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Population Dynamics of Corn Bacterial Endophytes

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Abstract

An investigation was conducted to gain essential information on the microbial ecology of endophytic bacteria in 17 corn plant varieties. Throughout the growing season, population dynamics of endophytic bacteria were discovered in the roots, leaves, and stems of corn plants. The bacterial density fluctuated from 10^4 to 10^5 colony-forming units per gram and increased as the plant grew, reaching the maximum at the heading stage. The mean bacterial populations were generally more in the roots and decreased in leaves and stems. This might be related to the soil serving as the source of endophytic bacteria and the colonization of roots by bacteria in the rhizosphere. These results collectively indicated that endophytic bacteria were not uniformly distributed in plant tissues. Moreover, the population was correlated with the growth period and plant parts. Our research would heighten interest in the research on endophytic bacteria, which may have potential value as a biofertilizer or biopesticide, thus providing a viable approach to sustainable agriculture.

Keywords: *Corn, Endophytic bacteria, Population dynamics*

Introduction

Zea mays L. is a staple food for half of the world's population [1]. However, the plant can be seriously infected with many diseases, such as Bipolaris maydis [2], northern corn leaf blight (Spot), Fusarium stalk rot [3], head smut [4] ,and corn rust [5]. Chemical pesticides are widely used to protect corn against diseases. However, they also have many adverse effects, including pesticide residue, environmental pollution, disease resistance enhancement. Thus, interest in using biological control as an alternative approach via antagonistic microorganisms is increasing.

Endophytic bacteria are a group of bacteria that can be isolated from surface-sterilized tissues of asymptomatic plants [6]. Since the 1940s, more than 200 genera of endophytic bacteria from different plant tissues have been successfully categorized and reported from healthy plants [7,8]. And with over 300,000 species of land plant on earth is likely to host to one or more endophyte species [7,8]. Generally, each endophytic bacterium has a wide host range where most commonly isolated bacterial genera include Bacillus, Enterobacteria, Pseudomonas, Kosakonia, Methylobacterium, Microbacterium, Nocardioides, Pantoea, and Burkholderia [9-11]. These endophytic bacteria are important members of plant microbiome living asymptomatically in plant tissues and have attracted considerable attention as potential agents in their beneficial activities in terms of nutrient availability, plant growth hormones, and control of soil-borne and systemic pathogens [12,13]. The endophytic bacteria widely distributed in plant different tissues including roots, stems, leaf, flower, fruits, seeds, and pollens [12]. And the community structure depends on various factors, such as soil conditions, biological and abiotic stresses, as well as the genotype and age of plant [8,14-17].

Despite the beneficial effects of endophytes on plant growth, little is known about the population dynamics corresponding to the growing stage. Therefore, this study aimed to determine the distribution of endophytic bacteria at different growth periods in the roots, stems, and leaves of 17 corn varieties.

Materials and Methods

Corn Varieties

A total of 17 varieties of corn were used in this study: Dafeng123, Diandu 8, Zhongdan 815, Yunjin 2, Zhengda 615, Wugu 2, Wugu 3861, Yunrui 2, Yunrui 7, Yunrui 8, Yunrui 47, Yunrui 88, Yunrui 99, Yunrui 220, Yunyou 105, YR6, YR7. They were obtained from Seed Management Stations in Yunnan Province. A single field plot on the Yunnan Agricultural University farm was used to plant the different corn varieties. Meanwhile, 20 plants of each variety were grown in two rows, with 20 cm spacing between neighboring plants and a 60 cm long and 30 cm wide space between the rows.

Sample Preparation and Surface-disinfestation

Plants were sampled at three growth stages: seedling, elongation, and heading. On specific dates, one plant from each variety was randomly selected, manually uprooted, and washed thoroughly in running tap water to remove the adherent soil particles. Then, the third leaf from the top to bottom was removed, and a section of stem 20 cm above the ground was cut off. One gram of roots (5-10 cm below the soil line) was obtained. The plant materials of different varieties were transported in separate plastic sample bags to a laboratory where they were immediately surface-disinfested immediately [18].

Leaf and Root Surface-disinfestation

For the isolation of leaf and root endophytes, 1 g of each sample was used and the plant parts were cut into small segments. The segments were surface sterilized by immersing them in 75% ethanol for 150 s, followed by immersion in sodium hypochlorite (1%, vol/vol) for 5 min. The samples were then rinsed with 10 mL sterilized distilled water to remove all chemical residues and were ground in a sterile mortar with 9 mL sterile distilled water (SDW).

Stem Surface-disinfestation

A stem section closest to the ground was surface-disinfected with 75% ethanol for 5 min and washed three times with SDW. After the epidermis was aseptically removed, 1 g of stem tissues was transferred to a sterile mortar and ground with 9 mL SDW.

To ensure that the plant surface had been thoroughly sterilized, each surface-disinfected stem, root, and leaf sample was first allowed to touch the surface of LB plates and coated with SDW before incubation at 30°C.

Bacterial Cultivation and Preservation

The ground tissues of each plant part were mixed with 9 mL SDW and ground further to obtain a tissue suspension. Bacteria from roots, stems, and leaves, respectively, were cultivated in 9-cm Petri dishes containing Lysogeny broth (LB) media (5 g/mL yeast extract, 10 g/mL tryptone, 10 g/mL sodium chloride, agar 15 g, 1000 mL ddH_2O, pH 7.0-7.2). An aliquot of 200 µL tissue suspension was plated on the LB plates and incubated at 30°C for 36-48 h. A total of three plates were used for each plant part. The population of endophytic bacteria was estimated by counting the colonies appearing on the agar plates. The endophytic density was determined using the following formula [18]:

Endophytic density (CFU/g)=average number of colonies × dilution ratio × 5 (CFU: colony-forming units).

One representative from the numerous bacterial colonies with similar morphological characteristics on the culture plates was transferred to a fresh LB plate to establish a pure culture line for each bacterium strain isolated. Individual bacterial strains were transferred to LB liquid media and shaker-cultured at 180 rpm at 25°C until the media turned milky. The bacteria suspension was then transferred to 2 mL centrifuge tubes containing 40% glycerol and stored at -80°C.

Results

Bacteria recovered from surface-disinfested leaves, stems, and roots throughout the growing season were isolated on LB plates after incubating for two days. The density of bacteria colonies on LB agar plates did not change much with the extension of incubation time. The population density of bacterial endophytes in corn was influenced by the variety, origin of plant tissues, and growing stage.

Distribution of Endophytic Bacteria Taxa during Different Growth Periods

A different number of bacteria taxa were consistently isolated from the healthy plant organs of 17 different corn varieties (Table 1).

Table 1: Distribution of endophytic bacterial taxa in 17 corn varieties during different growth periods.

Variety	Seedling stage	Elongation stage	Heading stage
	Root Stem Leaf	Root Stem Leaf	Root Stem Leaf
Dafeng123	1 - 3	3 2 1	1 2 2
Diandu 8	3 - 3	3 2 1	1 2 2
Zhongdan 815	1 - 3	3 2 2	1 2 2
Zhengda 615	1 - 3	2 1 1	1 2 1
Wugu 2	2 - 3	2 2 2	1 1 3
Wugu 3861	2 - 3	2 1 3	2 1 2
Yunjin 2	3 - 4	2 1 3	1 1 1
Yunyou 105	2 - 2	2 1 3	1 1 2
Yunrui 2	1 - 3	2 1 3	2 1 2
YR 6	0 - 2	3 1 1	1 1 2
YR7	1 - 2	2 1 3	1 1 2
Yunrui 7	1 - 3	2 1 2	2 1 3
Yunrui 8	1 - 3	2 1 5	1 1 3
Yunrui 47	1 - 2	2 1 2	1 1 2
Yunrui 88	1 - 2	2 1 2	1 1 1
Yunrui 99	2 - 3	2 2 2	1 1 1
Yunrui 220	2 - 2	2 1 1	1 1 2

In the stems and roots, there were more taxa (2-3 taxa per corn variety) of endophytic bacteria in the elongation stage than in the seedling and heading stages. The number of bacteria taxa on the leaves was more in the seedling stage. In general, there were more taxa of endophytic bacteria in the leaves, followed by the roots.

Population Dynamics of Endophytic Bacteria

Effect of the Growing Season on the Population Density

Endophytic bacteria were found during the entire growth period, and its population dynamics in different tissues changed accordingly. Based on the number of colonies on LB agar plates, the seedling bacterial population from field-grown corn was 10^3-10^4 CFU/g; however, it increased with the age of the plants, reaching up to 10^5 CFU/g in the heading stage (Figure 1).

Effect of Plant Tissues on the Population Density

In all corn varieties, the endophytic bacterial density of corn was the highest in the roots, followed by the leaves, and lowest in the stems (Figure 2).

Endophytic populations in corn leaves and stems remained at 10^3 CFU/g for most of the growing season and increased to 10^4 CFU/g in the heading or post-harvest stages. Root tissues harbored more endophytic bacteria than other plant tissues. For the remainder of the growing season, the root's bacteria population in the heading stage ranged from 10^4-10^5 CFU/g.

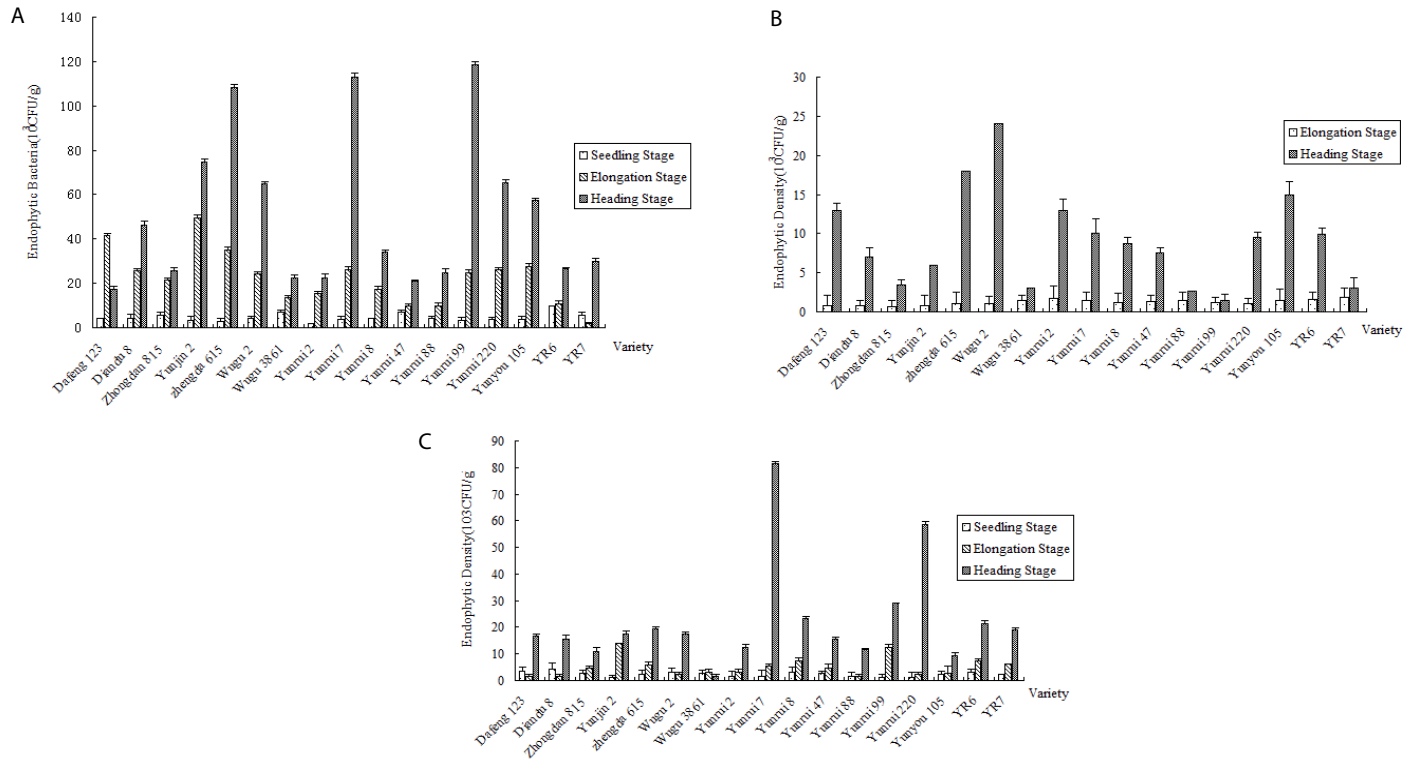

Figure 1: Population quantity of endophytic bacteria in three growth periods of corn. a: Endophytic bacteria in corn root. b: Endophytic bacteria in corn stem. c: Endophytic bacteria in corn leaf.

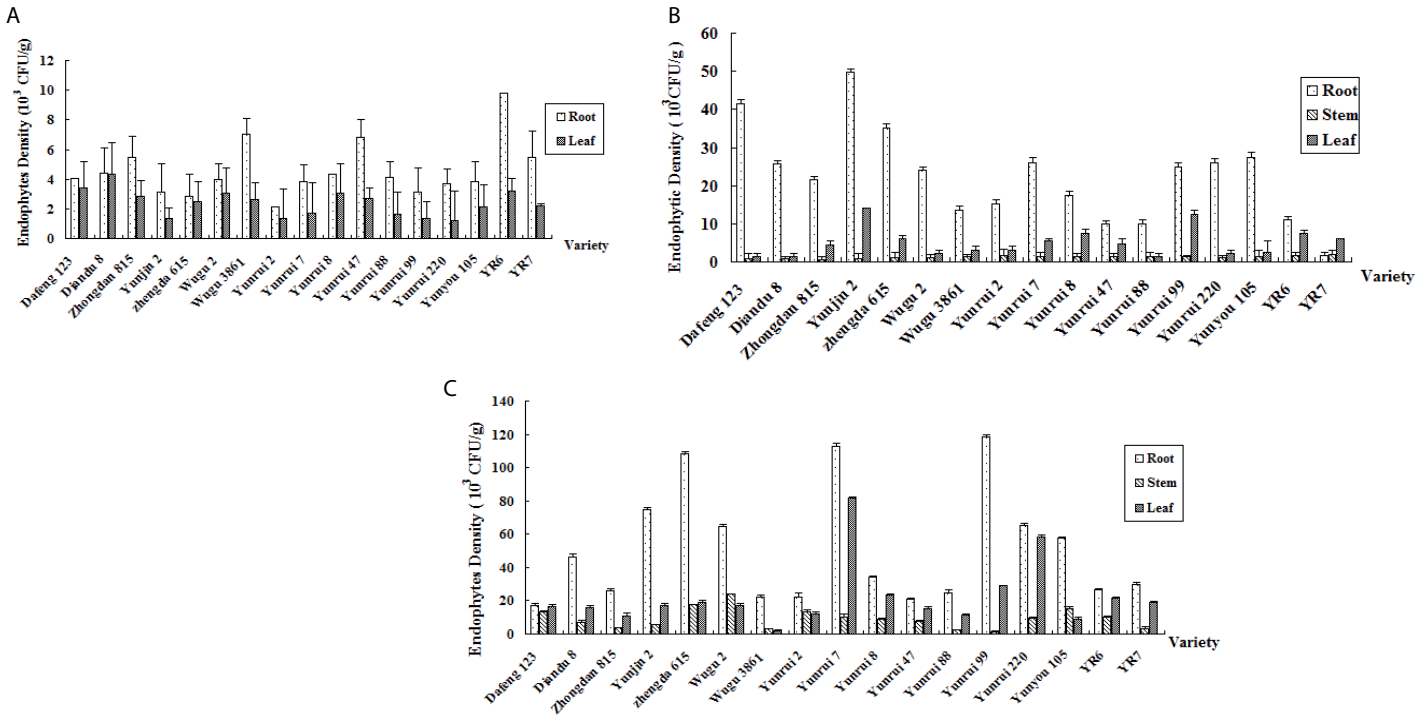

Figure 2: Population quantity of endophytic bacteria in different tissues. a: Endophytic bacteria in the seedling stage. b: Endophytic bacteria in the elongation stage. c: Endophytic bacteria in the heading stage.

Discussion

Extensive knowledge regarding the density and diversity of endophytic bacteria colonizing plant tissues is essential in understanding the indigenous endophytic bacterial community and the assessment of endophytes as potential sources for plant growth promotion and biological control of plant diseases. However, an accurate estimation of the total number of endophytic bacteria is often difficult. This is due to the heterogeneous distribution of bacteria within the plant tissues

and the tendency of some bacteria to clump together in their secreted mucilage [19] or adhere to various particles, including the plant cell wall components [20]. Researchers have also found that the density of indigenous endophytic bacteria was approximately 10^5 CFU/g in the root, but 10^4 CFU/g and 10^3 CFU/g, respectively, in the stem and leaf [18-20]. Similarly, the endophytic population in corn stem and root was 10^4-10^6 CFU/g for most of the growing season [18,21].

The present study systemically revealed the effects of tissue-types corresponding to different growing periods on structures and densities of endophytic bacteria. Obtained results showed that there was no significant difference in endophytic bacterial community structure among maize varieties. The overall trend was that the endophytic bacteria species were the most abundant in leaves and the least in stems. Previous studies have demonstrated that endophytic bacterial diversity variation is associated with physicochemical properties of soil and atmospheric conditions [18,22-24]. Soil microorganisms can migrate through the xylem track to colonize the plant tissues and the roots play an essential role for the retrieval of microbiome community from soil [25-27]. Therefore, this might be a response to the similar soil type, growing climate condition, as well as the agricultural practices. A dynamic infection process could begin in the rhizosphere (especially at the site of lateral root emergence), followed by endophytic colonization of the roots and subsequent ascending endophytic migration into the stem base, leaf sheath, and leaves [28]. However, it is still unknown if the vascular tissues only serve as a transport channel for endophytic bacteria or if bacteria multiply within the vascular system. In the latter case, many bacteria within the vessels might lead to plugging and, therefore, could induce plant pathogenicity. This may explain why endophytic bacteria are usually found in relatively low numbers within the vascular tissues. Therefore, the hypothesis is supported that bacterial endophytes originate in the rhizosphere and proceed into stem tissue and leaves via the vascular system. Moreover, our study found that the bacterial population from field-grown corn was increased as the plant grew, reaching the highest in the heading stage. The heading stage signals that the crops change from vegetative to reproductive growth and is in a critical period for determining crop yield. Endophytic bacteria have been demonstrated to improve plant growth by producing phytohormones, including IAA and gibberellic acid. In addition, endophytic bacteria have been shown to induce plant disease resistance [18,29]. These biological functions can provide a healthy growth environment for the plants and increase their ability to absorb water and minerals from the soil.

Our results provided more insights by demonstrating that the endophytic bacterial community was dynamic and influenced by biotic factors, with the plant itself being one of the significant factors. The total number and taxa of endophytic bacteria isolated from corn in this study suggest that internal corn tissues harbor diverse microbial flora. Screening endophytic bacteria adds to the list of bacteria as potential plant growth promotors and biological control agents. More useful endophytic bacteria are expected to be discovered as more crops are studied.

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Author Contributions

Yueqiu He conceived and designed the study and experiments; Liwei Guo and Pengfei He performed the experiments; Pengfei He analyzed the data; Liwei Guo wrote the manuscript; and all authors contributed to the final draft of the manuscript.

Competing Interests

The authors declare that they have no competing interests.

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