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Microbial resources management 微生物资源管理

中国农业微生物菌种保藏管理中心大豆根瘤菌精准评价

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摘　要：大豆(*Glycine max*)是世界上重要的粮食作物和油料作物。农业供给侧结构性改革需要增加优质食用大豆种植面积，但由于我国耕地资源的固有特性，大豆的国内产量远不能实现自给自足，亟需提高国内大豆种植面积和产量，摆脱对进口的依赖。根瘤菌(rhizobia)是研发最早的微生物肥料，但在我国的接种面积极低。【目的】本研究拟从库藏根瘤菌中筛选出大豆促生结瘤菌株，为缓解粮食问题提供种质资源保障。【方法】以中国农业微生物菌种保藏管理中心数十年收集的大豆根瘤菌为实验材料，结合 16S rRNA 基因和 *recA* 序列分析，复核菌种保藏信息；通过水培结瘤实验，综合考虑结瘤率、根瘤数量和根瘤质量以及植株株高和干重，评价菌株的结瘤和促生能力。【结果】共活化获得 213 株大豆根瘤菌菌株，其中 156 株隶属于慢生根瘤菌(*Bradyrhizobium*)、48 株隶属于中华根瘤菌属(*Sinorhizobium*)、9 株隶属于根瘤菌属(*Rhizobium*)。在这些菌株中，149 株能与本研究选用的大豆品种匹配结瘤，43 株能够显著促进大豆植株生长。【结论】本研究进一步明确了库藏大豆根瘤菌的分类地位，评价了其结瘤促生能力，为大豆根瘤菌菌剂的开发提供了丰富的菌质资源。

关键词：大豆；根瘤菌；结瘤促生；根瘤菌种质资源

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Accurate evaluation of soybean rhizobia preserved in the Agricultural Culture Collection of China

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Abstract: Soybean (*Glycine max*) is an important cereal and oil crop in the world, and the supply-side structural reform in the agricultural sector requires an increase in the planting area of high-quality edible soybean. Due to the inherent characteristics of arable land resources in China, the domestic production of soybean is far from self-sufficiency, and there is an urgent need to increase the planting area and production of soybean domestically, reducing the dependence on imports. Rhizobia are the earliest developed microbial fertilizer, while their application area is limited in China. **[Objective]** To select suitable strains from the stored rhizobia to provide germplasm resources for alleviating food issues. **[Methods]** We reviewed taxonomic status of rhizobia preserved in the Agricultural Culture Collection of China over decades based on the 16S rRNA and *recA* gene sequences. The hydroponic nodulation test was carried out to assess the nodulating and soybean growth-promoting effects based on comprehensive consideration of the nodulation rate, nodule number, nodule weight, plant height, and dry weight. **[Results]** A total of 213 strains of soybean rhizobia were activated and identified, including 156 strains of *Bradyrhizobium*, 48 strains of *Sinorhizobium*, and 9 strains of *Rhizobium*. Among them, 149 strains were able to nodulate with soybean cultivar selected in this study, and 43 strains significantly contributed to the growth of soybean plants. **[Conclusion]** This study further clarifies the taxonomic status of preserved soybean rhizobia and evaluates their nodulating and plant growth-promoting effects, providing abundant microbial resources for the development of soybean rhizobia-based agents.

Keywords: *Glycine max*; rhizobia; nodulating and growth-promoting effects; germplasm resources of rhizobia

微生物将 N_2 还原为 NH_3 的过程称为生物固氮(biological nitrogen fixation, BNF)。据固氮微生物(nitrogen-fixing microorganisms)与其他生物的关系，固氮微生物可分为：自生固氮菌(free-living nitrogen-fixer)、共生固氮菌(symbiotic nitrogen-fixer)以及联合固氮菌(associative nitrogen-fixer)。根瘤菌(rhizobia)和豆科(*Leguminosae*)植物的固氮体系是共生固氮的典型代表。全球每年生物固氮量约为 2.28×10^8 t，是工业固氮的 1.52 倍[1-2]，其中豆科植物与根瘤菌共生固氮量约 2.15×10^7 t[3-4]，占根瘤共生固氮量的 83.01%，占陆地生态系统参与的固氮量的

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24.43%[5]。

根瘤菌多指广泛存在于土壤中，能与豆科植物共生固氮的革兰氏阴性细菌[6]。绝大多数具有结瘤固氮能力的根瘤菌属于α-变形菌纲(*Alphaproteobacteria*)，包括根瘤菌属(*Rhizobium*)、剑菌属(*Ensifer*)、中华根瘤菌属(*Sinorhizobium*)[7]、异根瘤菌属(*Allorhizobium*)、副根瘤菌属(*Pararhizobium*)、新根瘤菌属(*Neorhizobium*)、申氏菌属(*Shinella*) (之前称为*Crabtreella*)、中慢生根瘤菌属(*Mesorhizobium*)、胺杆菌属(*Aminobacter*)、叶杆菌属(*Phyllobacterium*)、布鲁氏菌属(*Brucella*) (之前称为*Ochrobactrum*)、甲基杆菌属(*Methylobacterium*)[8]、微枝形杆菌属(*Microvirga*)[9]、慢生根瘤菌属(*Bradyrhizobium*)、固氮根瘤菌属(*Azorhizobium*)和德沃斯氏菌属(*Devosia*)。此外也有分类于β-变形菌纲[10](*Betaproteobacteria*)的副伯克霍尔德氏菌属(*Paraburkholderia*)和贪铜菌属(*Cupriavidus*)[11]，以及隶属于γ-变形菌纲(*Gammaproteobacteria*)的假单胞菌属(*Pseudomonas*)[12]。根瘤菌的多样性体现出共生固氮体系在自然界的广泛性，是研究豆科植物-根瘤菌共生体系进化、选育高效固氮系统以提高农业粮食产量的宝贵资源。

豆科植物与根瘤菌的共生固氮作用发生在植物根系。根瘤菌响应豆科植物根释放的黄酮类(flavonoids)物质，经过一系列生理反应后[13-14]与植物共生形成根瘤[15-16]。成熟根瘤在低氧微环境[17-18]中将N_2还原为NH_3供植物利用，植物则为根瘤菌提供营养物质实现共生。根瘤菌只能响应少数黄酮类物质，因此大多数根瘤菌具有较强的宿主特异性。除了共生固氮作用外，根瘤菌还具有各种植物根际促生菌(plant growth promoting rhizobacteria, PGPR)的能力，如溶磷[19-20]、解钾[21-22]，产生植物激素[23-27]、胞外多糖、铁载体[28]和生防植物病原体等[29-31]。

根瘤菌菌剂是最早研发的生物肥料产品，是世界上公认效果最稳定的微生物肥料，已在全世界范围内推广应用。据统计，根瘤菌在美国、巴西等大豆种植的主要国家接种率达到95%以上，然而我国主要豆科作物的根瘤菌接种面积极低，仅为1%–2%。

为了更好地配合大豆等油料作物的种植，有必要针对大豆等主要种植区的根瘤菌资源进行精准评价，以服务农业生产。中国农业微生物菌种保藏管理中心(Agricultural Culture Collection of China, ACCC)在根瘤菌研究方面已有60余年的积累，保藏根瘤菌菌株约3 700株，基本涵盖了能与我国播种豆科作物共生结瘤的优良菌种，并在20世纪70–90年代的生产实践中广泛应用。然而，因化肥的大量施用，从20世纪90年代起根瘤菌的应用面积骤减，导致对根瘤菌的研究主要关注基础理论，忽略了应用研究。由于ACCC保藏的菌株历史较长，大部分根瘤菌分类鉴定基于较为传统的互接种族和生理生化特性，且存在结瘤能力退化的风险。因此，本研究对1982年以来保藏的大豆根瘤菌采用现代分类方法和体系进行重新鉴定，并对其结瘤及促生功能进行评价，以期为大豆根瘤菌菌剂的开发提供种质资源。

1 材料与方法

1.1 供试作物和菌株

大豆种子购自哈尔滨团贸食品有限公司。基于以往农业生产实践经验筛选出241株供试菌株，均保藏于中国农业微生物菌种保藏管理中心，保藏信息详见表1。

表1　库藏根瘤菌保藏信息

Table 1　Preservation information of rhizobium in store

Number	Time	Source of strain	Number	Time	Source of strain
E. fredii			ACCC 15176#	2012.04	Changsha, Hunan, China
ACCC 15061	1989.05	No record	ACCC 15177	No record	Changsha, Hunan, China
ACCC 15067#	1989.04	America	ACCC 15178	2017.12	Changsha, Hunan, China
ACCC 15068#	1989.04	Shangdong, China	ACCC 15179	1994	Changsha, Hunan, China
ACCC 15069#	2021.10	America	ACCC 15180#	1994	Changsha, Hunan, China
ACCC 15070#	1989.04	Henan, China	ACCC 15182#	No record	Changsha, Hunan, China
ACCC 15071#	1989.04	Henan, China	ACCC 15184	No record	Changsha, Hunan, China
ACCC 15072	1989.04	Henan, China	ACCC 15185#	2018.02	Changsha, Hunan, China
ACCC 15075	1989.04	Henan, China	ACCC 15186#	1996	Changsha, Hunan, China
ACCC 15076#	1989.04	Henan, China	ACCC 15187#	1994	Changsha, Hunan, China
ACCC 15077	1989.04	Shanxi, China	ACCC 15189#	1995	Jingong, Jiangxi, China
ACCC 15082#	1989.04	Jiangsu, China	ACCC 15190#	1995	Jingong, Jiangxi, China
ACCC 15084#	1989.04	No record	ACCC 15191#	1995	Jingong, Jiangxi, China
ACCC 15085#	1989.04	Changping, Beijing, China	ACCC 15192#	1995	Jingong, Jiangxi, China
ACCC 15086#	2003.01	Changping, Beijing, China	ACCC 15193#	1995	Changsha, Hunan, China
ACCC 15090#	1989.05	Lingxian, Shandong, China	ACCC 15194#	1995	Changsha, Hunan, China
ACCC 15101#	2003.01	Changping, Beijing, China	ACCC 15195#	1995	Changsha, Hunan, China
ACCC 15102#	1989.05	Lingxian, Shandong, China	ACCC 15196#	1995	Changsha, Hunan, China
ACCC 15104#	2003.01	Manasi, Xinjiang, China	ACCC 15197#	1995	Changsha, Hunan, China
ACCC 15106#	2003.01	Ningxia, China	ACCC 15198#	1995	Nanchang, Jiangxi, China
ACCC 15107#	1990.11	Lingxian, Shandong, China	ACCC 15200	1995	Nanchang, Jiangxi, China
ACCC 15108#	1989.03	Jiaxiang, Shandong, China	ACCC 15201	1995	Nanchang, Jiangxi, China
ACCC 15109#	2003.01	Lingxian, Shandong, China	ACCC 15202#	1995	Jingong, Jiangxi, China
ACCC 15117#	1989.05	Changping, Beijing, China	ACCC 15203#	No record	Jingong, Jiangxi, China
ACCC 15118#	2003.01	Changping, Beijing, China	ACCC 15204#	1995	Jingong, Jiangxi, China
ACCC 15119#	2002.12	Changping, Beijing, China	ACCC 15205	1995	Jingong, Jiangxi, China
ACCC 15120#	2002.12	Changping, Beijing, China	ACCC 15206#	1995	Futian, Fujian, China
ACCC 15121#	2002.12	Changping, Beijing, China	ACCC 15212#	1995	Futian, Fujian, China
ACCC 15123#	2003.01	Changping, Beijing, China	ACCC 15214#	1995	Futian, Fujian, China
ACCC 15125#	2003.01	Changping, Beijing, China	ACCC 15215#	1995	Futian, Fujian, China
ACCC 15126#	1989.05	Lingxian, Shandong, China	ACCC 15216#	1995	Futian, Fujian, China
ACCC 15127#	1989.05	Lingxian, Shandong, China	ACCC 15217#	1995	Futian, Fujian, China
ACCC 15129#	1989.05	Lingxian, Shandong, China	ACCC 15218#	1995	Futian, Fujian, China
ACCC 15130#	1989.05	Lingxian, Shandong, China	ACCC 15220#	2010.10	Huiyang, Guangdong, China
ACCC 15131#	1989.05	Lingxian, Shandong, China	ACCC 15221#	2020.01	Huiyang, Guangdong, China
ACCC 15132	No record	Lingxian, Shandong, China	ACCC 15222#	1995	Huiyang, Guangdong, China
ACCC 15133#	1988.11	Manasi, Xinjiang, China	ACCC 15223#	1995	Huiyang, Guangdong, China
ACCC 15139#	1989.05	Manasi, Xinjiang, China	ACCC 15224	1995	Huiyang, Guangdong, China
ACCC 15140#	2003.01	Manasi, Xinjiang, China	ACCC 15225#	1995	Huiyang, Guangdong, China
ACCC 15142#	1988.11	Manasi, Xinjiang, China	ACCC 15226#	1995	Huiyang, Guangdong, China
ACCC 15143#	1989.05	Manasi, Xinjiang, China	ACCC 15228#	1995	Huiyang, Guangdong, China
ACCC 15145#	1989.03	Jiaxiang, Shandong, China	ACCC 15229#	1995	Huiyang, Guangdong, China
ACCC 15147#	2003.01	Shanghai, China	ACCC 15230	1995	Huiyang, Guangdong, China
B. japonicum			ACCC 15230#	1995	Huiyang, Guangdong, China
ACCC 15005#	2003.01	America	ACCC 15231#	1995	Huiyang, Guangdong, China
ACCC 15006#	1994	Heze, Shandong, China	ACCC 15235#	1995	Huiyang, Guangdong, China
ACCC 15007#	2003.01	No record	ACCC 15238#	1995	Huiyang, Guangdong, China
ACCC 15018#	1990.11	Shenyang, Liaoning, China	ACCC 15239#	1995	Huiyang, Guangdong, China
ACCC 15020#	1990.11	Guizhou, China	ACCC 15241#	2017.05	Huiyang, Guangdong, China
ACCC 15021#	1991.01	Shangdong, China	ACCC 15242	1995	Ningming, Guagnxi, China
ACCC 15022-1#	1982.12	Shangdong, China	ACCC 15243#	1995	Ningming, Guagnxi, China
ACCC 15022-2#	1982.12	Shangdong, China	ACCC 15245#	1995	Meizhou, Guangdong, China
ACCC 15023#	1985.04	Guizhou, China	ACCC 15246#	1995	Meizhou, Guangdong, China
ACCC 15027#	1982.12	America	ACCC 15247#	1995	Meizhou, Guangdong, China

(待续)

(续表1)

Number	Time	Source of strain	Number	Time	Source of strain
ACCC 15028#	2003.03	No record	ACCC 15248#	1995	Guangxi, China
ACCC 15032#	1990.11	America	ACCC 15250#	1995	Fujian, China
ACCC 15033#	2003.01	America	ACCC 15251#	1995	Fujian, China
ACCC 15034#	2003.01	America	ACCC 15252#	1995	Fujian, China
ACCC 15035#	2003.01	America	ACCC 15254#	1995	No record
ACCC 15036#	2003.01	America	ACCC 15255#	1995	Changsha, Hunan, China
ACCC 15037	2003.01	America	ACCC 15256#	1995	Nanchang, Jiangxi, China
ACCC 15038#	1990.11	America	ACCC 15257#	1995	Futian, Fujian, China
ACCC 15039	1990.11	America	ACCC 15258#	1995	Ningming, Guagnxi, China
ACCC 15041	2001.04	America	ACCC 15259#	1995	Meixian, Guangdong, China
ACCC 15042#	1991.03	America	ACCC 15260#	1995	Changsha, Hunan, China
ACCC 15043#	2003.01	Australia	ACCC 15262#	1995	Changsha, Hunan, China
ACCC 15043	2003.01	Australia	ACCC 15263#	1995	Changsha, Hunan, China
ACCC 15044	1990.11	America	ACCC 15264#	1995	Changsha, Hunan, China
ACCC 15045#	1985.04	America	ACCC 15269#	1995	Changsha, Hunan, China
ACCC 15046#	2003.01	Argentina	ACCC 15273#	1995	Nanchang, Jiangxi, China
ACCC 15047#	1991.01	America	ACCC 15275#	1995	Nanchang, Jiangxi, China
ACCC 15049	1990.11	America	ACCC 15276#	1995	Nanchang, Jiangxi, China
ACCC 15051	2020.06	America	ACCC 15277#	1995	Jingong, Jiangxi, China
ACCC 15052	1990.11	Yulin, Guangxi, China	ACCC 15279#	2003.01	Futian, Fujian, China
ACCC 15053	2003.03	Wuhan, Hubei, China	ACCC 15280#	2003.01	Futian, Fujian, China
ACCC 15055#	1982.12	Wuhan, Hubei, China	ACCC 15281#	2003.01	Futian, Fujian, China
ACCC 15057#	1991.01	Argentina	ACCC 15282#	2003.01	Futian, Fujian, China
ACCC 15058#	1991.01	Argentina	ACCC 15283#	2003.01	Futian, Fujian, China
ACCC 15059#	1990.11	America	ACCC 15284#	2003.01	Futian, Fujian, China
ACCC 15060#	1990.11	New Zealand	ACCC 15285#	2003.01	Futian, Fujian, China
ACCC 15062	2003.01	America	ACCC 15289#	2003.01	Ningming, Guagnxi, China
ACCC 15063	1990.11	Liaoning, China	ACCC 15291#	2003.01	Ningming, Guagnxi, China
ACCC 15064#	1982.12	India	ACCC 15293#	2003.01	Meixian, Guangdong, China
ACCC 15065#	1990.11	America	ACCC 15402#	No record	Guangzhou, Guangdong, China
ACCC 15081#	2003.01	No record	ACCC 15601#	2001.04	America
ACCC 15083#	2003.01	No record	ACCC 15603#	1991.01	America
ACCC 15095#	1990.11	America	ACCC 15605#	1991.04	America
ACCC 15096#	No record	America	ACCC 15606#	2003.01	America
ACCC 15097#	1991.01	America	ACCC 15608#	1990.11	No record
ACCC 15150#	1996.06	Wuxuan, Guangxi, China	ACCC 15610#	2003.01	America
ACCC 15156#	1994	Nanchang, Jiangxi, China	ACCC 15611#	1982.12	No record
ACCC 15157#	No record	Nanchang, Jiangxi, China	ACCC 15617	2006.12	Inner Mongolia, China
ACCC 15158#	No record	Nanchang, Jiangxi, China	ACCC 15618	2006.12	Inner Mongolia, China
ACCC 15159#	1994	Jingong, Jiangxi, China	ACCC 15619#	2006.12	Inner Mongolia, China
ACCC 15161#	No record	Futian, Fujian, China	ACCC 15620#	No record	Inner Mongolia, China
ACCC 15162#	2018.01	Futian, Fujian, China	ACCC 15621#	No record	Inner Mongolia, China
ACCC 15163#	1994	Futian, Fujian, China	ACCC 15622#	2017.12	Inner Mongolia, China
ACCC 15164	No record	Huiyang, Guangdong, China	ACCC 15623#	2006.12	Inner Mongolia, China
ACCC 15165#	1994	Huiyang, Guangdong, China	ACCC 15624#	2006.12	Inner Mongolia, China
ACCC 15166#	1994	Ningming, Guagnxi, China	ACCC 15627#	No record	Inner Mongolia, China
ACCC 15167#	1994	Ningming, Guagnxi, China	ACCC 15630#	2006.12	Inner Mongolia, China
ACCC 15168#	1994	Nanning, Guagnxi, China	ACCC 15631#	2006.12	Inner Mongolia, China
ACCC 15169#	1994	Nanning, Guagnxi, China	ACCC 15632#	2006.12	Inner Mongolia, China
ACCC 15170#	1994	Nanning, Guagnxi, China	ACCC 15633#	2006.12	Inner Mongolia, China
ACCC 15171#	1994	Meixian, Guangxi, China	ACCC 15634#	2006.12	Inner Mongolia, China
ACCC 15172#	No record	Changsha, Hunan, China	*S. meliloti*		
ACCC 15173#	No record	Changsha, Hunan, China	ACCC 15094#	1990.11	Gagong, Xizang, China
ACCC 15175	2018.04	Changsha, Hunan, China	ACCC 15660	No record	Zhenyuan, Gansu, China

The strains marked with "#" were conducted in nodule experiments.

1.2 菌种活化

在超净工作台中用浸过 75% 乙醇的脱脂棉球将干燥安瓿管表面擦净，用酒精灯外焰加热安瓿管顶端，将冷的无菌水滴至热的安瓿管顶端使其玻璃碎裂，用无菌镊子敲开安瓿管顶端。

慢生根瘤菌接种于豆芽汁培养基(bean sprouts extract medium, BSE)[32]：甘露醇/蔗糖 10.0 g，$MgSO_4·7H_2O$ 0.2 g，NaCl 0.1 g，K_2HPO_4 0.5 g，$CaCl_2$ 0.1 g，1% H_3BO_3 2.0 mL，1% Na_2MoO_4 2.0 mL，豆芽汁(500 g 黄豆芽/绿豆芽用 1 500 mL 水煮沸 30 min，四层纱布过滤 2 次定容至 1 000 mL) 1 000 mL，琼脂 20.0 g，自然 pH，115 ℃灭菌 20 min。快生根瘤菌接种于甘露醇酵母汁琼脂培养基(yeast extract mannitol agar, YMA)：甘露醇/蔗糖 10.0 g，酵母粉 1.0 g，$MgSO_4·7H_2O$ 0.2 g，NaCl 0.1 g，K_2HPO_4 0.5 g，$CaCO_3$ 3.0 g，1% H_3BO_3 2.0 mL，1% Na_2MoO_4 2.0 mL，琼脂 20.0 g，自然 pH，115 ℃灭菌 20 min。用无菌滴管吸取适量无菌水于安瓿管内，缓慢吹打使冻干菌体悬浮。另取无菌滴管吸取适量菌悬液接种于新鲜的培养基，涂匀，置于 30 ℃恒温培养箱避光培养 3−5 d，直至出现菌苔。挑取适量菌苔划线纯化，直至出现单菌落。将纯培养的菌株接种到 BSE/YMA 斜面中，4 ℃冰箱保存备用。

1.3 菌株信息复核

采用细菌基因组 DNA 提取试剂盒[天根生化科技(北京)有限公司]提取活化菌株的基因组。

PCR 反应体系(50 μL)：DNA 模板 6 μL，上、下游引物(10 μmol/L)各 2 μL，ddH_2O 15 μL、2×*Taq* PCR StarMix [康润景星(苏州)生物科技有限公司] 25 μL。用通用引物 27F (5′-AGAGTTTGATCMTGGCTCAG-3′)和 1492R (5′-GGTTACCTTGTTACGACTT-3′)扩增 16S rRNA 基因，扩增程序：94 ℃预变性 10 min；94 ℃变性 30 s，55 ℃退火 60 s，72 ℃延伸 90 s，循环 30 次；72 ℃终延伸 10 min。用特异性引物 41F (5′-TTCGGCAAGGGMTCGRTSATG-3′)和 640R (5′-ACATSACRCCGATCTTCATGC-3′)扩增 *recA*，扩增程序：95 ℃预变性 2 min；95 ℃变性 45 s，58 ℃退火 30 s，72 ℃延伸 90 s，循环 35 次；72 ℃终延伸 7 min。

扩增产物送至生工生物工程(上海)股份有限公司测序。16S rRNA 基因序列提交至 EzBioCloud 数据库(https://www.ezbiocloud.net/)进行序列比对，*recA* 序列提交至 NCBI 中的 GenBank 数据库(https://blast.ncbi.nlm.nih.gov/Blast.cgi)进行 BLAST 分析比对，对比结果在中国典型培养物保藏中心(http://cctcc.whu.edu.cn/portal/dictionary/index)查询菌株中文名。复核无误后的菌株保藏至 4 ℃ YMA 斜面和−80 ℃甘油管备用。利用 MEGA-X 和邻接法(neighbor-joining method)构建系统发育树。

1.4 结瘤实验

1.4.1 松本哲良营养液[33]

组分 A (g/L)：KH_2PO_4 8.8，KCl 62.0，$MgSO_4·7H_2O$ 100.0。组分 B (g/L)：$CaCl_2$ 86.0。组分 C (g/L)：柠檬酸铁 12.0。组分 D (g/L)：$NaNO_3$ 12.0，$MnSO_4·H_2O$ 0.4，$ZnSO_4$ 0.1，$CuSO_4·5H_2O$ 0.1，H_3BO_3 0.1，Na_2MoO_4 0.02。使用时 4 个组分等量混合后稀释 200 倍，自然 pH。

1.4.2 培养体系准备

采用滤纸桥法进行结瘤实验[34]，每个处理设置 3 次重复。将滤纸裁剪成 350 mm×40 mm 滤纸条，纵向对折后再横向对折，横向折痕处向内翻折 150 mm 制成“M”型，中间凹处根据种子大小剪出“V”型小孔，将滤纸桥放入 25 mm×200 mm 试管(图 1)。每支试管加入适量松本哲良营养液，液面约与“M”型桥底部平齐，用棉塞封口，121 ℃灭菌 30 min 备用。

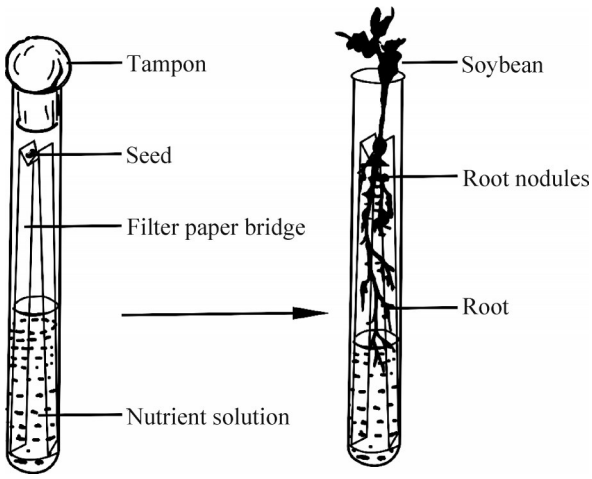

图1 滤纸桥法大豆结瘤实验示意图

Figure 1 Schematic diagram of soybean nodulation in filter paper bridge.

1.4.3 种子表面消毒

选取饱满、均一、健壮的大豆种子，放入干燥的 500 mL 或 1 000 mL 锥形瓶中(根据种子量选择)，加入浓硫酸没过种子表面，精确处理 3 min，其间不断摇晃。倒去浓硫酸，用无菌水冲洗至少 10 次，直至清洗废液经 pH 试纸检测呈中性。

1.4.4 种子准备

消毒后的种子用无菌水浸泡 6−8 h，无菌水的体积约为种子总体积的 250%−300%。吸水膨胀的种子播于铺有 4 层无菌湿润纱布的铝盒内，避光置于 25 ℃恒温培养箱内催芽 24−48 h，种子胚根伸长约 0.5−1.0 cm 为宜，其间保持萌发床湿润。

1.4.5 菌悬液准备

结合菌株入库时的菌株登记信息、农业生产应用效果以及菌种复核结果，选择 190 株菌株进行结瘤试验，菌株信息见表 1。供试菌株斜面长出大面积菌苔后，加入 5 mL 无菌水，用无菌接种环刮洗菌苔，涡旋仪振荡 20 s，制成菌悬液。

1.4.6 播种

挑选萌发状态相似的种子放入无菌培养皿中，倒入菌悬液，使种子均匀附着菌液，浸泡 30 min 后播种。

最先播种对照组。长柄镊子经火焰消毒后，用无菌水冷却，将种子播种于滤纸桥“M”型凹槽内，胚根嵌入“V”型小孔，菌悬液均匀加入每支试管，棉塞封口。在室温 25−28 ℃，14 h/10 h 光照培养箱中培养，待幼苗长出几片真叶后摘除棉塞，其间根据实际情况补充营养液。30 d 后统计实验结果。

1.4.7 植株结瘤情况和农艺性状调查

结瘤实验结束后，计算结瘤率，统计植株结瘤总数，并迅速摘下根瘤，在分析天平上称量鲜重。结瘤率计算如公式(1)所示。

$$\text{结瘤率}(\%)=\text{结瘤植株数量}/\text{实验植株总数}\times 100 \quad (1)$$

以第一子叶叶痕作为划分地上和地下部分的标准，测量地上部分茎尖生长点以下的长度作为株高；测量后将地上部分装入信封，置于烘箱中 105 ℃杀青 2 h，再 80 ℃烘干至恒重后称量干重。计算株高和干重的增长率，如公式(2)所示。

$$\text{增长率}(\%)=(\text{处理组平均指标}-\text{CK组平均指标})/\text{CK组平均指标}\times 100 \quad (2)$$

1.5 数据分析

原始数据经过 Excel 预处理后，用 R 和 GraphPad 进行显著性分析及图示，显著性水平设定为 $\alpha=0.05$。

2 结果与分析

2.1 根瘤菌菌种信息复核

本研究对库藏根瘤菌进行了信息复核。213 株经 16S rRNA 基因序列比对为根瘤菌(图 2)：156 株隶属慢生根瘤菌属(*Bradyrhizobium*)，

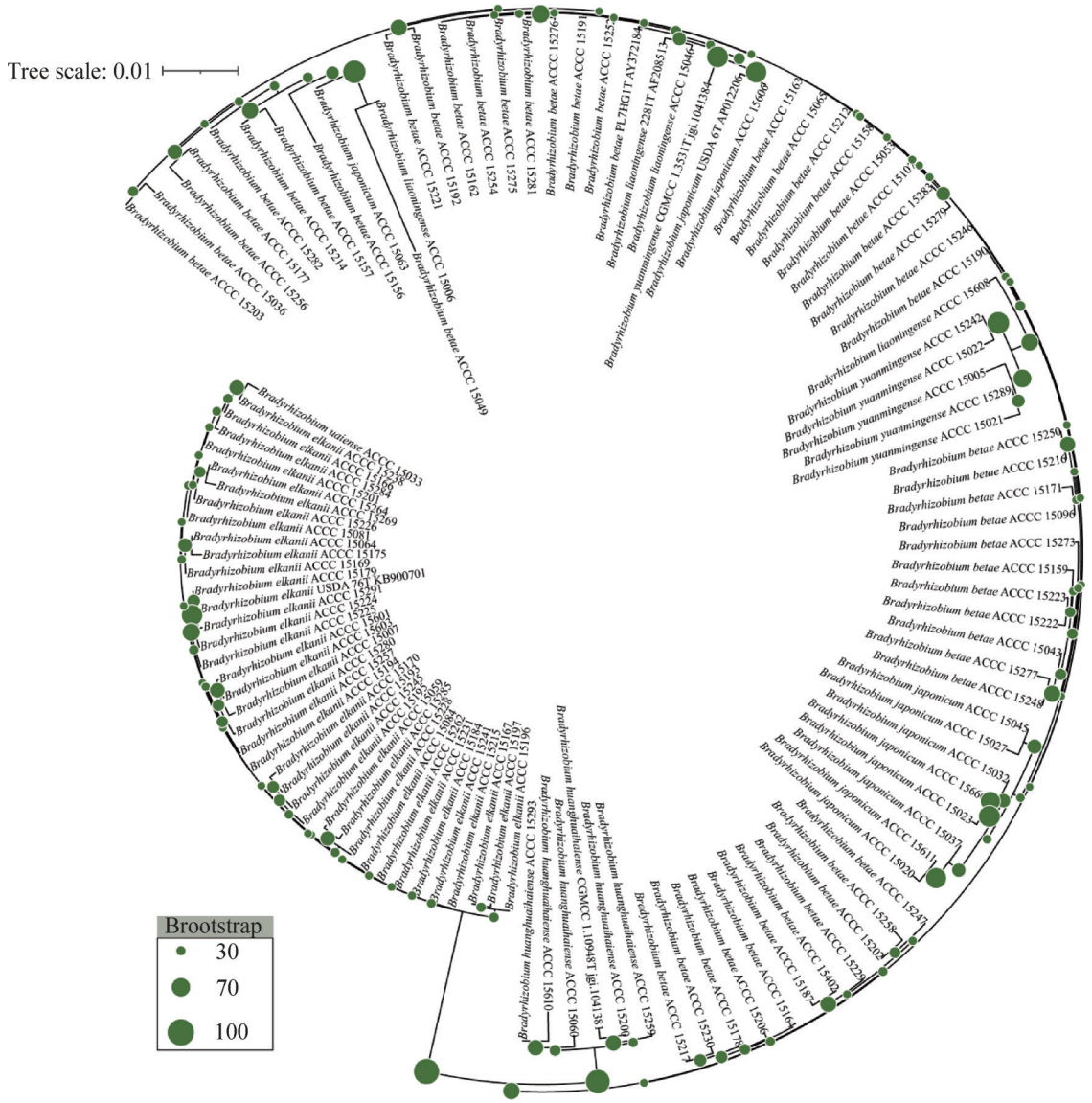

图2　基于本研究中部分根瘤菌16S rRNA基因序列构建的系统发育树

Figure 2　Neighbour-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences of a part of rhizobium in this study.

占比 73.24%；48 株隶属中华根瘤菌属(*Sinorhizobium*)，占比 22.54%；9 株隶属根瘤菌属(*Rhizobium*)，占比 4.22%。结合 *recA* 序列比对，慢生根瘤菌属中，57 株鉴定为埃氏慢生根瘤菌(*B. elkanii*)，占比 36.54%；34 株鉴定为高效固氮慢生根瘤菌(*B. diazoefficiens*)，占比 21.79%；23 株鉴定为甜菜慢生根瘤菌(*B. betae*)，占比 14.74%；19 株鉴定为日本慢生根瘤菌(*B. japonicum*)，占比 12.18%；8 株鉴定为渥太华慢生根瘤菌(*B. ottawaense*)，占比 5.13%；其余分别为 8 株黄淮海慢生根瘤菌(*B. huanghuaihaiense*)、7 株圆明慢生根瘤菌(*B. yuanmingense*)、1 株大庆慢生根瘤菌(*B. daqingense*)和 1 株辽宁慢生根瘤菌(*B.*

liaoningense)。中华根瘤菌属中，有 45 株弗氏中华根瘤菌(*S. fredii*)，占比 93.75%，2 株萨赫里中华根瘤菌(*S. saheli*)以及 1 株圣丰中华根瘤菌(*S. shofinae*)。根瘤菌属中，有 5 株线状根瘤菌(*R. viscosum*)和 4 株内蒙古根瘤菌黄土亚种(*R. mongolense* subsp. *loessense*)。菌株分类信息已在中国农业微生物菌种保藏管理中心官网(http://www.accc.org.cn/)更新。

213 株根瘤菌具有来源多样性(表 1)，其中 17.37% 的菌株来源于海外，剩余菌株主要来源于我国广东(11.27%)、湖南(11.27%)、福建(9.86%)、江西(9.39%)、山东(8.45%)、广西(6.57%)、内蒙古(6.57%)、北京(4.69%)等 19 个省(自治区、直辖市)。

2.2 与大豆匹配结瘤的根瘤菌筛选

本研究根据菌株信息复核结果以及前期实验结果，共选出 186 株根瘤菌进行结瘤实验：慢生根瘤菌属 138 株、中华根瘤菌属 43 株、根瘤菌属 5 株。实验规模较大，分为两部分完成，第一部分共 109 株，第二部分共 77 株。

在两部分实验中，选择根瘤数量和根瘤质量均排名前 10 的菌株，结瘤差异统计见表 2。第一部分实验筛选出 ACCC 15033、ACCC 15065、ACCC 15083、ACCC 15263 和 ACCC 15611 等 5 株菌，根瘤数量(P>0.23)和根瘤质量(P>0.34)之间均无显著差异；第二部分实验筛选出 ACCC 15023、ACCC 15090、ACCC 15254、ACCC 15276、ACCC 15279 和 ACCC 15282 等 6 株菌，根瘤数量之间无显著差异(P>0.45)，ACCC 15254 的根瘤质量最大，ACCC 15282 次之，但两者之间无显著差异(P=0.141)，ACCC 15254 的根瘤质量与除 ACCC 15282 外的菌株有显著差异(P<0.12)，ACCC 15282 的根瘤质量与其他菌株之间无显著差异(P>0.10)。11 株菌的结瘤率均为 100%。

2.3 根瘤菌对大豆植株农艺性状的影响

在第一部分植株样本中，仅 ACCC 15611 (0.254±0.007) g 处理的干重高于 CK (0.236±0.042) g，但差异不显著(P=0.667)。在第二部分植株样本中，ACCC 15108 (0.414±0.010) g、ACCC 15273 (0.409±0.178) g、ACCC 15118 (0.373±0.055) g 等 34 株菌处理的大豆植株干重高于 CK (0.255±0.019) g，但差异均不显著(P>0.06)。有 27 株菌处理的大豆植株株高高于 CK

表2 高效结瘤根瘤菌的筛选结果

Table 2 Screening results of efficient nodulation rhizobium

Preservation number	Strain	Average number of root nodules	Average weight of root nodules (g)
ACCC 15033	*B. elkanii*	10.33±3.68a	0.078±0.009a
ACCC 15065	*B. diazoefficiens*	11.67±5.44a	0.092±0.028a
ACCC 15083	*S. fredii*	14.00±2.16a	0.090±0.008a
ACCC 15263	*B. elkanii*	9.67±3.09a	0.079±0.006a
ACCC 15611	*B. japonicum*	9.67±0.94a	0.082±0.002a
ACCC 15023	*B. japonicum*	14.67±7.32a	0.015±0.007b
ACCC 15090	*S. shofinae*	16.00±1.41a	0.017±0.002b
ACCC 15254	*B. diazoefficiens*	12.67±6.65a	0.060±0.017a
ACCC 15276	*B. diazoefficiens*	12.00±3.27a	0.020±0.007b
ACCC 15279	*B. diazoefficiens*	19.00±15.12a	0.018±0.011b
ACCC 15282	*B. diazoefficiens*	17.67±12.28a	0.039±0.024ab

Perform univariate ANOVA on the data after passing the Shapiro-Wilk test, n=3, multiple comparisons by the LSD. The data were mean±SE, different lowercases represent significant differences in the treatment groups (α=0.05).

(20.87±1.89) cm，其中 ACCC 15055、ACCC 15243、ACCC 15197、ACCC 15176、ACCC 15206 和 ACCC 15611 与 CK 差异显著(表 3)，其中 ACCC 15055 极显著地促进植株生长(*P*<

表3　高效促生根瘤菌对大豆株高的影响

Table 3　Effects of efficient growth promoting rhizobium on soybean height

Preservation number	Strain	Stem length (cm)	Growth rate (%)
ACCC 15055	*B. yuanmingense*	30.63±0.82a	48.81***
ACCC 15176	*B. diazoefficiens*	28.35±0.35a	35.86**
ACCC 15197	*B. elkanii*	28.63±3.31a	37.22**
ACCC 15206	*B. betae*	26.00±1.42a	24.60*
ACCC 15243	*B. elkanii*	29.40±2.28a	40.89**
ACCC 15611	*B. japonicum*	25.80±2.05a	23.64*
ACCC 15006	*B. diazoefficiens*	36.46±1.67bcd	21.90*
ACCC 15007	*B. elkanii*	36.51±1.10bcd	22.09*
ACCC 15085	*S. fredii*	38.70±4.05abcd	29.42**
ACCC 15086	*S. fredii*	36.24±5.49bcd	21.18*
ACCC 15101	*S. fredii*	41.45±0.11abcd	38.61***
ACCC 15108	*S. fredii*	40.32±1.08abcd	34.81**
ACCC 15118	*S. fredii*	37.41±0.44abcd	25.11*
ACCC 15119	*S. fredii*	36.99±0.22abcd	23.69*
ACCC 15123	*S. fredii*	37.22±0.00abcd	24.44*
ACCC 15131	*S. fredii*	36.93±2.79abcd	23.50*
ACCC 15143	*S. fredii*	37.61±1.59abcd	25.76*
ACCC 15147	*S. fredii*	37.09±4.30abcd	24.03*
ACCC 15191	*B. ottawaense*	35.84±3.41d	19.86*
ACCC 15194	*B. elkanii*	43.00±2.80ab	43.80***
ACCC 15222	*B. diazoefficiens*	42.02±1.17abcd	40.51***
ACCC 15225	*B. elkanii*	35.92±2.81d	20.10*
ACCC 15250	*B. diazoefficiens*	39.80±1.35abcd	33.10**
ACCC 15252	*B. diazoefficiens*	42.83±2.81abc	43.22***
ACCC 15254	*B. diazoefficiens*	37.76±1.53abcd	26.27**
ACCC 15262	*B. elkanii*	38.26±2.31abcd	27.93**
ACCC 15264	*B. elkanii*	39.18±0.58abcd	31.03**
ACCC 15273	*B. ottawaense*	40.31±1.23abcd	34.80**
ACCC 15275	*B. ottawaense*	39.88±1.33abcd	33.37**
ACCC 15276	*B. diazoefficiens*	40.64±1.35abcd	35.89***
ACCC 15279	*B. diazoefficiens*	39.76±2.26abcd	32.94**
ACCC 15282	*B. diazoefficiens*	39.57±2.06abcd	32.31**
ACCC 15285	*B. elkanii*	37.18±4.53abcd	24.32*
ACCC 15291	*B. elkanii*	38.23±3.83abcd	27.85**
ACCC 15402	*B. diazoefficiens*	38.04±4.80abcd	27.19**
ACCC 15615	*B. japonicum*	43.37±0.65a	45.03***
ACCC 15622	*S. fredii*	38.90±0.83abcd	30.08**
ACCC 15623	*S. fredii*	40.30±2.12abcd	34.77**
ACCC 15624	*S. fredii*	38.02±2.56abcd	27.12**
ACCC 15630	*B. elkanii*	37.38±1.14abcd	24.98*
ACCC 15631	*R. mongolense* subsp. *loessense*	36.04±2.82cd	20.50*
ACCC 15632	*R. mongolense* subsp. *loessense*	35.88±2.89d	19.98*
ACCC 15634	*R. mongolense* subsp. *loessense*	36.16±1.82bcd	20.93*

Perform univariate ANOVA on the data after passing the Shapiro-Wilk test, n=3, multiple comparisons by the LSD. The data were mean±SE, different lowercases represent significant differences in the treatment groups (α=0.05), * represents significant differences between treatment group and CK. *: α<0.05; **: α<0.01; ***: α<0.001.

0.001)，相比 CK 株高增长了 48.81% (表 3，图 3A)。

在第二部分实验中，39 株菌处理的植株均高于 CK，除 ACCC 15117 (35.42±4.13) cm (P=0.065)和 ACCC 15619 (35.20±1.77) cm (P=0.076)处理的植株外，所有植株株高相较于 CK 均有显著差异(表 3)，其中 ACCC 15615、ACCC 15194、ACCC 15252、ACCC 15222、ACCC 15101 和 ACCC 15276 对植株的促生作用极显著(P<0.001)，相比 CK 分别增加了 45.03%、43.80%、43.22%、40.51%、38.61%、35.89% (表 3，图 3B−3G)。

2.4 根瘤菌的结瘤和促生特性差异

所有菌株的结瘤率见表 4。根据菌株生长速率不同，将慢生根瘤菌属归类为慢生型根瘤菌(slow-growing rhizobium)，中华根瘤菌属和根瘤菌属菌株归类为快生型根瘤菌(fast-growing rhizobium)。根据结瘤率将根瘤菌分成 4 类，以此进行列联分析，探究根瘤菌间的结瘤功能差异，分析结果见图 4A。快生型根瘤菌整体结瘤率较低，能够结瘤的菌株占比 33.33%，66.67%的快生型根瘤菌未检测到根瘤；而未与大豆共生形成根瘤的慢生型根瘤菌仅占 6.52%，55.07%的慢生型根瘤菌结瘤率达到 100%。卡方检验(Chi-square test)表明根瘤菌类型与结瘤率之间不相互独立(P<0.001)，Pearson 相关系数为−0.632 (P<0.001)，即慢生型根瘤菌相比快生型根瘤菌对宿主的选择性更低，能够更广谱地与宿主共

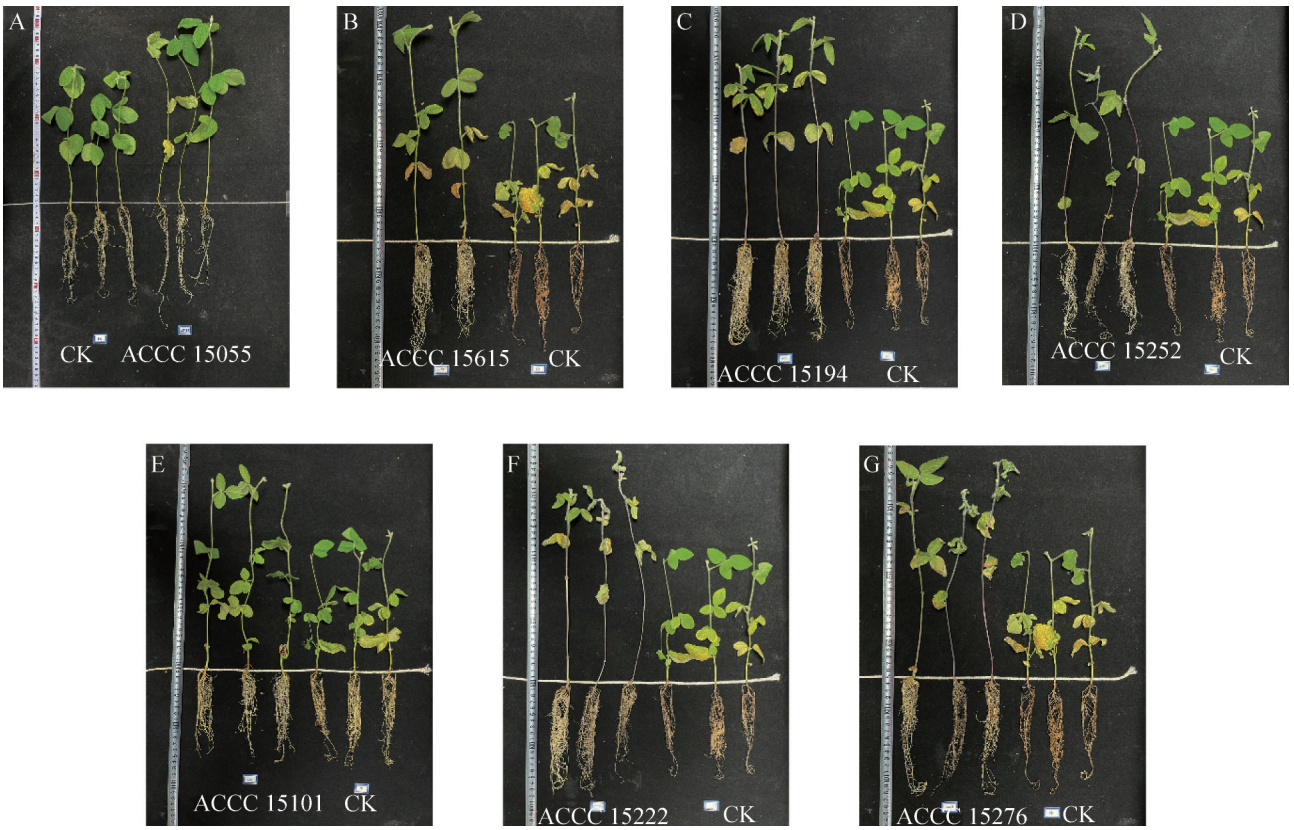

图3 接种高效促生根瘤菌的大豆长势

Figure 3 Soybean growth inoculated with efficient growth promoting rhizobium. A: ACCC 15055; B: ACCC 15615; C: ACCC 15194; D: ACCC 15252; E: ACCC 15101; F: ACCC 15222; G: ACCC 15276.

生结瘤。菌株保藏时间(P=0.225)或菌株来源(P=0.520)与结瘤率互相独立，无相关性。

结瘤实验结果表明，供试根瘤菌对大豆植株地上部分的干物质积累无显著影响，但共有93株根瘤菌能够增加大豆株高，其中41株菌有显著的促生效果(P<0.05)。根据P值将菌株分成4类进行列联分析，探究不同类型根瘤菌间促生特性的差异，分析结果见图4B。快生型根瘤菌

表4 结瘤实验结瘤率汇总

Table 4 Summary of nodulation rate in nodulation experiment

Nodulation rate (%)	Treatment					
0	ACCC 15005	ACCC 15067	ACCC 15068	ACCC 15069	ACCC 15070	ACCC 15071
	ACCC 15076	ACCC 15082	ACCC 15102	ACCC 15108	ACCC 15109	ACCC 15118
	ACCC 15120	ACCC 15123	ACCC 15125	ACCC 15126	ACCC 15127	ACCC 15129
	ACCC 15130	ACCC 15131	ACCC 15133	ACCC 15139	ACCC 15140	ACCC 15142
	ACCC 15143	ACCC 15291	ACCC 15402	ACCC 15615	ACCC 15621	ACCC 15622
	ACCC 15623	ACCC 15624	ACCC 15631	ACCC 15632	ACCC 15633	ACCC 15634
	ACCC 15064#	ACCC 15170#	ACCC 15196#	ACCC 15259#	ACCC 15281#	
33	ACCC 15007	ACCC 15101	ACCC 15104	ACCC 15106	ACCC 15119	ACCC 15145
	ACCC 15147	ACCC 15273	ACCC 15280	ACCC 15284	ACCC 15610	ACCC 15619
	ACCC 15620	ACCC 15627	ACCC 15630	ACCC 15022-1#	ACCC 15022-2#	ACCC 15027#
	ACCC 15042#	ACCC 15157#	ACCC 15158#	ACCC 15159#	ACCC 15163#	ACCC 15215#
	ACCC 15241#	ACCC 15603#	ACCC 15606#			
67	ACCC 15006	ACCC 15018	ACCC 15034	ACCC 15085	ACCC 15094	ACCC 15096
	ACCC 15121	ACCC 15147	ACCC 15165	ACCC 15185	ACCC 15204	ACCC 15264
	ACCC 15283	ACCC 15285	ACCC 15601	ACCC 15020#	ACCC 15028#	ACCC 15032#
	ACCC 15035#	ACCC 15046#	ACCC 15059#	ACCC 15081#	ACCC 15097#	ACCC 15150#
	ACCC 15162#	ACCC 15168#	ACCC 15176#	ACCC 15180#	ACCC 15189#	ACCC 15190#
	ACCC 15193#	ACCC 15195#	ACCC 15198#	ACCC 15238#	ACCC 15248#	ACCC 15257#
	ACCC 15258#	ACCC 15293#				
100	ACCC 15023	ACCC 15033	ACCC 15057	ACCC 15065	ACCC 15083	ACCC 15084
	ACCC 15086	ACCC 15090	ACCC 15095	ACCC 15107	ACCC 15117	ACCC 15169
	ACCC 15173	ACCC 15182	ACCC 15191	ACCC 15194	ACCC 15203	ACCC 15222
	ACCC 15222	ACCC 15223	ACCC 15225	ACCC 15226	ACCC 15228	ACCC 15229
	ACCC 15230	ACCC 15245	ACCC 15246	ACCC 15246	ACCC 15247	ACCC 15250
	ACCC 15252	ACCC 15254	ACCC 15255	ACCC 15256	ACCC 15262	ACCC 15263
	ACCC 15269	ACCC 15275	ACCC 15276	ACCC 15277	ACCC 15279	ACCC 15282
	ACCC 15611	ACCC 15021#	ACCC 15036#	ACCC 15038#	ACCC 15043#	ACCC 15045#
	ACCC 15047#	ACCC 15055#	ACCC 15058#	ACCC 15060#	ACCC 15156#	ACCC 15161#
	ACCC 15166#	ACCC 15167#	ACCC 15171#	ACCC 15172#	ACCC 15186#	ACCC 15187#
	ACCC 15192#	ACCC 15197#	ACCC 15202#	ACCC 15206#	ACCC 15212#	ACCC 15214#
	ACCC 15216#	ACCC 15217#	ACCC 15218#	ACCC 15220#	ACCC 15221#	ACCC 15231#
	ACCC 15235#	ACCC 15239#	ACCC 15243#	ACCC 15251#	ACCC 15260#	ACCC 15289#
	ACCC 15605#	ACCC 15608#				

The strains marked with "#" were inoculated in the first part, while the left was inoculated in the second. The nodulation rate in CK was 0 in both parts.

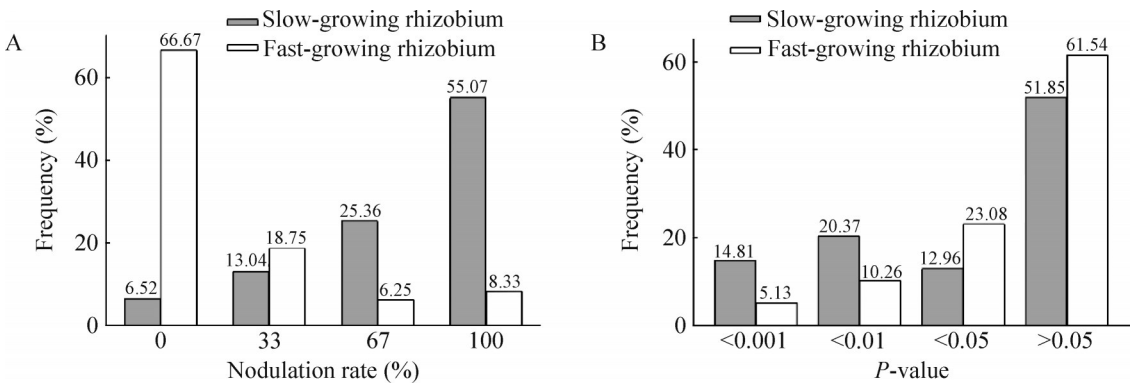

图4 不同类型根瘤菌结瘤促生差异比较。A：结瘤率；B：促生效果显著性。

Figure 4 Comparison of nodulation and growth promotion between different kinds of rhizobia. A: Nodulation rate; B: Significance of growth promotion effect.

中能够显著促进植株生长的菌株占 38.46%：10.26% 促生效果非常显著 (0.001≤*P*<0.01)，5.13% 促生效果极显著(*P*<0.001)；48.15% 的慢生型根瘤菌能显著促进植株生长：促生效果非常显著的菌株占比 20.37%，促生效果极显著的菌株占比 14.81%。虽然慢生型根瘤菌中促生效果非常显著和极显著的菌株均比快生型根瘤菌多，但卡方检验结果表明根瘤菌类型与促生能力之间相互独立(*P*=0.162)，即不同类型根瘤菌的促生能力无相关性。

3 讨论与结论

微生物菌种资源的复核是针对那些被正式收录和保藏的菌株所进行的纯度、分类地位和功能特性的核查。本研究基于 16S rRNA 基因和 *recA* 序列，对 241 株库藏大豆根瘤菌进行了信息复核，明确了其系统分类地位，并对其与大豆的共生结瘤活性进行了检测。

16S rRNA 基因序列比对与进化分析是研究原核生物系统发育地位的最常用方法。16S rRNA 基因在细菌中普遍存在，其基因中同时存在高度保守的区域和高度可变的区域，因此适合作为衡量细菌生命进化过程中亲缘关系的标准。如果 2 个菌株的 16S rRNA 基因相似性>99.0%，则被认为属于同一种；若<98.7%，则可能为一个新种。因此，16S rRNA 基因序列常用于根瘤菌的分类鉴定[35-36]，有些研究也会选择 23S rDNA[37-38] 或 16S-23S rDNA 内转录间隔区[39-41] (internal transcribed spacer, ITS)。然而，由于 16S rRNA 基因的高度保守性以及基因片段的互相转移现象[42-43]，仅基于 16S rRNA 基因的根瘤菌系统发育体系并不十分准确。根瘤菌中具有特殊功能的基因，如 *nodA*[44-46]和 *nifH*[47-48]，以及一些保守性较强的蛋白编码持家基因，如 *dnaK*[49-50]、*gln II*[51-53]、*gyrB*[39,54] 和 *recA*[55-56] 等可作为 16S rRNA 基因的替代或补充，用于在种属水平对细菌进行精准鉴定。本研究结合了 16S rRNA 基因与 *recA* 基因序列进行系统分类鉴定。

最初能与大豆结瘤的根瘤菌均被分类为 *B. japonicum*。Hollis 等[57]通过 DNA/DNA 杂交技术将其分类为 3 个类群，其中 Group Ⅱ被定义为一个新种，命名为 *B. elkanii*[58]。随着 *B. japonicum*[59] 和 *B. huanghuaihaiense*[60] 相继从我国大豆根瘤中分离，*B. yuanmingense*[61]、*B. liaoningense*[59]以及 *R. leguminosarum*[47,62]等菌种

也被发现具有与大豆结瘤固氮的能力。根瘤菌与宿主的共生关系具有选择性[63]。马中雨等[64]的研究发现，慢生型根瘤菌比快生型根瘤菌对宿主的选择性更为松弛，这一点与本研究的结论一致。尽管土壤中根瘤菌广泛分布，但其中大部分为无效或低效菌株，仅有一小部分能与大豆结瘤，且多集中在慢生根瘤菌属和中华根瘤菌属。因此，选择能有效与大豆匹配结瘤的根瘤菌是推广根瘤菌菌剂的前提。本研究筛选出一批能有效与大豆匹配结瘤的根瘤菌，其中包括 ACCC 15023、ACCC 15033、ACCC 15065、ACCC 15083、ACCC 15090、ACCC 15254、ACCC 15263、ACCC 15276、ACCC 15279、ACCC 15282、ACCC 15611 等 11 株能与大豆高效结瘤的菌株，也包含了宿主选择性严苛的快生型根瘤菌。此外，ACCC 15055、ACCC 15101、ACCC 15194、ACCC 15222、ACCC 15252、ACCC 15276、ACCC 15615 等 43 株根瘤菌能够显著促进大豆植株生长，为后期菌剂研发和功能菌株筛选创造了条件。

由于本研究的局限性，所使用的菌株在地理和时间维度上并未表现出显著的结瘤差异，未来可针对性地从不同来源以及不同保藏时间的根瘤菌中筛选出有应用前景的菌株，在丰富大豆根瘤菌种质资源库的同时，了解农耕环境和自然环境对根瘤菌种群数量、遗传背景以及结瘤固氮能力的影响。在根瘤菌分离鉴定过程中，可检验菌株与目前主要栽培大豆品种的结瘤能力，以完善菌种信息。此外，由于不同品种大豆的遗传差异，能够招募共生的根瘤菌各不相同。未来可将本研究筛选出的高效菌株与我国不同地区种植的大豆品种进行交叉结瘤实验，以筛选出更有应用前景的菌株，为育种以及微生物肥料的研发和推广提供参考。

作者贡献声明

韩嘉诚：水培实验，调查大豆植株农艺性状以及菌株结瘤形状，数据分析，撰写文章；朱宏图：负责菌株整理、活化与鉴定，完成水培实验；杨芾：协助数据处理；郭捷：协助完成实验；马晓彤：指导并协助完成水培实验，设计、指导实验；张晓霞：指导实验设计，提供研究所需的资源与材料，修订文章。

作者利益冲突公开声明

作者声明不存在任何可能会影响本文所报告工作的已知经济利益或个人关系。

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