我国主要生态区域绿豆慢生根瘤菌的遗传多样性和系统发育研究

作者简介：袁天英（），女，河南安阳人，硕士研究生，主要从事根瘤菌多样性和生物固氮遗传学方面的研究。

基金项目：国家‘’重点基础研究项目（）；国家微生物资源平台建设项目（）；农业微生物学国家重点实验室开放基金资助

通讯作者。
1.1.1

<table>
<thead>
<tr>
<th>Strains</th>
<th>Host plant</th>
<th>Geographical origin</th>
<th>16S rRNA genotype</th>
<th>16SrRNA RFLP patterns</th>
<th>IGS RFLP patterns</th>
</tr>
</thead>
<tbody>
<tr>
<td>LY1</td>
<td>Vigna radiata</td>
<td>Lianyang, Jiangsu, China</td>
<td>I</td>
<td>ABAC</td>
<td>1</td>
</tr>
<tr>
<td>LY2</td>
<td>Vigna radiata</td>
<td>Lianyang, Jiangsu, China</td>
<td>I</td>
<td>ABAC</td>
<td>11</td>
</tr>
<tr>
<td>LY3, LY4, LY5, LY6, LY7, LY8, LY9, LY10, LY11, XJ1, XJ2, XJ3, XJ4, XJ5, XJ6, XJ7, XJ8, XJ9, XJ10, XJ11, XJ12</td>
<td>Vigna radiata</td>
<td>Shizhe, Xinjiang, China</td>
<td>II</td>
<td>AAAA</td>
<td>9</td>
</tr>
<tr>
<td>HD1, HD2, HD3</td>
<td>Vigna radiata</td>
<td>Handan, Hebei, China</td>
<td>II</td>
<td>AAAA</td>
<td>6</td>
</tr>
<tr>
<td>HD4</td>
<td>Vigna radiata</td>
<td>Handan, Hebei, China</td>
<td>I</td>
<td>ABAC</td>
<td>15</td>
</tr>
<tr>
<td>SC2</td>
<td>Vigna radiata</td>
<td>Yangzhou, Jiangsu, China</td>
<td>I</td>
<td>ABAC</td>
<td>3</td>
</tr>
<tr>
<td>SC3G</td>
<td>Vigna radiata</td>
<td>Yangzhou, Jiangsu, China</td>
<td>II</td>
<td>AAAA</td>
<td>10</td>
</tr>
<tr>
<td>SX1</td>
<td>Vigna radiata</td>
<td>Guanlin, Guanzhou, China</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SX2</td>
<td>Vigna radiata</td>
<td>Guanlin, Guanzhou, China</td>
<td>IV</td>
<td>ACCA</td>
<td>5</td>
</tr>
<tr>
<td>SX3</td>
<td>Vigna radiata</td>
<td>Guanlin, Guanzhou, China</td>
<td>IV</td>
<td>ACCA</td>
<td>16</td>
</tr>
<tr>
<td>SX4</td>
<td>Vigna radiata</td>
<td>Guangzhou, Guangdong, China</td>
<td>IV</td>
<td>ACCA</td>
<td>16</td>
</tr>
<tr>
<td>SX5</td>
<td>Vigna radiata</td>
<td>Shiji, Shandong, China</td>
<td>I</td>
<td>ABAC</td>
<td>2</td>
</tr>
<tr>
<td>SX6</td>
<td>Vigna radiata</td>
<td>Shiji, Shandong, China</td>
<td>I</td>
<td>ABAC</td>
<td>2</td>
</tr>
<tr>
<td>Bradyrhizobium japonicum</td>
<td>Glicyne max</td>
<td>United States</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USDA110</td>
<td>Glicyne max</td>
<td>United States</td>
<td>IV</td>
<td>ACCA</td>
<td>19</td>
</tr>
<tr>
<td>Bradyrhizobium elkanii</td>
<td>Glicyne max</td>
<td>United States</td>
<td>IV</td>
<td>ACCA</td>
<td>20</td>
</tr>
<tr>
<td>USDA46, USDA78T</td>
<td>Glicyne max</td>
<td>United States</td>
<td>IV</td>
<td>ACCA</td>
<td>19</td>
</tr>
<tr>
<td>USDA86</td>
<td>Glicyne max</td>
<td>United States</td>
<td>IV</td>
<td>ACCA</td>
<td>20</td>
</tr>
<tr>
<td>Bradyrhizobium liaoningense</td>
<td>Glicyne max</td>
<td>China</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a The 16S rRNA genotype and IGS RFLP patterns represent combination of restriction patterns obtained by enzymes used. b Each letter refers to a restriction pattern obtained with enzymes Hae III || Hpa I || Hinf I || and Msp I || respectively and the restriction pattern of B. japonicum USDA110 was assigned AAAA pattern.

1.1.2

1.2

Table 1: Vigna radiata rhizobia and reference strains.

10% SDS 50μg/mL 37°C 1mol/L NaClO4 TE Bio-Rad E

1.2

Beckman DU-800

© 中国科学院微生物研究所期刊联合编辑部
http://journals.im.ac.cn
1.3 16S rRNA  PCR-RFLP  

20 μL  PCR 反应体系：
25 mmol/L dATP, dCTP 15 mmol/L dGTP, dTTP 10 mmol/L 0.3 μmol/L dNTP
5 μmol/L dNTP, 0.5 μmol/L dNTP, 0.5 μmol/L primer D1
5 μmol/L forward primer D1
5 μmol/L reverse primer D1

timer component 2 μL 10× buffer 2 μL MgCl2

1.4 16S-23S rRNA IGS PCR-RFLP

pH=5' 5'-TGGCGGCTGCAGCACCC
TCCTG-3'

p32SR01 5'-GGCTGCTCTAAGCC
AAC-3'

16S rRNA 23S rRNA

IGS PCR  PCR-RFLP  

Kodak 20%  Yang

1.5 16S-23S rRNA IGS Alignment

Alignment
Mega

Neighbor-joining

2.2 16S rRNA RFLP

16S rRNA RFLP

1.5 kb DNA 1 μL LYG10 1 μL GZ1

GenBank

DQ442912  DQ442913

2.3 16S-23S rRNA IGS PCR-RFLP

pH 6.1

p32SR01

PCR

1.8 kb

Hae III Hha I Hinf I Msp I

UPGMA

BioEdit

16S rRNA RFLP

1% B. elkanii

B. japonicum

B. liaoningense

85%

16S rRNA PCR-RFLP

Fig. 1 UPGMA dendrogram generated from the 16S rRNA gene RFLP fingerprints and the 16S rRNA PCR-RFLP patterns of the representatives produced by four enzymes. A\[HphI\] \[HaeIII\] \[ClaI\] HhoI \[MspI\]. Line 1: LYG1, 221, 3, USDA110, 4, XJ1, 5, GX1, 6, GX2, 7, USDA76.

Fig. 2 Dendrogram of strains generated by Neighbor-joining method. Kimura-2 distances were derived from a distance matrix to construct an optimal unrooted tree using the Neighbor-joining method. Numbers in the parenthesis are the GenBank accession number of the sequences.
3

3.1

16S-23S rRNA IGS RFLP

Fig. 3 UPGMA dendrogram generated from the 16S-23S rRNA IGS RFLP fingerprints and the 16S-23S rRNA IGS RFLP patterns of the representatives produced by four enzymes. A  Hae I  B  Hpa I  C  Hpi I  D  Msp I  E  Line F  LYGI

供试菌株的聚类与16S-23S rRNA IGS RFLP分析大体一致,与B. elkanii的代表菌株聚在一起的F群供试菌株均分离自南方如广西和广东,群B包括B. japonicum USDA110, B. liaoningense 22817和B. elkanii的其他供试菌株,相当于16S-23S rRNA IGS RFLP中的群A和群B。但由聚类结果可以看出:与16S-23S rRNA IGS RFLP相比,16S-23S rRNA IGS RFLP提供了更高的解析度,供试菌株和参比菌株共分为95种16S-23S rRNA IGS RFLP型,在系统发育上表现出更加丰富的遗传多样性。

地理位置和气候环境是影响根瘤菌多样性的主要因素,我国新疆属于典型的干旱和半干旱气候特征,每一绿洲农业生态系统相对独立,不同地区根瘤菌间很少有机会接触,由于受长期的地域隔离,很少有机会与其他地区的根瘤菌接触,也很难有遗传物质的交换。形成了分离自该地区的根瘤菌的遗传相似性很高,而与其它地区的菌株远缘的特性。
Studies on genetic diversity and phylogeny of slow-growing rhizobia isolated from Vigna radiata at main ecotypes of China

YUAN Tian-ying¹,², YANG Jiang-ke³, ZHANG Wei-tao³, ZHOU Jun-chu³ *

¹ State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, China
² College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, China

Abstract: Studies on genetic diversity and phylogeny of slow-growing rhizobia isolated from Vigna radiata at main ecotypes of China were conducted by using 16S rRNA gene PCR-RFLP, 16S rRNA gene sequencing and 16S-23S rRNA hybridization analysis. Results of 16S rRNA gene PCR RFLP analysis reveal that all the strains tested are clustered into three groups at the similarity of 76%. Group I contains 13 slow-growing rhizobia tested including LYG1. Group II consists of 21 strains tested and the type strains of B. japonicum and B. liaoningense and 10 tested strains isolated from Guangdong, Guangxi and the type strain of B. elkanii. Group III. The results of 16S-23S rRNA IGS PCR-RFLP show that strains tested could be divided into A and B groups which could be correspondently subdivided into A I I A II I A III I B I and B II subgroups at the similarity of 85%. Compared with 16S rRNA PCR-RFLP, IGS RFLP assay show higher resolution strains and reference strains tested can be divided into 21 IGS RFLP patterns. The strains isolated from Xinjiang, Guangdong and Guangxi regions show obvious geographical effect on genetic diversity.

Keywords: Slow-growing rhizobia, 16S rRNA PCR-RFLP, 16S-23S rRNA IGS PCR-RFLP

Foundation items: Key Project of Chinese National Programs for Fundamental Research and Development (001CB1089), Open Foundation of National Laboratory of Agriculture Microbiology

* Corresponding author. Tel: 86-27-87281685; Fax: 86-27-87280670; E-mail: zoujunchu@mail.hzau.edu.cn
Received 16 March 2006/Accepted 31 May 2006/Revised 4 June 2006

© Chinese Academy of Agricultural Sciences