

# 不同宿主植物根瘤*Frankia*及其生物学特性\*

谢一青\*\*

(福建省林业科学研究院 福州 350012)

**摘要** 对从木麻黄、杨梅、桤木和胡颓子等宿主植物根瘤中获得的19株分离菌的离体培养形态特征、生理特性和交叉侵染特性等进行了比较分析。结果表明,各分离菌均有*Frankia*属所特有的分枝状菌丝、孢囊、泡囊或串珠状菌丝等形态结构;细胞壁类型多属胞壁Ⅲ型,生理类型多属B型,无氮诱导培养下都具有固氮酶活性,在BAP、JA或S培养液中菌体生长较好,以吐温-80和酪蛋白水解物为最佳碳、氮源。但不同宿主分离菌的形态和培养特征差异明显,木麻黄属分离菌菌丝较粗,孢囊数量较少,在BAP培养液中多呈荔枝肉白絮状颗粒沉淀;杨梅属菌丝较细,孢囊数量较多,在BAP培养液中多是浅红色颗粒沉淀;桤木属和胡颓子属的菌体形态特征在BAP培养液中与木麻黄属的相似,但桤木属菌丝较细,胡颓子属的菌丝较粗。根据回接及交叉侵染特性可将分离菌分为2个宿主特异类群:能侵染木麻黄苗木的木麻黄类群;只侵染原宿主并能在杨梅属、胡颓子属和桤木属间相互侵染,但不能使木麻黄属苗木结瘤的杨梅-桤木-胡颓子类群。图2表5参19

**关键词** *Frankia*菌; 生物学特性; 交叉侵染; 宿主特异性

CLC S718

## Biological Characteristics of *Frankia* Isolated from Different Actinorhizal Plants\*

XIE Yiqing\*\*

(Fujian Academy of Forestry, Fuzhou 350012, China)

**Abstract** 19 strains were isolated from the root nodules of actinorhizal plants *Casuarina*, *Myrica*, *Elaeagnus* and *Alnus* by squashing, and their morphological, physiological and cross-infection characteristics were studied. The results showed that all the isolates had a typical *Frankia* morphology with filamentous hyphae, sporangia and vesicle. The reproductive torulose hyphae were observed in some isolates (FCc64, FCe33 and FMr43). Most of the *Frankia* isolates were characterized in that their cell walls were type III and the physiological groups belonged to B. Furthermore, it is deduced that these strains all had nitrogenase activity for their growth in the nitrogen-free medium. They were also found growing well in liquid media BAP, JA and S. Tween-80 and casein were the optimum carbon and nitrogen sources. However, some obvious differences in morphological and cultural characteristics occurred among the strains isolated from different actinorhizal plants. Most of the isolates from *Casuarina* had thick hyphae and few sporangia, and formed Lichee's meat white flocculent granular precipitate in the BAP liquid medium, while most of the isolates from *Myrica rubra* had thin hyphae and many sporangia, and formed rosiness granular precipitate. The morphological characteristics of the isolates from *Alnus* and *Elaeagnus* were similar to the isolates from *Casuarina* in the same medium, but the isolates from *Alnus* had thin hyphae while the isolates from *Elaeagnus* had thick hyphae. The *Frankia* isolates were divided into two host-specific groups, according to back inoculation experiment and cross-infection of pure culture with seedlings of *Casuarina cunninghamiana*, *C. equisetifolia*, *C. glauca*, *Myrica rubra*, *Alnus cremastogym* and *Elaeagnus angustifolia*. The strains of group I which were all isolated from *Casuarina* could not only infect *Casuarina*, but also *Myrica*, *Elaeagnus* and *Alnus*, and those of group II from *Myrica*, *Elaeagnus* and *Alnus* had the ability to infect the unoriginal host plants except *Casuarina*. Fig 2, Tab 5, Ref 19

**Keywords** *Frankia*; biological characteristics; cross-infection; host specificity

CLC S718

*Frankia* (弗兰克氏菌)是一类能与非豆科植物结瘤共生固氮的放线菌,具有很强的共生固氮能力,能为陆地农林农业生产提供重要的氮素,在培肥土壤和改善生态环境中发挥重要作用。与*Frankia*共生固氮的宿主植物较多,据统计有

收稿日期: 2008-08-04 接受日期: 2008-10-24

\*福建省科技厅自然科学基金项目(No. B993001)和国家林业局南方山地用材林培育重点实验室资助 Supported by the Natural Science Foundation of Fujian Province, China (No. B993001) and the Key Laboratory of Timber Forest Cultivation in South China Mountains of the Ministry of Forestry of China

\*\*通讯作者 Corresponding author (E-mail: xielq@yahoo.com.cn)

8科、25属、279种<sup>[1, 2]</sup>。目前国内外已从桤木、杨梅、马桑、旱冬瓜、木麻黄、沙棘等多种放线菌结瘤植物根瘤中分离出许多弗兰克氏菌,研究内容涉及*Frankia*的形态特征、生物学特性、固氮酶活性、回接侵染特性和遗传多样性等<sup>[3~8]</sup>。但因*Frankia*分离成功率低,大部分属放线菌结瘤植物仍未分离到纯培养,分离到的菌株数量也极有限,加上*Frankia*生长缓慢、生物学特性多样等因素,使得整个*Frankia*研究进展依然缓慢,*Frankia*属以下的分类至今还缺乏统一的指标。本研究对木麻黄、杨梅、桤木、胡颓子等不同宿主植物根瘤*Frankia*的分离培养、生理特性及交叉侵染特性进行了比较,报道如

下,以期探讨不同宿主植物间根瘤*Frankia*的生物学差异,为*Frankia*属的进一步分类提供有益资料。

## 1 材料与方法

### 1.1 菌株来源及分离

用根瘤匀浆法<sup>[7]</sup>对采自福建的木麻黄、杨梅、桤木和胡颓子植物根部的新鲜根瘤进行分离,在S<sup>[9]</sup>、BAP<sup>[10]</sup>或JA<sup>[11]</sup>液体培养基中培养,共获得19株根瘤内生菌纯培养物,其基本情况见表1。

主分离菌菌丝粗细差异明显,胡颓子属和木麻黄属分离菌菌丝较粗(直径在0.35~1.0 μm之间),桤木属的较细,约0.35~0.8 μm,杨梅属的多小于0.35 μm。在杨梅属菌株FMrl6和木麻黄属的FCc64(图1右)、FCe33中还发现有长度不等的被Diem等<sup>[13]</sup>称之为串珠状生殖菌丝的菌丝结构。从表2还可看出,在BAP、S和JA培养液中,杨梅属分离菌的孢囊数量相对较多,木麻黄属的相对较少,而孢囊形状和大小宿主间差异不大,形状有球型、草莓型、纺锤型、豆荚型、棒型或不规则型,长约2.67~46.5 μm,宽约0.3~31.3 μm;在离体有氮或无氮诱导培

表1 供试的不同宿主植物根瘤内生菌  
Table 1 The isolates from different actinorhizal plants used in the test

分离菌 Isolate	宿主植物 Host plant	采集地点 Location	土壤 Soil
FCg07	粗枝木麻黄 <i>Casuarina glauca</i>	东山 Dongshan	滨海沙地 Coastal sand
FCg08	粗枝木麻黄 <i>C. glauca</i>	东山 Dongshan	滨海沙地 Coastal sand
FCe02 <sup>AC</sup>	细枝木麻黄 <i>C. cunninghamiana</i>	福州 Fuzhou	珍珠岩 Perlite
FCc64	细枝木麻黄 <i>C. cunninghamiana</i>	福鼎 Fuding	滨海盐渍地 Coastal tidal flat
FCc91	细枝木麻黄 <i>C. cunninghamiana</i>	惠安 Huian	滨海沙地 Coastal sand
FCe19	短枝木麻黄 <i>C. equisetifolia</i>	东山 Dongshan	滨海沙地 Coastal sand
FCe33	短枝木麻黄 <i>C. equisetifolia</i>	惠安 Huian	滨海沙地 Coastal sand
FCe42	短枝木麻黄 <i>C. equisetifolia</i>	菜舟 Laizhou	红壤 Red soil
FMrl6	杨梅 <i>Myrica rubra</i>	菜舟 Laizhou	山地红壤 Red soil on hill
FMrl31	杨梅 <i>M. rubra</i>	长汀 Changting	果园红壤 Red soil in orchard
FMrl43	杨梅 <i>M. rubra</i>	武夷山 Wuyishan	山地红壤 Red soil on hill
FMrl61	杨梅 <i>M. rubra</i>	福鼎 Fuding	果园红壤 Red soil in orchard
FMrl72	杨梅 <i>M. rubra</i>	福鼎 Fuding	山地红壤 Red soil on hill
FAc01	四川桤木 <i>Alnus cremastogyne</i>	菜舟 Laizhou	珍珠岩 Perlite
FAc03	四川桤木 <i>A. cremastogyne</i>	福州 Fuzhou	山地红壤 Red soil on hill
FAf07	台湾桤木 <i>A. formosana</i>	菜舟 Laizhou	山地红壤 Red soil on hill
FEo01	福建胡颓子 <i>Elaeagnus oldhami</i>	惠安 Huian	滨海沙地 Coastal sand
FEg17	蔓胡颓子 <i>E. glabra</i>	梅花山 Meihuashan	山地黄壤 Yellow soil on hill
FEg18	蔓胡颓子 <i>E. glabra</i>	梅花山 Meihuashan	山地黄壤 Yellow soil on hill

### 1.2 形态特征观察

将供试菌株分别等量接种于S、BAP和JA培养液中,28℃静置培养4~8 wk后,肉眼观察菌体生长量和色素,并在光学显微镜和电子显微镜下观察菌丝、孢囊和泡囊等形态结构。

### 1.3 细胞壁类型和固氮酶活性测定

菌体细胞壁类型和固氮酶活性参见谢一青方法<sup>[7]</sup>和胡传炯等方法<sup>[5]</sup>测定。

### 1.4 生理特性测定

生理类型划分和碳氮源利用参考Lechevelier等方法<sup>[12]</sup>测定。

### 1.5 回接及交叉侵染特性测定

将供试*Frankia*菌培养10 wk后,离心收集菌体,匀浆制备成菌液接种剂。用接种剂分别浸泡杨梅、细枝木麻黄、短枝木麻黄、粗枝木麻黄、四川桤木和沙枣等无菌幼苗根部2 h后,将苗木移栽至袋装的灭菌珍珠岩内,接种量为0.1 mg湿菌体/株苗。同时,以不接种*Frankia*的杨梅、木麻黄、四川桤木和沙枣苗木为对照,6 mo后调查苗木结瘤状况。

## 2 结论与分析

### 2.1 不同宿主*Frankia*的形态特征

在显微镜下观察到19个分离菌株均具有*Frankia*属所特有的分枝状菌丝、孢囊或泡囊等形态特征(图1左)。但不同宿

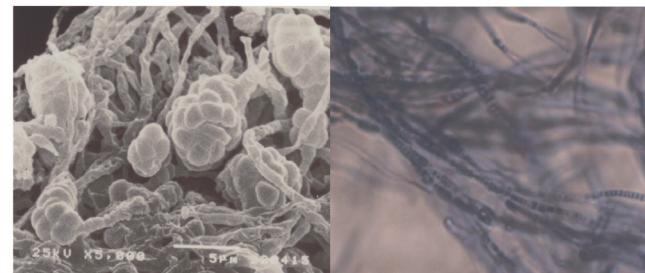


图1 FAf07形态特征(左)和FCc64的串珠状菌丝(右)  
Fig. 1 Morphological characteristics of FAf07 (Left) and reproductive torulose hyphae of FCc64 (Right)

养下,所有分离菌都能形成数量不等的直径约0.7~2.0 μm的球形孢囊,经测定均具有固氮酶(乙炔还原)活性,但宿主间差异不大,其中FMrl6的固氮酶活性远高于其他菌株,可能与孢囊数量多有关。在BAP培养液中,所有菌株不分泌可溶性色素,菌体都生长在试管的底部,但不同菌株在菌体形态和颜色上却有所不同,木麻黄属分离菌菌体多呈荔肉白絮状颗粒沉淀,杨梅属的多为浅红色颗粒状沉淀,桤木属和胡颓子属的多是荔肉白絮状沉淀(表2)。

### 2.2 不同宿主*Frankia*的细胞壁类型

由表3可知,测定分离菌的细胞壁都含有meso-DAP(内消旋二氨基庚二酸)、甘氨酸、谷氨酸和丙氨酸,但不同菌株细胞壁的4种氨基酸含量差异较大。从以甘氨酸的摩尔数

表2 分离菌的形态特征及固氮酶活性  
Table 2 Morphological characteristics and nitrogenase activity of *Frankia* isolates

分离菌 Isolate	菌丝直径 Hypha diameter	可溶性色素 Soluble pigment	孢囊形成 Sporangia formation			孢囊Vesicle <sup>1)</sup> Growth Size		菌体培养特征 <sup>2)</sup> Mycelium cultural characteristics	固氮酶活性 Nitrogenase activity [λ(C <sub>2</sub> H <sub>4</sub> )/μmol mg <sup>-1</sup> h <sup>-1</sup> ]
			BAP	S	JA				
FCc02 <sup>AC</sup>	B	-	+	++	++	+	D	荔枝肉白颗粒状 Lichee's meat white granular	0.443
FCc64	A	-	+++	++	+++	++	D	浅白絮状颗粒 Light white flocculent granular	1.923
FCc91	A	-	+	++	++	+	D	荔枝肉白絮状颗粒 Lichee's meat white flocculent granular	0.132
FCe19	A	-	+	+	+++	+	D	荔枝肉白絮状颗粒 Lichee's meat white flocculent granular	0.519
FCe33	A	-	++	++	+++	+	D	荔枝肉白颗粒状 Lichee's meat white granular	0.115
FCe42	A	-	+++	++	++	++	D	荔枝肉白絮状 Lichee's meat white flocky	1.241
FCg07	B	-	++	++	++	+	D	荔枝肉白絮状颗粒 Lichee's meat white flocculent granular	0.314
FCg08	B	-	++	++	++	+	D	荔枝肉白颗粒状 Lichee's meat white granular	0.252
FMr16	C	-	+++	+++	+++	+++	D	荔枝肉白絮状 Lichee's meat white flocky	12.760
FMr31	C	-	+++	+++	+++	+	D	浅红颗粒状 Rosiness granular	0.008
FMr43	B	-	+++	+++	+++	+	D	荔枝肉白絮状颗粒 Lichee's meat white flocculent granular	0.086
FMr61	C	-	+++	+++	+++	+	D	浅红颗粒状 Rosiness granular	0.045
FMr72	C	-	+++	+++	+++	+	D	浅红絮状颗粒 Rosiness flocculent granular	0.078
FAc01	B	-	+	++	++	+	D	荔枝肉白絮状颗粒 Lichee's meat white flocculent granular	0.045
FAc03	B	-	+++	+++	++	+	D	荔枝肉白絮状颗粒 Lichee's meat white flocculent granular	0.561
FAf07	B	-	+++	++	++	++	D	浅白絮状 Light white flocky	1.407
FEo01	A	-	+++	+	+++	+	D	浅白颗粒状 Light white granular	0.279
FEg17	A	-	++	++	+++	+	D	荔枝肉白絮状 Lichee's meat white flocky	0.102
FEg18	A	-	+++	++	++	+	D	荔枝肉白絮状 Lichee's meat white flocky	0.093

A, 0.55~1.0 μm; B, 0.35~0.8 μm; C, <0.35 μm; D, 0.7~2.0 μm

-: 不形成; +: 少数; ++: 中等; +++: 多。<sup>1)</sup>在BAP无氮液体培养基中; <sup>2)</sup>在BAP液体培养基中

-, Not formed; +, Few; ++, Some; +++, Many. <sup>1)</sup> In BAP nitrogen-free liquid medium; <sup>2)</sup> In BAP liquid medium

为1来换算其它3种氨基酸的分子比(表3)来看, 多数菌株的甘氨酸和丙氨酸之比均在1:3.2~16.5之间(胞壁Ⅲ型), 而菌株FCc02<sup>AC</sup>和FMr16细胞壁中的甘氨酸和丙氨酸分子比大于1:3.2~16.5, 根据Benson等<sup>[14]</sup>和胡传炯等<sup>[5]</sup>对*Frankia*属细胞壁类型的划分方法, 多数分离菌细胞壁类型应归为胞壁Ⅲ型(含meso-DAP), 而木麻黄属菌株FCc02<sup>AC</sup>和杨梅属的FMr16应属胞壁Ⅱ型(含meso-DAP和较高含量的甘氨酸), 说明不同宿主间分离菌的细胞壁类型差异不大, 同一宿主中也有不同细胞壁类型的菌株存在。

### 2.3 不同宿主*Frankia*的生理特性

根据Lechevelier等<sup>[12]</sup>和胡传炯等<sup>[5]</sup>方法对分离菌的生理类群进行划分的结果(表4)表明, 不同宿主间分离菌的生理类群没有差异, 同宿主植物有生理A型(加入Tween-80后*Frankia*不利用葡萄糖)、B型(加入Tween-80后*Frankia*能更好地利用葡萄糖)或AB混合型(Tween-80不影响*Frankia*对葡萄糖的利用)3个类群的菌株存在, 但多数分离菌生理类群属B型, 这与国际上报道的*Frankia*菌株多为生理A类群有异<sup>[9, 14]</sup>, 图2为3个不同生理类群分离菌在6种不同培养基中的生长状况。

况, 碳氮源和有机酸利用试验表明, 分离菌对碳氮源和有机酸的利用与生理类群之间没有很明显的对应关系(表4), 都不利用果糖和蛋白胨, 能利用吐温-80、乙酸钠、丙酸钠、丙酮酸钠和酪蛋白水解物, 并分别以吐温-80和酪蛋白水解物为最佳碳氮源, 而在甘露糖、麦芽糖、苹果酸钠及尿素等其它碳氮源和有机酸的利用上不同菌株有一定差异, 但与绝大多数的实验结果一致<sup>[15, 16]</sup>。

### 2.4 不同宿主*Frankia*的侵染特异性

将分离自不同宿主的*Frankia*回接苗木6 mo后调查发现, 对照苗木都没结瘤, 所有菌株均能成功回接侵染原宿主苗木并结瘤, 但不同宿主间分离菌交叉侵染结果差异明显(表5). 其中, 木麻黄属除菌株FCg07和FCg08分别不能侵染杨梅和沙枣结瘤外, 多数分离菌不仅能在木麻黄属内进行交叉侵染, 还能侵染杨梅、桤木属的四川桤木和胡颓子科的沙枣结瘤; 杨梅属分离菌能侵染沙枣和四川桤木结瘤, 但都不能感染木麻黄属的细枝木麻黄、短枝木麻黄和粗枝木麻黄苗木结瘤; 楫木属的能侵染杨梅和沙枣, 但不能使木麻黄结瘤; 胡颓子属的能侵染杨梅和四川桤木, 但不感染木麻黄。根据

表3 各菌株全细胞壁氨基酸含量及分子比  
Table 3 Content and molar ratios of four characteristic amino acids in cell wall of *Frankia* strains

分离菌 Isolate	氨基酸含量 Content of amino acid (b/μmol g <sup>-1</sup> mycelia)				氨基酸分子比 Molar ratio of amino acid				细胞壁类型 Cell-wall type
	Glu	Gly	Ala	meso-DAP	Glu	Gly	Ala	meso-DAP	
FCc02 <sup>AC</sup>	0.141	0.169	0.293	0.624	0.834	1.00	1.734	3.692	II
FCc64	0.203	0.267	0.918	0.994	0.760	1.00	3.438	3.723	III
FCe33	2.186	1.341	5.507	4.040	1.630	1.00	4.107	3.015	III
FMr16	0.104	0.619	1.094	1.900	0.168	1.00	1.767	3.069	II
FMr61	1.227	0.436	3.434	3.152	2.814	1.00	7.876	7.229	III
FMr72	3.732	1.265	9.174	6.890	2.950	1.00	7.252	5.447	III
FAc01	0.817	0.449	2.403	2.249	1.820	1.00	5.352	5.009	III
FAc03	1.404	0.747	3.718	3.542	1.880	1.00	4.977	4.742	III
FEo01	2.621	0.907	6.466	5.316	2.890	1.00	7.129	5.861	III

表4 各菌株对碳源、有机酸和氮源的利用  
Table 4 Utilization of carbon sources, organic acids and nitrogen sources by *Frankia* strains

分离菌 Isolate	生理类群 Physiological group	碳源 Carbon source								有机酸 Organic acid					氮源 Nitrogen source							
		Gl	So	Xy	Man	Fr	La	Su	Mal	Tw	Ac	Pr	Py	Su	Ma	Be	NS	Ur	Pe	Bee	Ca	
FCc02 <sup>AC</sup>	AB	1	0	0	0	0	0	0	0	2	2	3	3	2	0	0	0	0	0	0	2	2
FCc64	B	V	V	0	V	0	0	0	0	2	3	2	2	0	1	0	1	0	0	0	2	3
FCc91	B	0	0	0	0	0	0	0	0	2	3	3	3	1	0	0	1	0	0	0	2	2
FCe19	AB	0	0	0	0	0	0	0	0	1	2	2	3	V	V	1	0	0	0	0	0	3
FCe33	B	0	0	0	0	0	0	0	0	2	3	3	2	0	0	0	1	0	0	0	2	2
FCe42	A	0	0	0	V	0	0	0	0	1	2	3	3	1	0	0	1	0	0	0	2	2
FCg07	B	V	0	0	0	0	0	0	0	2	3	3	2	0	0	0	0	0	0	0	2	3
FCg08	B	V	0	0	0	0	0	0	0	2	2	3	3	3	V	0	1	0	0	2	3	
FMr16	A	V	1	1	1	0	1	0	1	2	2	3	3	1	0	0	3	0	0	0	3	3
FMr31	B	0	0	0	0	0	0	0	0	2	2	3	3	0	0	0	2	0	0	0	1	2
FMr43	B	0	0	0	0	0	0	0	0	2	2	3	2	0	0	0	1	0	0	0	V	3
FMr61	B	0	0	0	0	0	0	0	0	1	2	3	2	2	0	0	1	V	0	0	2	3
FMr72	B	0	0	0	0	0	0	0	0	2	2	3	2	0	0	0	1	0	0	1	1	1
FAc01	B	0	0	0	1	0	0	1	0	2	3	3	3	2	0	0	2	0	0	0	2	3
FAc03	B	0	0	0	V	0	0	0	0	3	3	3	2	0	0	0	2	0	0	0	1	3
FAf07	B	0	0	0	1	0	0	0	1	3	1	3	2	0	0	0	0	0	0	0	V	2
FEo01	AB	0	0	0	0	0	0	0	0	2	1	2	3	0	0	0	2	1	0	0	V	1
FEg17	B	0	0	0	0	0	0	0	0	2	2	2	2	0	0	0	1	0	0	0	2	2
FEg18	A	0	0	0	0	0	0	0	0	2	2	2	2	0	0	0	1	0	0	0	2	2

Gl, 甘油; So, 山梨醇; Xy, 木糖; Man, 甘露糖; Fr, 果糖; La, 乳糖; Su, 蔗糖; Mal, 麦芽糖; Tw, 吐温-80; Ac, 乙酸钠; Pr, 丙酸钠; Py, 丙酮酸纳; Su, 丁二酸纳; Ma, 苹果酸纳; Be, 苯甲酸纳; NS, 硫酸铵; Ur, 尿素; Pe, 蛋白胨; Bee, 牛肉膏; Ca, 酪蛋白。

0, 不生长; 1, 生长差; 2, 生长较好; 3, 生长好; V, 不确定; AB, 生理AB型; A, 生理A型; B, 生理B型

Gl, Glycerol; So, Sorbitol; Xy, Xylose; Man, Mannitol; Fr, Fructose; La, Lactose; Su, Sucrose; Mal, Maltose; Tw, Tween-80; Ac, Acetate; Pr, Propionate; Py, Pyruvate; Su, Succinate; Ma, Malate; Be, Benzoinate; NS,  $(\text{NH}_4)_2\text{SO}_4$ ; Ur, Urea; Pe, Peptone; Bee, Beef extract; Ca, Casein.

0, No growing; 1, Poorly growing; 2, Moderately growing; 3, Well growing; V, Uncertainty; AB, Physiological group tape AB; A, Physiological group tape A; B, Physiological group tape B

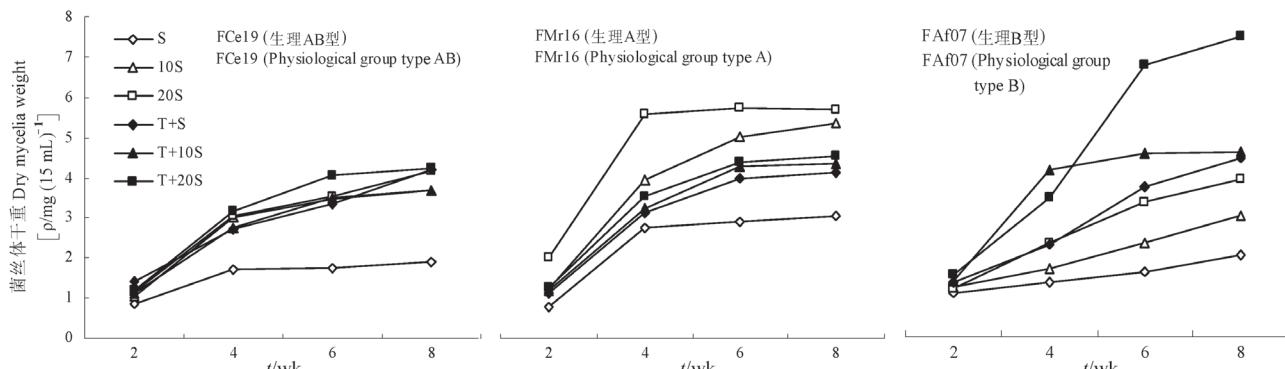


图2 分离菌FCe19、FMr16和FAf07在6种培养基中的生长状况  
Fig. 2 Growths of FCe19, FMr16 and FAf07 in six media

上述侵染结果可初步将分离菌划分为2个宿主特异类群, 能侵染木麻黄属苗木的木麻黄类群; 只侵染原宿主, 并能在杨梅属、胡颓子属和桤木属间相互侵染, 但不能使木麻黄属苗木结瘤的杨梅-桤木-胡颓子类群。这种宿主特异类群的划分结果与Bosco的研究结果<sup>[17]</sup>一致, 而与杜大至所划分的类群<sup>[18]</sup>有一定差异。此外, 3个生理A类群的菌株FCe42、FMr16和FEg18均能使宿主植物感染结瘤, 这与Lechevelier等<sup>[19]</sup>和Benson等<sup>[14]</sup>认为生理A类群菌株不能感染宿主植物结瘤的结果相异。

### 3 结论

形态指标一直是作为*Frankia*鉴别的主要依据。本研究中, 从木麻黄、杨梅、桤木和胡颓子根瘤中分离的菌株均具有*Frankia*属所特有的分枝状菌丝、孢囊、泡囊等结构, 杨梅

属分离菌FMr43和木麻黄属的FCc64、FCe33还有串珠状菌丝; 分离菌细胞壁类型多属胞壁III型, 生理类群多为生理B类群; 在离体无氮诱导下, 能形成数量不等的泡囊, 经乙炔还原活性测定有固氮酶活性; 在BAP、S和JA培养液中菌体生长较好, 能利用乙酸钠、丙酸钠和丙酮酸钠等有机酸, 以吐温-80和酪蛋白水解物为最佳碳氮源。据*Frankia*属定义<sup>[19]</sup>, 分离自不同宿主的菌株应为*Frankia*属的成员。但依现有的材料还无法进行种的划分, 其遗传学背景的明确还有待进一步研究。

不同宿主*Frankia*在主要形态特征及交叉侵染特性上有明显差异。其中木麻黄属*Frankia*菌丝较粗(直径约0.35~1.0 μm), 菌体多为荔肉白絮状颗粒沉淀, 能侵染木麻黄、杨梅、四川桤木和沙枣结瘤; 杨梅属*Frankia*菌丝较细(直径多小于0.35 μm), 菌体多为浅红色颗粒状沉淀, 能侵染沙枣和四川桤木结瘤, 但不能使木麻黄结瘤; 楫木属*Frankia*菌丝也较

表5 各分离菌侵染不同放线菌植物苗木的结果  
Table 5 Inoculation of the seedlings of different actinorhizal plants with *Frankia* strains

分离菌 Isolate	接种苗木(结瘤株数/回接株数) Plant tested (Number of plants nodulated/ number of plants inoculated)					
	<i>C. cunninghamiana</i>	<i>C. equisetifolia</i>	<i>C. glauca</i>	<i>M. rubra</i>	<i>A. cremastogym</i>	<i>E. angustifolia</i>
FCc02 <sup>AC</sup>	8/8	5/5	6/6	8/9	4/4	9/9
FCc64	6/6	6/6	11/11	6/9	6/6	8/8
FCc91	9/9	12/12	8/8	9/9	3/3	8/8
FCE19	6/9	5/8	6/8	9/9	6/6	7/9
FCE33	8/8	7/7	8/11	6/6	9/9	9/9
FCE42	11/11	6/10	6/10	9/9	6/9	6/8
FCg07	11/11	7/7	7/7	0/8	9/9	9/9
FCg08	3/8	7/7	6/12	7/9	3/4	0/8
FMr16	0/7	0/11	0/11	6/6	6/6	8/8
FMr31	0/8	0/6	0/9	8/8	8/11	8/10
FMr43	0/11	0/10	0/10	6/6	8/8	6/6
FMr61	0/8	0/11	0/11	9/9	5/8	8/8
FMr72	0/11	0/10	0/10	8/8	8/8	8/8
FAc01	0/8	0/10	0/11	8/9	8/8	6/6
FAc03	0/7	0/11	0/10	9/9	8/10	6/6
FAf07	0/11	0/11	0/11	8/8	8/8	8/8
FEo01	0/11	0/11	0/6	6/6	6/6	6/6
FEg17	0/7	0/5	0/6	6/6	6/8	6/6
FEg18	0/10	0/10	0/11	8/8	8/8	8/8

细(约0.35~0.8 μm), 菌体多为荔肉白絮状沉淀, 只感染杨梅和沙枣, 不能使木麻黄结瘤; 胡颓子属*Frankia*菌丝较粗(约0.55~1.0 μm), 菌体多为荔肉白絮状沉淀, 能感染杨梅和四川桤木, 但不感染木麻黄。这种差异是否与宿主的生境及地理区域有关, 尚需进一步研究证实。根据交叉侵染特性, 初步可将19株分离菌划分为2个宿主特异类群: 能感染木麻黄属苗木的木麻黄类群; 只感染原宿主, 并能在杨梅属、胡颓子属和桤木属间交叉侵染, 但不能使木麻黄属苗木结瘤的杨梅-桤木-胡颓子类群。本研究结果将为分离*Frankia*菌在农林业生产上的推广应用提供参考价值, 同时对*Frankia*属的进一步分群具有重要意义。

**致谢** 感谢李志真、王志洁和李朝晖在野外采样、菌体分离培养和生理测定等方面作了大量工作。

#### References

- Lechevalier MP. Taxonomy of the genus *Frankia* (*Actinomycetales*). *Int J Syst Bacteriol*, 1994, **44** (1): 1~8
- Baker DD, Mullin BC. Actinorhizal symbioses. In: Stacey G, Burns RH, Evans HJ ed. Biological Nitrogen Fixation. New York, USA: Chapman and Hall, 1992. 259~292
- Xiong Z (熊智), Tang XM (唐晓萌), Dai YM (代玉梅), Zhang CG (张成刚), Zhang ZZ (张忠泽), Xu LH (徐丽华). Genetic diversity of *Frankia* strains in *Alnus nepalensis* nodules in Yunnan revealed by rep-PCR. *Chin J Appl Environ Biol* (应用与环境生物学报), 2006, **12** (5): 623~627
- He XH, Chen LG, Hu XQ, Tori SA. Natural diversity of nodular microsymbionts of *Myrica rubra*. *Plant & Soil*, 2004, **262**: 229~239
- Hu CJ (胡传炯), Zhou PZ (周平贞), Zhou Q (周启). Identification of a pure culture of nodular endophyte from *Coriaria nepalensis*. *Acta Microbiol Sin* (微生物学报), 1997, **37** (6): 417~422
- Wu SH (吴少慧), Liu Z (刘忠), Zhang CG (张成刚), Zhang ZZ (张忠泽). Purification and identification of trace *Frankia* and from nodules. *Chin J Appl Environ Biol* (应用与环境生物学报), 2001, **7** (1): 76~78
- Xie YQ (谢一青). Isolation and characterization of a *Frankia* strain from *Casuarina glauca*. *Microbiology* (微生物学通报), 2004, **31** (5): 9~13
- Zhang CG (张成刚), Zhang ZZ (张忠泽), Li WG (李维光), Wang YY (王育英), Su FY (苏凤岩). Infectiveness of *Frankia* strains from *Hippophae rhamnoides*. *Chin J Appl Ecol* (应用生态学报), 1994, **5** (3): 299~302
- Lechevalier MP, Lechevalier HA. Systematics, isolation and culture of *Frankia*. In: Schwintzer CR, Tjepkema JD ed. The Biology of *Frankia* and Actinorhizal Plants. San Diego, USA: Academic Press Inc, 1990. 35~60
- Murry MA, Lopez MF. Interaction between hydrogenase, nitrogenase and respiratory activities in a *Frankia* isolate from *Alnus rubra*. *Can J Microbiol*, 1989, **35**: 636~641
- Akkermans ADL, Roelofsen W, Blom J, Huss DK, Harkink R. Utilization of carbon and nitrogen compounds by *Frankia* in synthetic media and in root nodules of *Alnus glutinosa*, *Hippophae rhamnoides*, and *Datisca cannabina*. *Can J Bot*, 1983, **61**: 2793~2800
- Lechevalier MP, Baker D, Horriere F. Physiology, chemistry, serology, and infectivity of two *Frankia* isolates from *Alnus incana* subsp. *rugosa*. *Can J Bot*, 1983, **61**: 2826~2833
- Diem HG, Dommergues YR. In vitro production of specialized reproductive torulose hyphae by *Frankia* strain ORS021001 isolated from *Casuarina junghuhiana* root nodules. *Plant & Soil*, 1985, **187**: 1~29
- Benson DR, Silvester WB. Biology of *Frankia* strains actinomycete symbionts of actinorhizal plants. *Microbiol Rev*, 1993, **57**: 293~319
- Wang CG (王晨光), Song SZ (宋尚直), Ruan XS (阮继生). Studies on biological characteristics of *Frankia*. *Acta Microbiol Sin* (微生物学报), 1993, **33** (4): 297~303
- Benson DR, Schultz NA. Physiology and biochemistry of *Frankia* in culture. In: Schwintzer CR, Tjepkema JD ed. The Biology of *Frankia* and Actinorhizal Plants. San Diego, USA: Academic Press Inc, 1990. 107~127
- Bosco M, Fernandez MP, Simonet P, Materassi R, Normand P. Evidence that some *Frankia* sp. strains are able to cross boundaries between *Alnus* and *Elaeagnus* host specificity groups. *Appl Environ Microbiol*, 1992, **58**: 1569~1576
- Du DZ, Baker DD. Actinorhizal host-specificity of Chinese *Frankia* strains. *Plant & Soil*, 1992, **144**: 113~116
- Lechevalier MP, Lechevalier HA. Genus *Frankia* Brunchorst 1886, 174AL. In: Williams ST, Sharpe ME, Holt JG ed. Bergeys Manual of Systematic Bacteriology. Baltimore, USA: The Williams Wilkins Co, 1989, **4**: 2410~2417