diversity. The most abundant class, ML635J-21, further highlights the dominance of certain Cyanobacteria lineages in muscovite, with 225 clones identified. The presence of an unclassified category emphasizes the complexity and potential novelty of Cyanobacteria in this environment.

The distribution of orders and families within Cyanobacteria provides valuable insights into the microbial composition of muscovite. Notably, the absence of highly abundant orders or families suggests a more even distribution of diversity within this phylum. This diversity is further highlighted by the presence of orders and families categorized as intermediate and rare, signifying a moderate to lower prevalence within Cyanobacteria. In total, five distinct orders and five families were identified, further underscoring the complexity and diversity of Cyanobacteria in this unique environment. These classifications lay the foundation for future studies to explore the ecological roles and contributions of these specific Cyanobacteria orders and families in the muscovite ecosystem.

The distribution of orders and families within Cyanobacteria provided valuable insights into the microbial composition of Muscovite. Notably, no orders or families were classified as abundant, suggesting an even distribution of diversity within the phylum. Instead, three orders and three families were categorized as intermediate, indicating a moderate level of diversity and representation. Additionally, two orders and two families were classified as rare, indicating their lower prevalence. In total, five distinct orders and five families were identified, underscoring the complexity and diversity of Cyanobacteria in this unique environment. These classifications lay the foundation for future investigations into the ecological roles and contributions of these specific Cyanobacteria orders and families in the Muscovite ecosystem.

This study sheds light on the intricate microbial diversity within the Muscovite ecosystem, emphasizing the importance of comprehensive molecular approaches in understanding the ecology of this unique environment.

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