荒漠土壤中慢生根瘤菌
地理分布及分子进化机制

Geographic Distribution and Molecular Evolutionary Mechanism for *Mesorhizobium* Strains in Desert Soil

冀照君◆著

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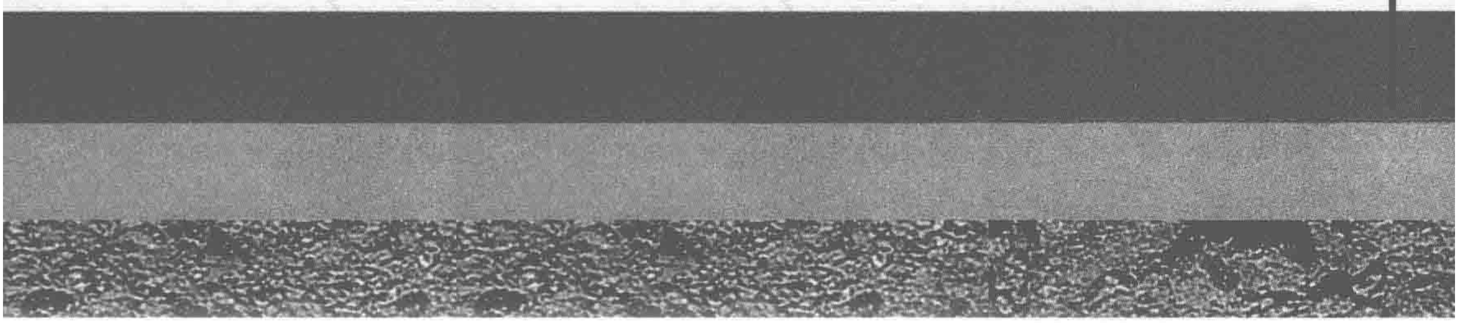

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Preface

Mesorhizobium species are the main microsymbionts associated with the medicinal or sand-fixing plants *Astragalus membranaceus* and *Caragana intermedia* (AC) in desert soil in China, while all the *Mesorhizobium* strains isolated from each of these plants could nodulate both of them. Biogeography and molecular evolution of rhizobia influenced by soil environments and selected by legumes have been investigated extensively.

In this book, microevolution of *Mesorhizobium* strains nodulating *Caragana* in semi-fixed desert belt at north China was investigated and investigations on the nodulation of three cultivated medicinal legumes, *Astragalus mongholicus*, *Astragalus membranaceus* and *Hedysarum polybotrys* were performed. Then the whole genomes of two (*M. silamurunense* CCBAU01550, *M. silamurunense* CCBAU45272) and five representative strains (*M. septentrionale* CCBAU01583, *M. amorphae* CCBAU01570, *M. caraganae* CCBAU01502, *M. temperatum* CCBAU01399 and *R. yanglingense* CCBAU01603) originally isolated from AC plants were sequenced, respectively.

Conclusively, the *Caragana*-associated mesorhizobia have divergently evolved according to their geographic distribution, and have been selected not only by the environmental conditions but also by the host plants. The soil fertility may be the main determinants for the distribution of rhizobia associated with these cultured legume plants. The multifactorial features of the rhizobia that may be associated with their host specificity at cross-nodulation group, including *nodE*, *nodZ*, T1SS as the possible main determinants; and *nodO*, hydrogenase system and T3SS as factors regulating the bacteroid formation or nitrogen fixation efficiency.

Zhaojun Ji

2018.11

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Chapter Ⅰ Genetic divergence and gene flow among *Mesorhizobium* strains nodulating the shrub legume *Caragana* growing along the semi-fixed desert belt in north China

Introduction

In 14 north provinces of China with an area of 124 043 774 hm^2, deserts, gobi and sand dune have a great influence on the agriculture and livelihood of people and animals dwelling around these regions. Sandstorms are the main natural calamities in these regions especially during the spring. Legumes such as *Caragana*, *Hedysarum scoparium*, *Hedysarum mongolicum*, *Alhagi sparsifolia* and *Sophora alopecuroides* are excellent windproof and sand-fixing plants which widely distribute in arid and semi-arid deserts. Nitrogen fixation of these legumes by establishing symbiosis with rhizobia plays an important role in their healthy growth, especially in the barren sand soil with low content of nutrients. Of the above psammophytic vegetations, *Caragana* distributes more widely and is planted artificially and broadly in the semi-fixed deserts. *Mesorhizobium* spp., the predominant microsymbionts nodulating the legumes, such as *Caragana* in particular, have a good fitness in alkaline sands due to their capability of acid production, high- or low-temperature and drought resistance. It has been documented that *Mesorhizobium septentrionale*, *Mesorhizobium amorphae*, *Mesorhizobium gobiense*, *Mesorhizobium mediterraneum*, *Mesorhizobium temperatum*, *Mesorhizobium caraganae*, *Mesorhizobium* huakuii, *Mesorhizobium tianshanense*, *Mesorhizobium metallidurans*, and *Mesorhizobium shangrilense* could nodulate *Caragana* in extreme arid and semi-arid deserts, or mountains with low temperature perennially.

So far, the existence of rhizobial biogeographic patterns has been well evidenced, but little is known about the processes underlying them. A central goal of biogeography is to understand the mechanisms that generate and maintain the rhizobial diversity, the natural selection, genetic drift, dispersal and gene mutation on the microevolution of these rhizobia, although a series of interventions on soil stability and preservation of biodiversity have provided tools and strategies to address this problem. Rhizobia nodulating *Caragana* may be used as a model to answer the question because they can survive in stressful environments of the nutrient-poor deserts or inhabit in the root nodule of *Caragana*. Rhizobial genes involved in nodulation and heat-shock play important roles in the symbiosis with legumes and adaptation in stressful environments. Analyses of the genetic divergence and gene flow (the movement and successful establishment of genotypes from one to another) may reveal the adaptively molecular evolution. In order to study the microevolutionary mechanism, six heat-shock factor genes (*clpA*, *clpB*, *dnaK*, *dnaJ*, *grpE*, *hlsU*), five nodulation genes (*nodA*, *nodC*, *nodD*, *nodG*, *nodP*), and five core genes (*atpD*, *glnII*, *gyrB*, *recA*, *rpoB*) were chosen to investigate genetic diversity, gene recombination and mutation events, divergence and gene flow among the *Mesorhizobium* nodulating *Caragana*.

Materials and methods

Rhizobial strains

A total of 724 *Caragana*-nodulating rhizobial strains were selected according to our previous studies. These rhizobial strains originated from 21 sites in 6 areas, including southeast of Tengger Desert (area A, 11 strains), south Mu Us Desert (area B, 447 strains), east Kubuqi Desert (area C, 65 strains), southeast Hunshandake Desert (area D, 72 strains), south Horqin Desert (area E, 113 strains), and mountains of northwest Yunnan (area F, 16 strains). The areas A, B, C, D and E distribute along the northern semi-fixed desert belt in China where the sandstorms blow in spring frequently. All these strains are preserved in the Culture Collection of Beijing Agricultural University (CCBAU) in TY medium with 20% glycerol for a long time.

Of these 724 strains, 72 were selected as representative strains to analyze the molecular evolution (Tab. 1-1). Besides, eight strains including *Mesorhizobium amorphae* ACCC 19665^T, *M. gobiense* CCBAU 83330^T, *M. tianshanense* CCBAU 3306^T, *M. mediterraneum* USDA 3392^T, *M. temperatum* SDW 018^T, *M. metallidurans* LMG 24485^T, *M. huakuii* CCBAU 2609^T and *M. septentrionale* SDW 014^T whose host plants are not *Caragana*, were used as the reference to determine the phylogenetic positions of the *Caragana* mesorhizobia.

In addition, 447 strains obtained from three neighboring sites (B-1, B-2 and B-3) of the area B were analyzed as a subset to study the influence of geographic distance on the gene exchanges among the mesorhizobia.

Tab. 1-1 A total of 72 representative strains isolated from *Caragana* used in this study

Strains (CCBAU No.)	Species	Area	Collector	Province/ Autonomous Region	City	Town/District/ Banner	Latitude/(°)	Longitude/(°)
75059	*M. amorphae*	A	Li Mao	Ningxia	Wuzhong	Wuzhong	38.127 2	105.917 5
75061	*M. temperatum*	A	Li Mao	Ningxia	Zhongwei	Shaopotou	37.451 1	105.023 0
75063	*M. gobiense*	A	Li Mao	Ningxia	Dujun	Gantang	37.426 1	104.625 8
01570	*M. septentrionale*	B	Li Mao	Inner Mongolia	Ordos	Dongsheng	39.7892	110.129 4
01602	*M. amorphae*	B	Li Mao	Inner Mongolia	Ordos	Dongsheng	39.789 2	110.129 4
01583	*Mesorhizobium* sp. Ⅰ	B	Li Mao	Inner Mongolia	Ordos	Dongsheng	39.789 2	110.129 4
01577	*Mesorhizobium* sp. Ⅵ	B	Li Mao	Inner Mongolia	Ordos	Dongsheng	39.789 2	110.129 4
01582	*M. temperatum*	B	Li Mao	Inner Mongolia	Ordos	Dongsheng	39.789 2	110.129 4
01722	*M. septentrionale*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.193 8	109.791 5
01655	*Mesorhizobium* sp. Ⅲ	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.193 8	109.791 5
01757	*M. amorphae*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.193 8	109.791 5
01731	*M. septentrionale*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.193 8	109.791 5
01647	*M. amorphae*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.193 8	109.791 5
01790	*Mesorhizobium* sp. Ⅱ	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.193 8	109.791 5

Continued

Strains (CCBAU No.)	Species	Area	Collector	Province/ Autonomous Region	City	Town/District/ Banner	Latitude/(°)	Longitude/(°)
01718	*Mesorhizobium* sp. Ⅳ	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.193 8	109.791 5
01764	*Mesorhizobium* sp. Ⅳ	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.193 8	109.791 5
01800	*Mesorhizobium* sp. Ⅶ	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.193 8	109.791 5
01656	*M. septentrionale*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.193 8	109.791 5
01753	*M. septentrionale*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.193 8	109.791 5
01645	*Mesorhizobium* sp. Ⅵ	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.193 8	109.791 5
01810	*M. septentrionale*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.193 8	109.791 5
01634	*M. septentrionale*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.193 8	109.791 5
01662	*Mesorhizobium* sp. Ⅲ	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.193 8	109.791 5
01819	*M. mediterraneum*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.193 8	109.791 5
01701	*M. septentrionale*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.193 8	109.791 5
01660	*M. septentrionale*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.193 8	109.791 5
01687	*M. amorphae*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.193 8	109.791 5
01669	*M. amorphae*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.193 8	109.791 5

Continued

Strains (CCBAU No.)	Species	Area	Collector	Province/ Autonomous Region	City	Town/District/ Banner	Latitude/(°)	Longitude/(°)
01646	*M. septentrionale*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.193 8	109.791 5
01788	*M. septentrionale*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.193 8	109.791 5
01820	*M. amorphae*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.852 2	109.901 1
01821	*M. temperatum*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.852 2	109.901 1
01643	*M. septentrionale*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.852 2	109.901 1
01728	*M. septentrionale*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.852 2	109.901 1
01739	*M. septentrionale*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.852 2	109.901 1
01670	*M. septentrionale*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.852 2	109.901 1
01822	*Mesorhizobium* sp. XII	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.852 2	109.901 1
01668	*M. amorphae*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.852 2	109.901 1
01641	*M. septentrionale*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.852 2	109.901 1
01754	*M. septentrionale*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.852 2	109.901 1
01654	*Mesorhizobium* sp. III	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.852 2	109.901 1
01751	*M. septentrionale*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.852 2	109.901 1

Continued

Strains (CCBAU No.)	Species	Area	Collector	Province/ Autonomous Region	City	Town/District/ Banner	Latitude/(°)	Longitude/(°)
01752	*M. septentrionale*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.852 2	109.901 1
01648	*M. amorphae*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.852 2	109.901 1
01661	*M. septentrionale*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.852 2	109.901 1
01750	*M. amorphae*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.852 2	109.901 1
01636	*M. septentrionale*	B	Ji Zhaojun	Inner Mongolia	Ordos	Ejin Horo	39.852 2	109.901 1
01405	*M. temperatum*	C	Lu Yangli	Inner Mongolia	ulangab	Feng Chin	40.558 3	113.308 7
01597	*M. amorphae*	C	Li Mao	Inner Mongolia	Ordos	Qingshuihe	39.952 8	111.676 7
03299	*M. septentrionale*	C	Lu Yangli	Shanxi	Xinzhou	Pianguan	39.464 6	111.671 9
03254	*Mesorhizobium* sp. Ⅵ	C	Lu Yangli	Shanxi	Xinzhou	Pianguan	39.464 6	111.671 9
01499	*M. caraganae*	D	Lu Yangli	Inner Mongolia	Xilingol League	Duolun	42.196 6	116.498 6
01502	*M. caraganae*	D	Lu Yangli	Inner Mongolia	Chifeng	Balinyou	43.684 8	118.946 1
01477	*M. amorphae*	D	Lu Yangli	Inner Mongolia	Xilingol League	Sanggendalai	42.682 3	115.945 3
01461	*M. loti*	D	Lu Yangli	Inner Mongolia	Xilingol League	Sanggendalai	42.682 3	115.945 3
11185	*M. huakuii*	E	Yan Xuerui	Liaoning	Fuxin	Zhangwu	42.523 8	122.474 2

Continued

Strains (CCBAU No.)	Species	Area	Collector	Province/Autonomous Region	City	Town/District/Banner	Latitude/(°)	Longitude/(°)
11196	*M. caraganae*	E	Yan Xuerui	Liaoning	Fuxin	Zhangwu	42.523 8	122.474 2
11206	*Mesorhizobium* sp. Ⅴ	E	Yan Xuerui	Liaoning	Yingkou	Dashiqiao	40.646 9	122.571 6
11208	*M. amorphae*	E	Yan Xuerui	Liaoning	Yingkou	Dashiqiao	40.646 9	122.571 6
11214	*Mesorhizobium* sp. Ⅺ	E	Yan Xuerui	Liaoning	Yingkou	Dashiqiao	40.646 9	122.571 6
11217	*M. caraganae*	E	Yan Xuerui	Liaoning	Yingkou	Dashiqiao	40.646 9	122.571 6
11226	*M. caraganae*	E	Yan Xuerui	Liaoning	Yingkou	Gaizhou	40.668 7	122.233 3
11231	*Mesorhizobium* sp. Ⅷ	E	Yan Xuerui	Liaoning	Yingkou	Gaizhou	40.668 7	122.233 3
11242	*M. amorphae*	E	Yan Xuerui	Liaoning	Chaoyang	Jianping	41.387 6	119.620 3
11244	*M. septentrionale*	E	Yan Xuerui	Liaoning	Chaoyang	Jianping	41.387 6	119.620 3
11257	*M. temperatum*	E	Yan Xuerui	Liaoning	Chaoyang	Jianping	41.387 6	119.620 3
11270	*M. huakuii*	E	Yan Xuerui	Liaoning	Tieling	Kaiyuan	42.285 9	124.225 9
11299^{T}	*M. caraganae*	E	Yan Xuerui	Liaoning	Chaoyang	Beipiao	41.936 0	121.130 2
65328	*Mesorhizobium* sp. Ⅸ	F	Lu Yangli	Yunnan	Diqing	Deqin	28.351 4	99.037 55
65333	*Mesorhizobium* sp. Ⅹ	F	Lu Yangli	Yunnan	Diqing	Shangri-la	27.907 4	99.831 60

Continued

Strains (CCBAU No.)	Species	Area	Collector	Province/ Autonomous Region	City	Town/District/ Banner	Latitude/(°)	Longitude/(°)
65318	*M. huakuii*	F	Lu Yangli	Yunnan	Diqing	Heqing	26.335 5	100.277 2
65327^T	*M. shangrilense*	F	Lu Yangli	Yunnan	Diqing	Deqin	28.351 4	99.037 55

Note: CCBAU, Culture Collection of Beijing Agricultural University

Gene amplification and sequencing

Template DNA from each strain was extracted as described previously. Five core genes including *atpD*, *glnII*, *gyrB*, *recA* and *rpoB*, encode ATP synthase beta subunit (AtpD), glutamine synthetase II (GlnII), DNA gyrase subunit B (GyrB), recombinase A (RecA), DNA-directed RNA polymerase subunit beta (RpoB), respectively. Six heat-shock factor genes covering *clpA*, *clpB*, *dnaK*, *dnaJ*, *grpE* and *hlsU*, are involved in encoding the ATP-dependent chaperone protein (ClpA and ClpB), molecular chaperone Hsp40 (DnaK and DnaJ), Hsp70 cofactor (GrpE) and ATP-dependent protease (HslU), respectively. Five nodulation genes comprising *nodA*, *nodC*, *nodD*, *nodG* and *nodP*, encode acyltransferase (NodA), N-acetylglucosaminyl transferase (NodC), transcriptional regulator (NodD), 3-oxoacyl- (acyl-carrier-protein) reductase nodulation protein (NodG) and sulfate adenylyltransferase (NodP), respectively.

The PCR amplification protocols of some genes were performed referring the procedures described previously (Tab. 1-2). Primers for the other genes were designed referring to the corresponding homologous regions of the whole genome of *M. amorphae* CCBAU 01578, *M. silamurunense* CCBAU 01550^T, *M. mediterraneum* CCBAU 01399 and *M. caraganae* CCBAU 01502 using the Primer 5.0 software in this study (Tab. 1-2). The annealing temperatures (Tm) for PCR amplification for these heat-shock factors and nodulation genes were also listed in Tab. 1-2. PCR products were purified and commercially sequenced by ABI 3730XL sequencer in Beijing, China, using their corresponding primers. All obtained sequences were checked using ChromasPro (Ver. 1.7.6, Technelysium) and were manually edited using DNAMAN (Ver. 7.212, Lynnon Corp., Quebec, Canada).