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新疆特殊生境岩石内生细菌末端限制性片段长度多态性技术分析

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摘要：【目的】了解新疆特殊生境不同类型岩石内生细菌的组成及多样性。【方法】采用末端限制性片段长度多态性技术（Terminal Restriction Fragment Length Polymorphism, T-RFLP），分析新疆乌苏花岗岩（1号样）、一冰川和木垒变质岩（2, 3号样）、裕民和托克逊岩石漆（4, 5号样）内生细菌群落。【结果】样品间多样性指数变化不大；聚类分析表明岩石类型相同，其相似性较高，2号样和3号样聚为一支并与1号样再聚为一支，4号样与5号样聚为一支；各样品共有种群为厚壁菌门（Firmicutes）、放线菌门（Actinobacteria）、变形菌门（Proteobacteria）、浮霉菌门（Planctomycetes）；4号样、5号样优势类群为放线菌门（29.3%），其它4个样品均为变形菌门，只是所占比例略有不同。【结论】生境不同的同类型岩石的内生细菌群落组成存在差异，各类岩石中可能存在大量未知细菌新种。

关键词：岩石内生细菌，T-RFLP，多样性
T-RFLP, PCR, DNA, T-RF, Hha I, Msp I

1

1.1

1.1.1

PCR, DNA, T-RF, Hha I, Msp I

72°C, 2 min; 95°C, 1 min; 30 min, 10°C

1.1.6

T-RFLP, Hha I, Msp I

37°C, 9 h, 1 min; 80°C, 20 min

1.1.7

T-RF, Shannon index, Evenness index, Simpson index

Cs = 2N_{AB}/N_{A} + N_{B} - N_{AB}

1.1.8

MiCA PAT′ (http://mica.ibest.uidaho.edu/) TRFs

Hha I, Msp I

4%

2

2.1

T-RFLP, Hha I, Msp I

5

Shannon-weiner (H′), Simpson (D), Evenness (E)

0.5 μL[x], 1 μLaq polymerase, 0.1 μL[y], 1 μL[x]

25 μL; PCR, 95°C, 5 min; 95°C, 45 s

55°C, 45 s; 72°C, 1 min, 30 cycles; 72°C, 10 min

4

Hha I, Msp I

Hha I, Msp I

37°C, 9 h, 1 min

80°C, 20 min

9

Hha I, Msp I

37°C, 9 h, 1 min

80°C, 20 min

9

Hha I, Msp I
牟文婷等：新疆特殊生境岩石内生细菌末端限制性片段长度多态性技术分析

Fig. 1 T-RFLP profile of bacterial Community. Numbers 1-5 mean sample 1-sample 5. H and M are different restriction endonuclease [HhaI and MspI].
Table 1  Diversity analysis of bacterial community

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hha I</th>
<th>Msp I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T-RFs</td>
<td>Shannon-Weiner index ($H'$)</td>
</tr>
<tr>
<td>sample1</td>
<td>25</td>
<td>2.33</td>
</tr>
<tr>
<td>sample2</td>
<td>30</td>
<td>2.32</td>
</tr>
<tr>
<td>sample3</td>
<td>31</td>
<td>2.51</td>
</tr>
<tr>
<td>sample4</td>
<td>35</td>
<td>2.61</td>
</tr>
<tr>
<td>sample5</td>
<td>29</td>
<td>2.37</td>
</tr>
</tbody>
</table>

2. 2  Samples similarity and cluster analysis

Clustering analysis can reflect the similarity of the bacterial community in different samples. Sample 2 and sample 3 clustered into a branch, and then clustered with sample 1 as a branch (Figure 2). Sample 4 and sample 5 clustered into a branch; this indicates that samples from different habitats have similar bacterial communities.

2. 3  Dominant microbial community analysis

According to the T-RFLP fingerprinting analysis of Hha I and Msp I restriction enzymes, the dominant bacteria in each sample are shown in Figure 3. There are five types of bacteria in the five samples: Firmicutes, Actinobacteria, Proteobacteria, Bacteroidetes, and Planctomycetes. The dominant bacteria in each sample are as follows: sample 1 is Bacillus (18.7%); sample 2 is Pseudomonas (21.2%); sample 3 is Micrococcus (13.6%); sample 4 is Bacillus (14.0%); and sample 5 is Arthrobacter (16.3%).
表2 优势细菌

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hha I observed</th>
<th>Msp I observed</th>
<th>Area/%</th>
<th>Accession</th>
<th>Similar group</th>
<th>Phylum</th>
</tr>
</thead>
<tbody>
<tr>
<td>sample 1</td>
<td>210.74</td>
<td>147.77</td>
<td>6.7</td>
<td>EF592490</td>
<td>Bacillus</td>
<td>Firmicutes</td>
</tr>
<tr>
<td>61.74</td>
<td>88.88</td>
<td>6.1</td>
<td>EF452662</td>
<td>Paenibacillus</td>
<td>Proteobacteria</td>
<td></td>
</tr>
<tr>
<td>176.96</td>
<td>129.23</td>
<td>5.3</td>
<td>EU131161</td>
<td>Acetobacter</td>
<td>Actinobacteria</td>
<td></td>
</tr>
<tr>
<td>61.74</td>
<td>462.81</td>
<td>21.2</td>
<td>AM411057</td>
<td>Pseudomonas</td>
<td>Proteobacteria</td>
<td></td>
</tr>
<tr>
<td>179.02</td>
<td>142.27</td>
<td>16.9</td>
<td>HQ327127</td>
<td>Arthrobacter</td>
<td>Actinobacteria</td>
<td></td>
</tr>
<tr>
<td>96.31</td>
<td>141.81</td>
<td>8.5</td>
<td>FJ529274</td>
<td>Acidobacterium</td>
<td>Acidobacteria</td>
<td></td>
</tr>
<tr>
<td>91.08</td>
<td>138.71</td>
<td>7.6</td>
<td>FJ481334</td>
<td>Chloroflexus</td>
<td>Chloroflexi</td>
<td></td>
</tr>
<tr>
<td>104.47</td>
<td>96.42</td>
<td>4.8</td>
<td>AB470321</td>
<td>Bacteroides</td>
<td>Bacteroidetes</td>
<td></td>
</tr>
</tbody>
</table>

讨论

同为花岗岩内生细菌，乌苏的优势类群为变形菌门（21.2%）和厚壁菌门（18.1%），美国犹他州的为变形菌门（54.5%）和厚壁菌门（27.3%），两者结果类似；而美国南洛基山的为放线菌门（41%），厚壁菌门仅为9%。一号冰川和木垒变质岩的优势类群均为变形菌门，分别占整个细菌种群的21.4%和22.1%，但种属不同，一号冰川为Azotobacter、Rhizobium和Sphingomonas，木垒为Pseudomonas、Rhizobium和Flavobacterium。此外降雨量、岩石渗透率及元素组成也可影响微生物多样性和丰度。
Chloroflexus, Flavobacterium, Bacillus, Arthrobacter, Rhodobacter.

(24.5%)(62%), Whipple, pH, Mn^{2+}, Mn^{4+}, Fe, Al, Si, Mn, Bacillus, Arthrobacter, Rhodobacter.


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Terminal restriction fragment length polymorphism analysis of endolithic bacteria community at special habitats in Xinjiang

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Abstract:

Objective: In order to investigate the composition and diversity of endolithic bacteria at special habitats in Xinjiang.

Methods: Five rock samples were collected including Wusu’s granite (sample 1), Glacier No. 1 and Mulei’s metamorphic rock (sample 2 sample 3), Yumin and Tokesun’s Rock varnish (sample 4 sample 5). Endolithic bacterial community composition and diversity were analyzed by the method of Terminal Restriction Fragment Length Polymorphism.

Results: Differences in diversity indexes among samples were not apparent. Clustering analysis suggested that similarity coefficient was higher in same rock type, sample 2 and sample 3 were grouped together, then sample 1 clustered with them and sample 4 and sample 5 were classified together. All samples harbored these phyla such as Firmicutes, Actinobacteria, Proteobacteria and Bacteroidetes. Acidobacteria and Planctomycetes existed in sample 1 and sample 2 respectively; Sample 5 was dominated by Actinobacteria, while other samples were dominated by Proteobacteria.

Conclusion: The endolithic bacterial composition of same rock type collected at various habitats was different. Meanwhile, a diversity of novel species and lineages maybe existed in rocks.

Keywords: endolithic bacteria, T-RFLP (Terminal Restriction Fragment Length Polymorphism), diversity

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