Systematic and Applied Microbiology 39 (2016) 211–219

Contents lists available at ScienceDirect

Systematic and Applied Microbiology

journal homepage: www.elsevier.de/syapm

Biodiversity and biogeography of rhizobia associated with common bean (*Phaseolus vulgaris* L.) in Shaanxi Province

Li Wang[a,1], Ying Cao[a,1], En Tao Wang[b], Ya Juan Qiao[a], Shuo Jiao[a], Zhen Shan Liu[a], Liang Zhao[a], Ge Hong Wei[a,*]

[a] State Key Laboratory of Crop Stress Biology in Arid Areas, College of Life Sciences, Northwest A&F University, Yangling, Shaanxi 712100, China
[b] Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, 11340 México, D.F., Mexico

ARTICLE INFO

Article history:
Received 23 November 2015
Received in revised form 28 January 2016
Accepted 1 February 2016

Keywords:
Rhizobia
Bean
Phylogeny
Diversity
Biogeography
recA
glnII
atpD
nodC
nifH

ABSTRACT

The biodiversity and biogeography of rhizobia associated with bean in Shaanxi Province were investigated. A total of 194 bacterial isolates from bean nodules collected from 13 sampling sites were characterized based on phylogenetic analyses of the 16S rRNA gene, the housekeeping genes *recA*, *glnII* and *atpD*, and the symbiotic genes *nodC* and *nifH*. Fifteen genospecies belonging to the genera *Rhizobium*, *Agrobacterium*, *Ensifer*, *Bradyrhizobium* and *Ochrobactrum* were defined among the isolates, with *Rhizobium* sp. II, *Agrobacterium* sp. II, *E. fredii* and *R. phaseoli* being the dominant groups. Four symbiotic gene lineages corresponding to *Rhizobium* sp. I, *Rhizobium* sp. II, *R. phaseoli* and *B. liaoningense* were detected in the *nodC* and *nifH* sequence analyses, indicating different origins for the symbiotic genes and their co-evolution with the chromosome of the bacteria. Moreover, the *Ensifer* isolates harbored symbiotic genes closely related to bean-nodulating *Pararhizobium giardinii*, indicating possible lateral gene transfer from *Rhizobium* to *Ensifer*. Correlation of rhizobial community composition with moisture, temperature, intercropping, soil features and nutrients were detected. All the results demonstrated a great diversity of bean rhizobia in Shaanxi that might be due to the adaptable evolution of the bean-nodulating rhizobia subjected to the diverse ecological conditions in the area.

Introduction

Common bean (*Phaseolus vulgaris* L.) originated and was domesticated in the Andes and Mexico. It is an excellent food crop cultivated worldwide and represents 50% of leguminous grain products for direct human consumption. This plant was introduced from Latin America to China 600 years ago [42], and its wide cultivation has made China an important producer and one of the secondary centers of diversity for common bean [54]. Like other legumes, common bean is able to fix nitrogen by forming root and/or stem nodules with rhizobia symbiotic bacteria. Currently, different rhizobial species nodulating with common bean plants grown in various areas of the world have been described, including *R. etli* [36], *R. tropici* [27], *P. giardinii* and *R. gallicum* [1,32], R. *lusitanum* [41], *R. phaseoli* [35], *R. azibense* [30], *R. freirei* [9], *E. meliloti* [56], *E. americanus* [31], and *Bradyrhizobium* sp. [17], among others. Furthermore, *E. fredii*-like salt-tolerating bacteria and *Mesorhizobium* spp. have been isolated from common bean plants in Spain [18] and Brazil [16], respectively. The existence of biogeographic patterns in the common bean-nodulating rhizobia has been shown in previous studies, and was thought to be a result of interaction between environmental factors, host plants and the symbiotic bacteria [43].

Shaanxi Province in China is the center of the Asian section of the second Eurasian continental bridge with a total area of 205,800 km^2. It is an arid-humid transition zone with a continental climate divided into three geographic/ecological regions by the Beishan Mountains and Qinling Mountains. The northern Shaanxi (Plateau of Northern Shaanxi) is to the north of Beishan Mountains, and classifies as a temperate semi-arid area with an annual average temperature of 7–12 °C and an altitude of 800–1300 m. The central area of Shaanxi (Guanzhong Plain) is between the two mountain ranges, and is a sub-humid warm temperate region accompanied by high temperature and drought in the summer, with an altitude of 325–800 m. The region of southern Shaanxi, to the south of Qinling Mountains, has a typical subtropical climate with a higher than annual average temperature and precipitation than the other two regions. In addition, the soil types in Shaanxi Province are very

* Corresponding author at: College of Life Sciences, Northwest A&F University, Yangling, Shaanxi 712100, China. Tel.: +86 2987080009; fax: +86 2987091175.
E-mail address: weigehong@nwafu.edu.cn (G.H. Wei).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.syapm.2016.02.001
0723-2020/

diverse, with more than 400 soil species in total, and common bean is widely cultivated as one of the vegetables in all three regions. Based on the diverse climate and soil conditions, various rhizobia associated with common bean could be expected, which represent an excellent model for estimating the correlation between rhizobial diversification and specific environmental conditions. However, the diversity of rhizobia associated with common bean has not been studied thoroughly in Shaanxi.

Considering the existence of complex ecological conditions in Shaanxi and the strong effects of the ecological and geographical factors on the distribution/diversity of rhizobia, this study (i) investigated the diversity of common bean rhizobia in the three ecoregions of Shaanxi; and (ii) estimated the ecological drivers for the distribution of common bean rhizobial species.

Materials and methods

Sampling strategy and rhizobial isolation

Based on the geographical and climatic conditions, 13 sampling sites were selected (Fig. 1). A total of 8 sampling sites were chosen from N32° to N38° at E109°, and 5 sampling sites were chosen on the N34° line, ranging from E107° to E109°. The sites from N38° to N36° corresponded to the Shaanbei (northern) Region, N35° to N34° to the Guanzhong (central) Region, and N33° to N32° to the Shaannan (southern) Region (Supplementary Table S1). All the sampling sites were located in intercropping fields without a rhizobial inoculation history. At least 15 plants were randomly chosen from each site during July to August in 2010. Roots of common beans excavated from the soil were transported to the laboratory together with soil samples collected from the surface layer of the same sites at a depth of 0–20 cm. The soils were subsequently used for physiochemical characterization (pH, organic matter, available nitrogen, available phosphorus and available potassium) using routine methods. The geographical conditions (longitude, latitude, altitude, landforms, soil profile structure, groundwater level), climatic conditions (rainfall, average geothermal at a depth of 20 cm, annual effective accumulative temperature, monthly average temperature, monthly average relative humidity, sunshine duration), soil features (soil types, parent materials), cropping system (number of crops per annum) and intercropping (type of crop) for each sampling site were obtained from the Department of Agriculture and Meteorological Bureau of Shaanxi Province (Table S2).

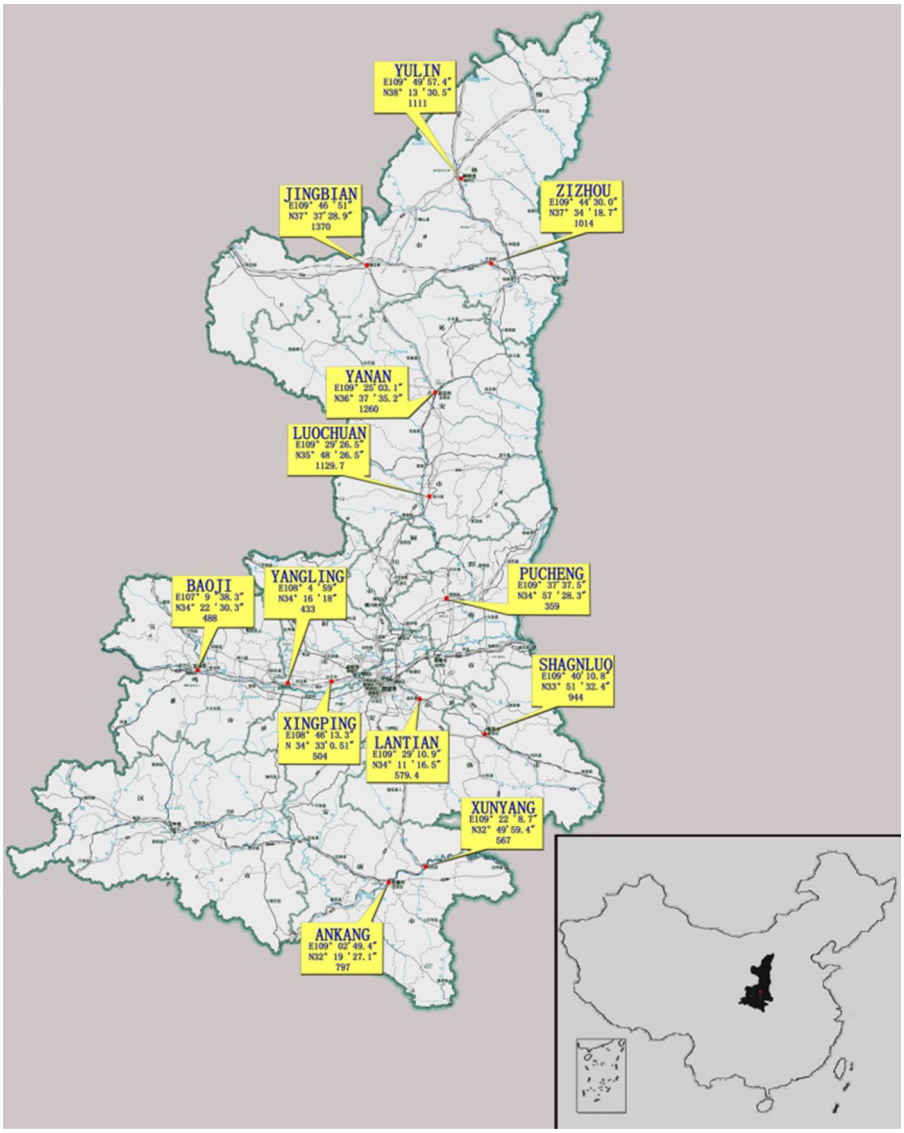

Fig. 1. Map of Shaanxi Province showing the sampling sites (·). The corresponding position of Shaanxi Province in China is shown in the inset. The two maps were created using DIVA-GIS software (http://www.diva-gis.org), and the sampling sites were added according to GPS records.