



Diversity and Biological Characteristics of Seed-borne Bacteria of *Achnatherum splendens*

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Abstract: As a high-quality plant resource for ecological restoration, *Achnatherum splendens* has strong adaptability and wide distribution. It is a constructive species of alkaline grassland in north-west China. The close relationship between seed-borne bacteria and seeds causes a specific co-evolutionary effect, which can enhance the tolerance of plants under various stresses. In this study, 272 bacterial isolates were isolated from the seeds of *Achnatherum splendens* in six different provinces of China. 41 dominant strains were identified, and their motility, biofilm formation ability and antibiotic resistance were analyzed. The results showed that the bacteria of *Achnatherum splendens* belonged to 3 phyla and 14 genera, of which Firmicutes was the dominant phylum and *Bacillus* was the dominant genus. The motility and biofilm formation ability of the isolated strains were studied. It was found that there were 6 strains with a moving diameter greater than 8 cm. There were 16 strains with strong biofilm formation ability, among which *Bacillus* with biofilm formation ability was the most, accounting for 37.5 %. The analysis of antibiotic resistance showed that sulfonamides had stronger antibacterial ability to strains. Correlation analysis showed that the resistance of strains to aminoglycosides (kanamycin, amikacin, gentamicin) was significantly positively correlated with their biofilm formation ability. This study provides fungal resources for improving the tolerance of plants under different stresses. In addition, this is the first report on the biological characteristics of bacteria in *Achnatherum splendens*.

Keywords: *Achnatherum splendens*; seed-borne bacterial diversity; motility; biofilm; antibiotic resistance

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Last-name

Received: date

Revised: date

Accepted: date

Published: date



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1. Introduction

Seeds are essential throughout the life cycle of plants, which can exist in the environment for a long time in a dormant state until the growth conditions are suitable and then grow into complete plants [1]. Species-borne bacteria refer to all bacteria that parasitize inside and on the surface of seeds, and their colonization plays different roles in plant growth and health at different growth stages [2,3]. Seed-borne bacteria can exist in the whole life cycle of seeds, including seed germination and development to growth results and post-harvest storage and processing. It can also ensure the growth and reproduction of seed-borne bacteria in the next generation through vertical transmission and rhizosphere colonization. It is precisely because seed and seed-borne bacteria affect seed germination and plant growth in plant-bacterial interactions and other forms[4], it is that great significance to clarify the diversity of seed-borne bacteria. *A.splendens* is a perennial tussock herb belonging to *Achnatherum* genus of Gramineae. It is widely distributed in

China and grows as a constructive species in the arid grassland of Northwest China [5,6]. As the dominant population of temperate grassland, *A.splendens* can be used as winter and spring forage by livestock. *A.splendens* growing along the Qinghai-Tibet Railway can not only prevent wind and sand, stabilize embankment, but also play a good role in soil and water conservation. Therefore, it is of great significance to explore the diversity and characteristics of seed-borne bacteria of *A.splendens* to alleviate the stress of plants under adversity and the further utilization of seed-borne bacteria.

At present, antibiotic resistance, as one of the global problems that threaten human health, has received extensive attention from scholars in different fields around the world. In 2019, scholars analyzed antibiotic resistance-related cases in more than 200 countries and regions around the world, and found that nearly 6.2 million people died directly or indirectly from antibiotic resistance [7]. Since the discovery and application of penicillin, the antibiotic industry rise rapidly [8], a variety of different antibiotics have been found and widely used in medical, agricultural and animal husbandry. Avermectins (AVMs) are commonly used to control grasshoppers and caterpillars in grasslands [9,10]. Livestock in Northwest China rely on grassland-based natural vegetation, and the use of antibiotics is limited to livestock disease management. Although the dose of antibiotics administered to grassland animals is significantly lower than that of centralized animal feeding (CAFOs), extensive grazing still has the potential to cause antibiotic resistance in animals and environmental reservoirs [11–13]. The extensive use of antibiotics has led to bacterial resistance. As the problem of bacterial resistance continues to emerge, the number of multi-antibiotic resistant bacteria continues to increase. It is believed that this may cause common infections that cannot be effectively treated and increase mortality [14]. The food chain [15], the horizontal spread of antibiotic resistance genes (ARGs), inadequate sanitary conditions, population mobility, and trade in live animals and meat make antibiotic resistance spread throughout the ecosystem [16]. In large-scale studies, it has been found that there is a significant correlation between antibiotic use and antibiotic resistance in livestock and human populations [17,18]. One of the reasons for bacterial resistance is the formation of biofilms. Biofilms can help bacteria better adapt to the living environment, and have certain resistance to fungicides such as antibiotics and harsh environments. Bacterial motility is crucial to the adhesion period of biofilm formation [19–21]. Different regions have bred different microbial resources due to differences in climatic conditions and geographical locations. As a reproductive organ of forage grass, the bacteria carried by seeds can not only transmit them by vertical transmission[22], but also bring them out by seed dispersal. If the bacteria carry the antibiotic resistance gene, the gene will also spread, and the antibiotics deposited in the environment will accumulate.

Therefore, in this study, *A.splendens* species from Xinjiang Uygur Autonomous Region, Inner Mongolia Autonomous Region, Ningxia Hui Autonomous Region, Qinghai Province, Gansu Province, Jiangsu Province and other places were used as research materials. Through the isolation, purification, phenotypic and molecular identification of the species-borne bacteria, the bacterial motility, biofilm formation ability and antibiotic resistance were determined, and the relationship between the three was analyzed. In order to preliminarily determine the diversity of culturable bacteria in *A.splendens*, and to clarify the biological characteristics of bacteria in *A.splendens*. Screening out strains with strong resistance and applying them to plants under antibiotic pollution, observing whether bacteria can improve the sensitivity of plants to antibiotics and slow down the persecution of antibiotics on plants can provide a theoretical basis for biological means to control antibiotic pollution, which is also our follow-up work.

2. Materials and Methods

2.1. Experimental materials

The information of grass species is shown in Table 1.

Table 1. Related information of *Achnatherum splendens* species.

Origin	Grass species	Production time	Source
Xinjiang Uygur Autonomous Region	<i>Achnatherum splendens</i>	2020	Qinghai Womiao Ecological Technology Company Ltd
Inner Mongolia Autonomous Region	<i>Achnatherum splendens</i>	2021	
Qinghai province	<i>Achnatherum splendens</i>	2022	
Gansu province	<i>Achnatherum splendens</i>	2021	
Ningxia Hui Autonomous Region	<i>Achnatherum splendens</i>	2021	
Jiangsu province	<i>Achnatherum splendens</i>	2020	

Reagents and medium: TSA medium, TSB medium, Swimming medium, Crystal violet staining solution.

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2.2. Methods

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2.2.1. Isolation of bacteria

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The prepared TSA medium was placed in the incubator for 1 day, and used after confirming the pollution-free. 1 g of wild *A.splendens* from six different regions were weighed and placed in sterile pots. The disinfection treatment group was first disinfected with 75 % alcohol for 2 min, and then disinfected with 1% NaClO for 3 min. After disinfection, it was washed four times with sterile water to remove the disinfectant on the surface. Add 10 mL sterile water to the disinfected seeds and the unsterilized seeds for full grinding. After standing for 10 min, the diluent was prepared: 100 μL supernatant was taken and diluted 10 times in 900 μL sterile water to obtain 10-1,10-2,10-3,10-4,10-5 diluents. 100 μL was evenly coated on TSA medium and cultured in darkness at 28 ± 1 °C constant temperature incubator. After 3 days, the single colony was purified and numbered, and then the strain was selected in TSB medium supplemented with 15% glycerol and stored at – 80 °C

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2.2.2.16. S rDNA molecular identification of bacteria

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TIANamp Bacteria DNA Kit (Tiangen Biochemical Technology (Beijing) Co., Ltd.) bacterial genomic DNA extraction kit was used to extract DNA from the isolated and purified bacteria. The operation steps were carried out according to the kit instructions.

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After DNA extraction, PCR amplification was performed. The amplified primer sequences were: 1 forward primer 27F: 5' -AGAGAG TTTGATCMTGGCTCAG-3', 2 reverse primer 1492R: 5' -GGYTACCTTGTTA CGACTT-3'. Among them, 1492R is a universal primer and 27F is a bacterial specific primer. The combination of two primers can effectively amplify the 16 Sr DNA of all bacterial groups. PCR amplification procedure: 95 °C, 4 min; 94 °C, 1 min; 50 °C, 1 min; 72 °C, 2 min; 34 cycle; 72 °C, 10 min. After PCR amplification, the obtained products were sent to Shanghai Parsons Biotechnology Co., Ltd. for sequencing. The sequences were aligned by BLAST in NCBI database (<http://www.ncbi.nlm.nih.gov/blast.cgi>). The phylogenetic tree was constructed by using the software MEGA 11.0 with the highest homologous strain sequence as the reference object.

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2.2.3. Determination of swimming motility

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According to the method of Alías-Villegas[23], the bacteria were picked up with sterile toothpicks and cultured in a swimming plate at 28 °C for 20 h. The diameter of the turbid area formed by the diffusion of the bacteria was observed and measured.

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2.2.4. Determination of biofilm formation ability

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According to the method of Stepanovich S,[24] the isolated and purified strains were inoculated on fresh TSA plates for 24 hours, and the strains were selected and cultured in

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sterilized TSB medium overnight. The concentration of the bacterial solution was adjusted to 0.5 McFarland standard (1.5×10⁸ CFU / mL) turbidity. The 150 µL bacterial solution was transferred to a 96-well plate and aerobically cultured at 37 °C for 48 h. After the culture was completed, the contents of the wells were gently removed, and the wells were rinsed three times with 0.9% sterile 250 µL normal saline. After washing, 150 µL 0.1% crystal violet was added to each well for staining. After 15 min, the color was gently rinsed and dried in an oven at 60 °C, and then washed three times with 250 µL distilled water. Finally, 150 µL 96% ethanol was added. The optical density value at 570 nm was measured by quantitative spectrophotometry. According to the following criteria: (4OD_c < OD₅₇₀ = strong biofilm-forming bacteria, 2OD_c < OD₅₇₀ ≤ 4OD_c = medium biofilm-forming bacteria, OD_c < OD₅₇₀ ≤ 2OD_c = weak biofilm-forming bacteria, OD₅₇₀ ≤ OD_c = no biofilm-forming bacteria, OD_c = negative control of the average OD₅₇₀ + (3 × negative control standard deviation), the isolates were divided into strong, medium, weak or no biofilm-forming bacteria.

2.2.5. Determination of antibiotic resistance

According to the agar dilution method of Lavallée et al [25], the prepared antibiotics with exponential concentration growth were added to the agar medium to make an antibiotic plate, and then the strain was inoculated to the surface of the medium. After 48 hours of culture, the growth status of the strain was observed, the minimum inhibitory concentration (MIC) of the strain was determined, and the antibiotic resistance of the strain was judged.

2.3. Statistical Analysis

All data indicators are expressed as mean ± standard error of three replicates. Data were collated using Excel 2021, analyzed by one-way ANOVA using Graphpad Prism9.50, and separated by means using Duncan's test. p < 0.05 indicates significance. The heatmap was carried out using the Chiot platform (<https://www.chiot.online/>).

3. Results

3.1. Bacterial diversity of seed-borne bacteria of A.splendens

A total of 41 strains of bacteria were isolated from the seeds of wild A.splendens from 6 regions. The number and species of bacteria isolated from various samples were different. (Table 2)

Table 2. Identification results and morphological description of seed-borne bacteria of Achnatherum splendens.

strain code	Gram	16S rDNA identification	Accession number
XJ1	-	<i>Klebsiella</i> sp.	OR889436
XJ2	-	<i>Enterobacter</i> sp.	OR889437
XJ3	-	<i>Atlantibacter</i> sp.	OR889438
XJ7	-	<i>Brevundimonas</i> sp.	OR889439
XJ12	-	<i>Klebsiella</i> sp.	OR889440
XJ16	-	<i>Enterobacter</i> sp.	OR889441
XXJ1	-	<i>Lysinibacillus</i> sp.	OR889445
XXJ6	+	<i>Bacillus</i> sp.	OR889446
XXJ9	+	<i>Bacillus</i> sp.	OR889447
XXJ17	-	<i>Enterobacter</i> sp.	OR889448
XNJ10	+	<i>Bacillus</i> sp.	OR889455
XNJ16	+	<i>Bacillus</i> sp.	OR889456

XNJ22	+	<i>Bacillus</i> sp.	OR889466
QJ1	-	<i>Pseudomonas</i> sp.	OR889452
QJ2	-	<i>Erwinia</i> sp.	OR889453
XQJ2	+	<i>Bacillus</i> sp.	OR915404
QJ14	-	<i>Stenotrophomonas</i> sp.	OR889451
XQJ4	-	<i>Pseudomonas</i> sp.	OR889467
GJ6a	-	<i>Pseudomonas</i> sp.	OR889449
GJ18	-	<i>Siccibacter</i> sp.	OR889450
XGJ1a	+	<i>Paenibacillus</i> sp.	OR889463
XGJ9a	+	<i>Bacillus</i> sp.	OR889468
XGJ4	-	<i>Pseudomonas</i> sp.	OR889464
XGJ8	+	<i>Bacillus</i> sp.	OR889454
XGJ12	+	<i>Bacillus</i> sp.	OR889465
NM6	-	<i>Siccibacter</i> sp.	OR889429
NM8	+	<i>Bacillus</i> sp.	OR889430
NM10	+	<i>Bacillus</i> sp.	OR889431
NM15	-	<i>Enterobacter</i> sp.	OR889432
NM27	-	<i>Lelliottia</i> sp.	OR889433
NM31	-	<i>Pseudomonas</i> sp.	OR889434
NM42	-	<i>Erwinia</i> sp.	OR889435
XNM11	+	<i>Bacillus</i> sp.	OR889442
XNM15	+	<i>Bacillus</i> sp.	OR889443
XNM19	-	<i>Enterobacter</i> sp.	OR889444
JS-1	-	<i>Mixta</i> sp.	OR889457
JS-4	+	<i>Zhihengliuella</i> sp.	OR889458
JS-10	+	<i>Bacillus</i> sp.	OR889459
JS-19	-	<i>Siccibacter</i> sp.	OR889460
JS-25	+	<i>Bacillus</i> sp.	OR889461
JS-38	+	<i>Bacillus</i> sp.	OR889462

Note : + : Gram-positive bacteria; -: Gram-negative bacteria

3.1.1. Taxonomic status of bacteria

According to the determination of potassium hydroxide wire drawing test with the same nature of Gram staining method, there were 17 strains of Gram-positive bacteria, accounting for 41.46%. There were 24 strains of Gram-negative bacteria, accounting for 58.54%. The 41 strains belonged to 3 phyla and 14 genera, of which 18 strains belonged to Firmicutes, accounting for 43.90%, 16 strains belonged to *Bacillus* sp., 1 strain belonged to *Lysinibacillus* sp., and 1 strain belonged to *Paenibacillus* sp. . 22 strains of Proteobacteria, accounting for 53.66%, 2 strains of *Klebsiella* sp., 5 strains of *Enterobacter* sp., 1 strain of *Atlantibacter* sp., 5 strains of *Pseudomonas* sp., 1 strain of *Brevundimonas* sp., 3 strains of *Siccibacter* sp., 1 strain of *Lelliottia* sp., 2 strains of *Erwinia* sp., and 1 strain of *Mixta* sp., 1 strain of *Stenotrophomonas* sp. . There was 1 strain of bacteria belonging to Actinobacteria, accounting for 2.44%, and one strain of *Zhihengliuella* sp.

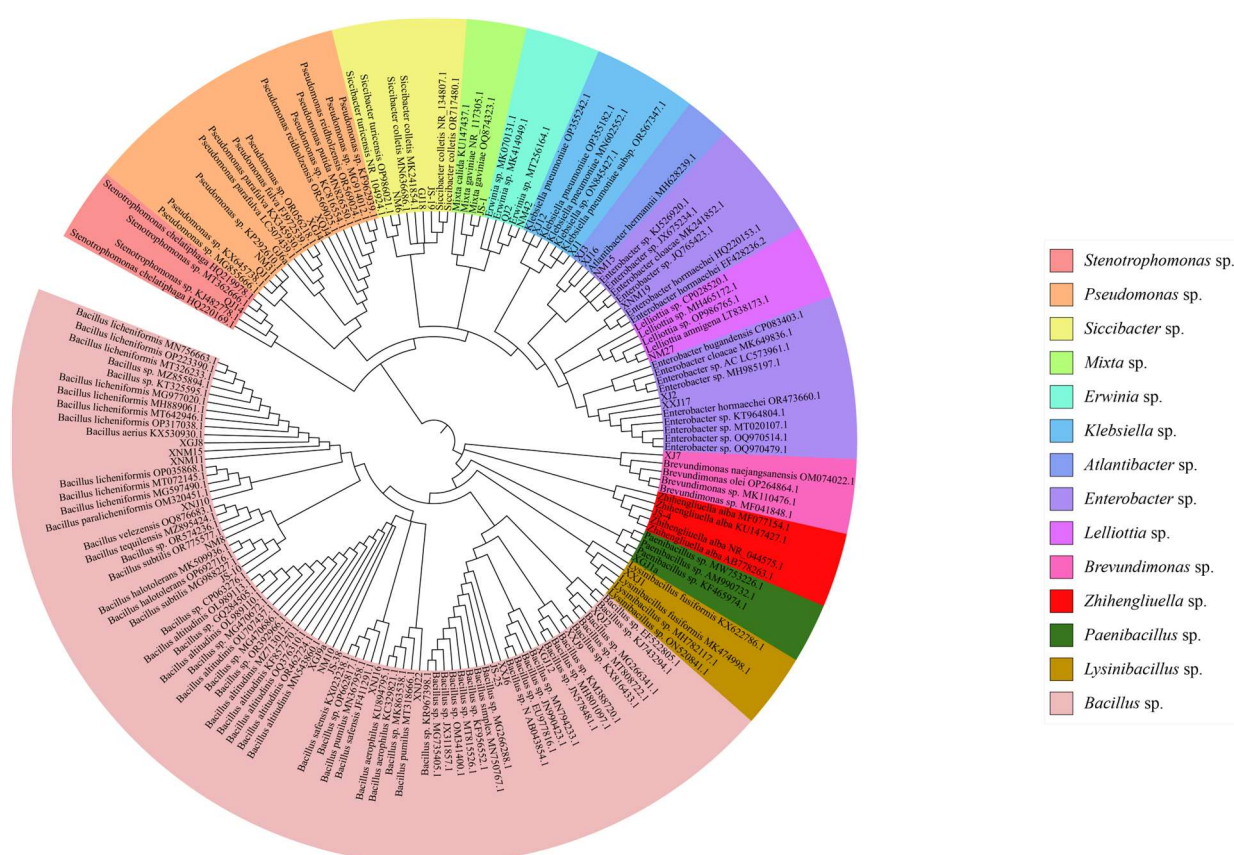


Figure 1. Phylogenetic tree of the species of seed-borne bacteria of *Achnatherum splendens*.

3.1.2. Bacterial diversity analysis of seed-borne bacteria of *A.splendens*

The diversity of seed-borne bacterial of *A.splendens* was analyzed. Through Wayne analysis, it was found that the common genus of bacteria isolated from six regions of Xinjiang, Inner Mongolia, Qinghai, Gansu, Ningxia Hui Autonomous Region and Jiangsu was *Bacillus* sp. (Fig.2-A). The Circos species relationship diagram intuitively presents the information of the samples and classification levels of each bacteria. The first circle showed the grouping information of the samples and the classification level of the bacteria. The second circle clearly showed the percentage of the relative abundance of bacteria in the samples. The third circle was distinguished by different colors, indicating the absolute abundance of bacteria. The central position of the fourth circle is connected between the sample and the classification level, echoing the outer ring (Fig.2-B). As another presentation of Venn diagram, UpSet diagram visually shows the common and unique parts among different regions. Using upset diagram to analyze the composition of bacterial genera in *A.splendens* seed zone in different regions, it is found that there is only one common genus in six regions, which is *Bacillus* sp., and there are endemic genera in Xinjiang, Inner Mongolia, Ningxia, Gansu and other places. The number of endemic genera is 4,3,1 and 1 respectively (Fig.2-C). The random forest model was constructed with the genus of *A.splendens* as the independent variable, and the %IncMSE was used to score and rank the characteristics. The top three genera were *Bacillus* sp., *Enterobacter* sp., *Siccibacter* sp., indicating that the species was more important for the identification of species in the group, indicating that the bacteria of *Bacillus* sp., *Enterobacter* sp., *Siccibacter* sp. were the marker species of the difference between groups (Fig.2-D).

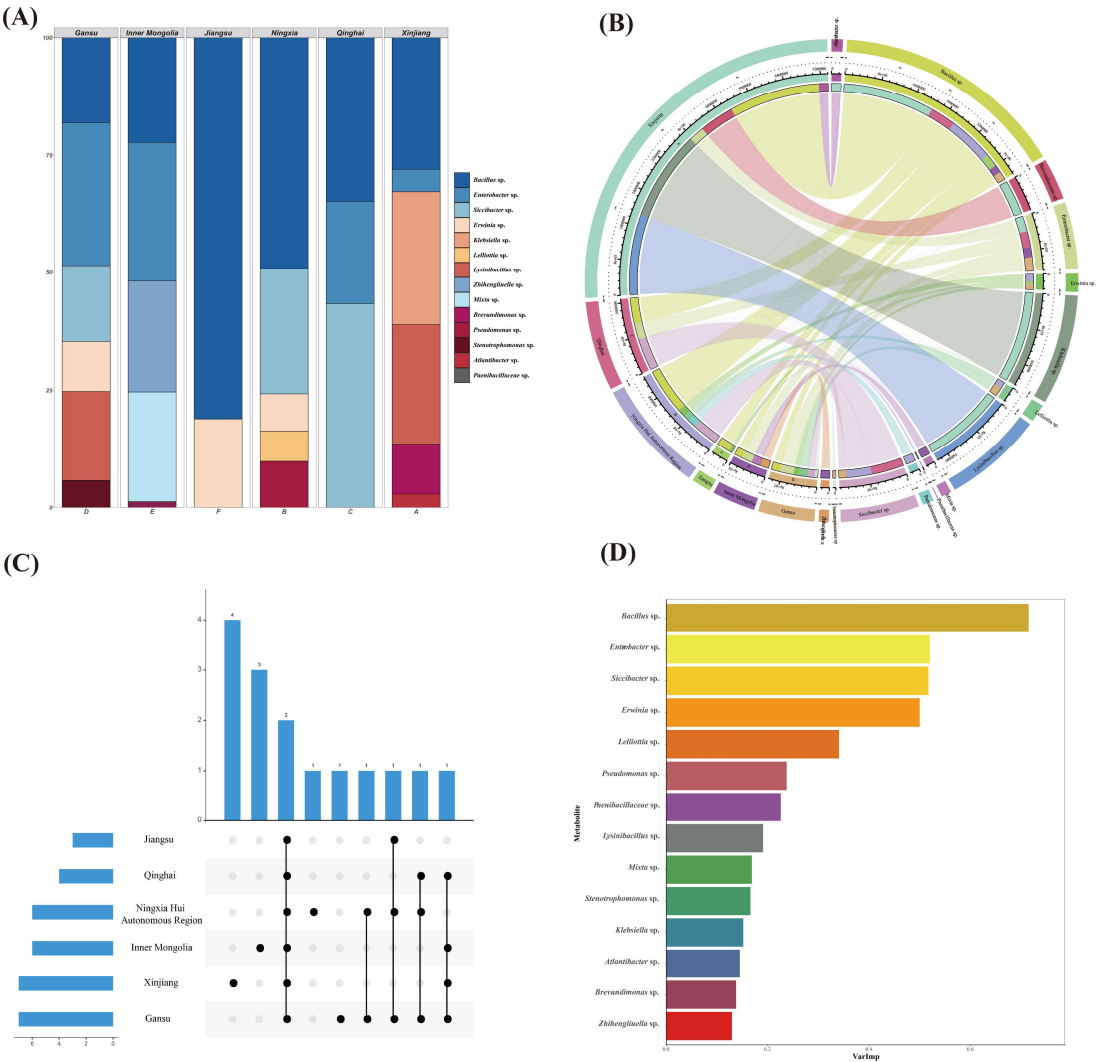
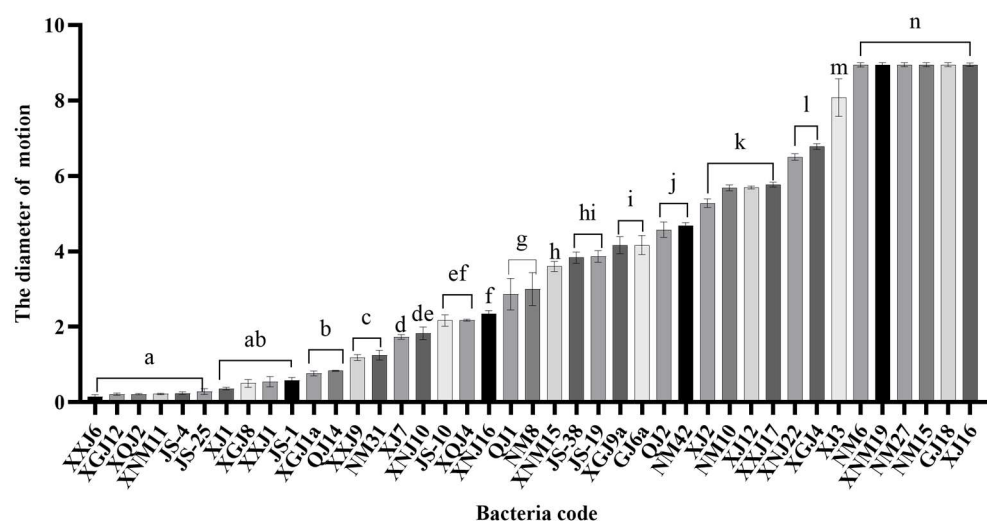


Figure 2. Analysis of bacterial diversity of seed-borne bacteria of *Achnatherum splendens*. (A) Describes the classification and proportion of bacteria contained in each region; (B) describes the bacteria contained in each region and the relationship between regions and bacteria; (C) Intuitively describes the common and unique genera among bacteria in each region; (D) describes the importance of species-borne bacteria in the host.

3.2. Mobility analysis

By inserting the strain into the swimming medium by acupuncture method, it was found that it could spread and grow on the surface of the plate, but its diffusion diameter was different. The results showed as Figure.3 that the moving diameter of strains XJ16, GJ18, NM15, XNM19, NM6, QJ16 and XJ3 on the surface of semi-solid agar plate was close to 9 cm. The diffusion surface of the strain was about to or had reached the inner edge of the petri dish, and its mobility was strong, accounting for 15.56%. There were 3 strains of bacteria with a moving diameter of 6-8cm, accounting for 6.67%, of which the diameter of XJ3 was 7.97cm ; there were 8 strains of bacteria with a moving diameter of 4-6 cm, accounting for 17.78%. There were 9 strains of bacteria with a moving diameter of 2-4 cm, accounting for 20%. There were 4 strains of bacteria with a moving diameter of 1-2 cm, accounting for 8.88%. There were 13 strains of bacteria with a moving diameter of less than 1 cm, accounting for 22.22%. There were 3 strains of bacteria without diffusion diameter, accounting for 6.67%. The results showed that most of the bacteria carried by *A.splendens* could diffuse on the surface of the swimming medium, indicating that the bacteria carried by *A.splendens* had certain mobility and could migrate under suitable conditions. Because of this, bacteria with excellent performance could play a role in seeds.



3.3. Analysis of biofilm formation ability

The biofilm formation ability was determined by crystal violet staining, ODC = 0.092. The results of biofilm formation are shown in the Figure.4. Strains XJ1, XJ2, XJ3, XNJ10, XNJ22, QJ1, XQJ4, GJ2, GJ6a, XGJ1a, XGJ4, XGJ8, XGJ12, NM8, XNM11, XNM15, JS-3, JS-25 and other 18 strains were strong biofilm-forming bacteria. The 15 strains of XJ7, XJ12, XXJ6, XXJ9, XXJ17, XNJ16, QJ16, GJ15, GJ18, XGJ9a, NM6, NM10, NM15, JS-1, JS-19 were medium biofilm forming bacteria. The 11 strains of XJ16, QJ2, QJ14, XQJ2, NM27, NM31, NM42, XNM19, JS-4, JS-10 and JS-38 were weak biofilm-forming bacteria. Strain XXJ1 is a non-biofilm-forming bacterium. Among them, the proportion of bacteria with strong, medium, weak and no biofilm formation ability was 45%, 37.5%, 27.5% and 2.5%, respectively.

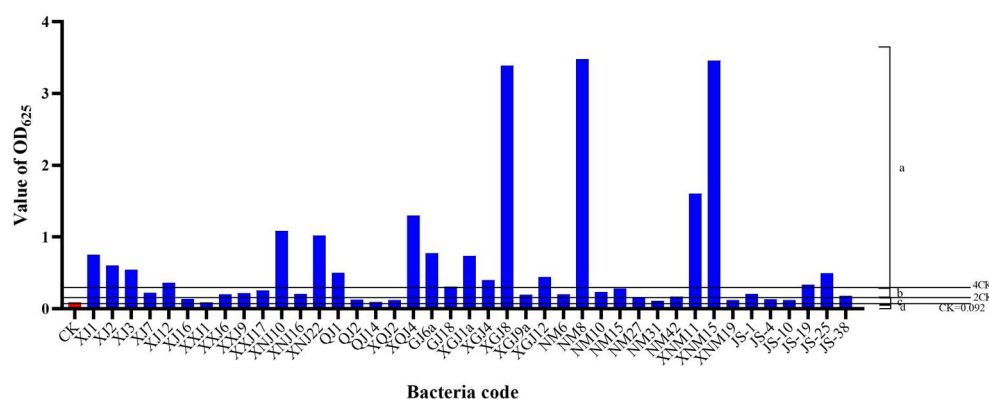


Figure 4. The Biofilm formation capacity of the *Achnatherum splendens* with seed-borne bacteria. Note: a is a strong biofilm formation ability; b is moderate biofilm formation ability; c is weak biofilm formation ability; d is no biofilm formation ability.

3.4. Antibiotic resistance analysis

The agar dilution method was used to determine the bacterial resistance of *A.splendens*. The results showed as Figure.5 that the bacteria of *A.splendens* were more resistant to sulfadiazine, sulfamethoxazole and other sulfonamides, and the resistance to ampicillin and erythromycin was also more intuitive. Through the determination of the minimum inhibitory concentration of 41 strains of *A.splendens* seed-borne bacteria, it was found that the MIC of sulfadiazine was 2560μg/mL, and the strains that could reach MIC were XJ1, XJ2, XJ3, XJ12, XXJ17, NM6, NM27, XNM19, XNJ10, XNJ22, QJ1, QJ2, XQJ4, XGJ4, JS-1, JS-19. The MIC of sulfamethoxazole was 1280μg/mL, and the strains that could reach MIC

were XXJ1, XXJ6, QJ14, GJ18, XGJ1a, JS-4, JS-38. The MIC of ampicillin was 1280µg/mL, only XXJ17 could reach MIC, the MIC of erythromycin was 1280µg/mL, only XJ1 could reach MIC. The MIC of kanamycin was up to 10 µg/mL. The bacteria that could reach MIC were XJ1, XJ2, XJ3, XJ12, XXJ17, NM27, XNM19, QJ2, QJ14, JS-4, JS-19. The MIC of rifampicin was up to 80µg/mL, and only XXJ17 could reach MIC. The MIC of gentamicin was up to 20µg/mL, and only QJ14 could reach MIC. The MIC of ceftazidime was up to 640µg/mL, and the bacteria that could reach MIC were NM10, XGJ1a and XGJ9a.

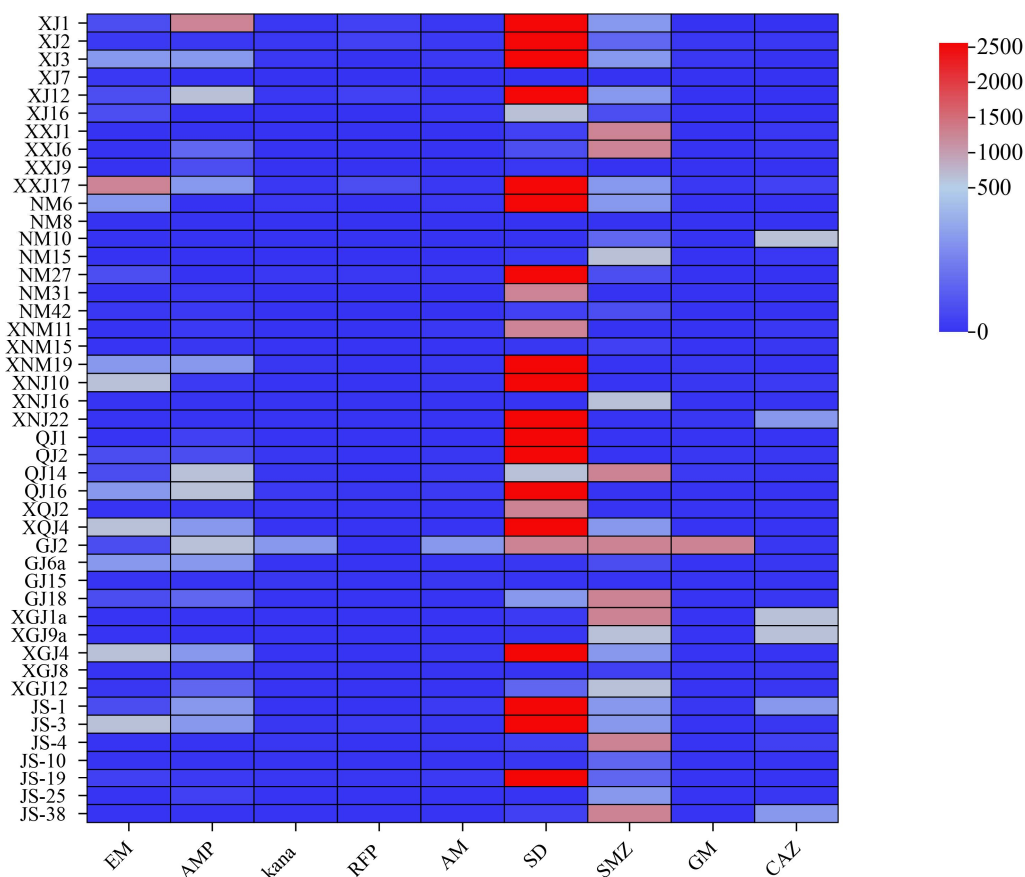


Figure 5. The antimicrobial resistance of the *Achnatherum splendens* with seed-borne bacteria.

3.5. Correlation analysis

Through the analysis of bacterial motility, biofilm formation ability and antibiotic resistance of *A.splendens*. As shown in Figure.6, the results showed that there was no correlation between bacterial motility and biofilm formation ability and antibiotic resistance. The biofilm formation ability was significantly correlated with the resistance of bacteria to aminoglycosides such as kanamycin, amikacin and gentamicin ($P < 0.01$), and there was no correlation between antibiotic resistance and motility and biofilm formation ability.

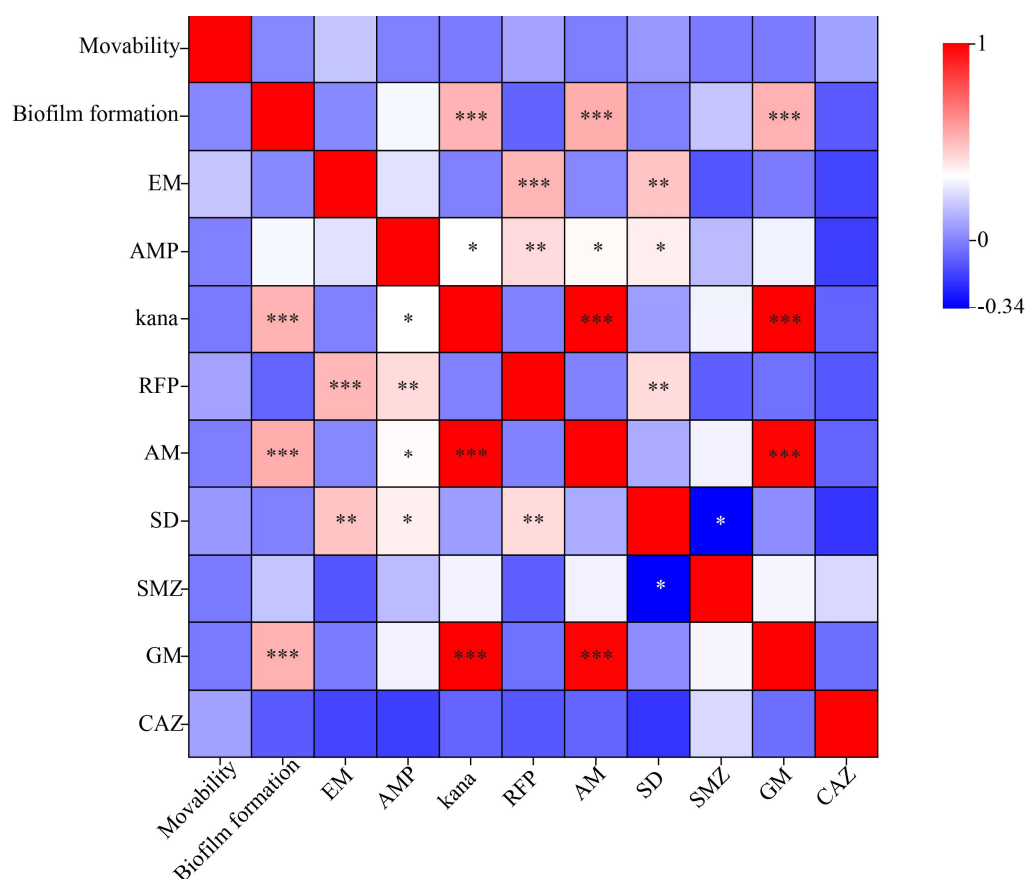


Figure 6. Correlation analysis of biological characteristics. Note: $|r| < 0.3$ is low correlation, $0.3-0.8$ is moderate correlation, $|r| > 0.8$ is highly correlated. * means $P < 0.05$, ** means $P < 0.01$, *** means $P < 0.001$, there is statistical significance.

4. Discussion

Seed is not only a production tool in agricultural production, but also a carrier of various microorganisms. Recent studies have shown that the vast majority of the planet's approximately 300,000 plant species contain endophytes [26]. Plant-associated bacterial communities are common in agroecosystems. Studies have shown that 131 genera of natural endophytic bacteria have been identified in the seeds of 25 common plants. Most of the endophytic bacteria in seeds belong to Proteobacteria, among which Enterobacteriaceae is the dominant endophytic bacteria in a variety of plant seeds [27,28]. In addition, Actinobacteriota, Firmicutes and Bacteroidetes are also found in plant seeds [29]. In the past two decades, many researchers have found that Gramineae and Leguminosae seeds carry bacteria, among which Proteobacteria, Actinobacteria and Firmicutes are the most abundant [30,31]. In the study of different seeds such as *Medicago sativa* [32], *Trifolium repens* L [28], *white clover* [33] etc., it was found that the microorganisms carried by the seeds were diverse. For the study of *Achnatherum inebrians* [34], *Elymus sibiricus* and *Elymus dahuricus* [33], *Avena sativa* L [35], etc., it was found that the dominant bacteria of most plants were *Bacillus*. In this study, 41 dominant bacteria were selected from 272 species-borne bacteria isolated from *A.splendens* in different regions for preliminary study. The analysis showed that the 41 strains belonged to 3 phyla and 14 genera, mainly Proteobacteria and Firmicutes, followed by Actinobacteria, which was consistent with previous research results. At the same time, through the molecular identification of the 41 strains in this experiment, it was found that the bacteria carried by the seeds in different regions were significantly different in species and quantity, which may be related to the storage life of the seeds and the ecological environment of the year. *Bacillus* was the dominant genus among the 41 strains, which was detected in the six regions of Xinjiang Uygur Autonomous

Region, Inner Mongolia Autonomous Region, Ningxia Hui Autonomous Region, Qinghai Province, Gansu Province and Jiangsu Province. This conclusion is consistent with the results of previous reports Truyens [28], indicating that the microorganisms in the *A.splendens* seed belt also have diversity. The phenotype of most plants is the product of synergistic and highly co-regulated expression of plant genes and microbial genes. The close connection between the seed-borne bacteria and the seed causes a specific co-evolutionary effect, and the bacteria that mutually benefit from the seed also continue due to the vertical transmission of the seed-borne bacteria [36]. Studies have shown that the bacteria carried by seeds can not only be transmitted across generations by vertical transmission, but also can be transmitted through vascular bundles from parent plants into seeds [37]. This mode of transmission of bacteria ensures that they can exist in the natural environment for a long time without being eliminated. In addition, studies have found that under the influence of biotic and abiotic stresses, the presence of seed-borne bacteria can improve the viability, germination and resilience of seeds, improve the growth and development of plants, and thus enhance the resistance of plants to adverse effects [38,39]. In this paper, through the study of bacterial resistance of *A.splendens*, it was found that the bacteria carried by *A.splendens* seeds had different degrees of resistance to different kinds of antibiotics. The presence of bacteria in the seed band could alleviate the stress of seeds in the antibiotic environment to a certain extent, which laid a foundation for the germination and growth of seeds in the antibiotic environment.

Bacteria carried by different seeds have different mechanisms of action on plants. According to the reports of Xu [4] and Walitang [40] et al, seed-borne bacteria can directly or indirectly promote the growth of host seeds and seedlings by producing hydrolases, regulating hormones and other abilities and their own growth-promoting characteristics such as nitrogen fixation, phosphorus solubilization and siderophore production. Compared with the studies on the growth-promoting functions of seed-borne bacteria and plant growth-promoting rhizobacteria (PGPR), such as nitrogen fixation, phosphorus solubilization and hydrolase production, there are few reports on the characteristics of seed-borne bacteria [41]. Seed-borne bacteria can not only affect the germination and growth of seeds and even plants by their unique functions, but also affect the host due to their potential uses. It has been reported that there is a great correlation between undiscovered microbial diversity and potential uses. Smith et al [26] found that the newly discovered bacteria can produce bioactive substances through the investigation of endophytic bacteria in tropical plants, which has potential significance in the fields of medicine, agriculture and industry. In 2013, Shi et al [34] studied the insecticidal activity of endophytic bacteria from *A.splendens*, and found that the isolates had good insecticidal activity against cotton aphids, which could be used to develop new natural insecticides. In this study, the characteristics of motility, biofilm formation and antibiotic resistance of 41 strains isolated from the seeds of *A.splendens* were qualitatively or quantitatively studied. It was found that the motility, biofilm formation and antibiotic resistance of each bacteria were different. In the future, more in-depth studies will be carried out on strains with excellent characteristics, aiming to provide high-quality bacterial sources for plant disease prevention, antibiotic pollution control, seed coating, new species breeding, and microbial fertilizer development and utilization.

Motility is one of the important characteristics of bacterial physiology and plays a vital role in microbial ecology. The motility of bacteria enables them to establish contact with animals and plants. If the nutritional conditions of the parasitic environment are good, the bacterial motility will be stronger, and the colonization area covered by their migration will be larger [42]. In addition, exercise can improve the efficiency of nutrient acquisition and avoid toxicity [43]. It has been reported that motility enables bacteria to migrate to favorable environments and stay away from the effects of toxic substances and their predators [44,45]. Samad et al [42] found that although the motility of *Staphylococcus* bacteria is limited, it can still migrate with other mobile bacteria as carriers, reach and survive and reproduce in its preferred niche, indicating that non-mobile bacteria can

migrate under the influence of mobile bacteria. In addition, studies have shown that flagella-mediated bacteria not only have strong motility, but also participate in the formation of bacterial biofilms due to the physical adsorption of flagella. As early as 1987, Lawrence et al [46] demonstrated that flagella, motility, and chemotaxis play an important role in the formation of bacterial biofilms; bacterial motility makes it spread to the appropriate niche to adhere and proliferate, thus promoting the formation of biofilm [47]. In this study, the motility of *A.splendens* seed-borne bacteria on the surface of swimming medium containing 0.3% agar was investigated. It was found that six strains of NM6, XNM19, NM27, NM15, GJ18 and XJ16 had strong motility. They belonged to *Siccibacter* sp., *Enterobacter* sp., *Atlantibacter* sp. and *Lelliottia* sp., and their diffusion area almost covered the whole surface of the medium. It may be that the nutritional conditions of the swimming medium were suitable for the movement and diffusion of these six strains. It may be that bacterial motility plays a role in promoting the formation of biofilm. In addition, studies have shown that biofilms can make bacteria better adapt to the living environment, especially strains with poor antibiotic resistance, and even if the biofilm formation ability of bacteria with high antibiotic resistance is weak, it can still protect strains from persecution [48]. However, in 1998, George A et al [49] found that the adhesion ability of flagellar-defective mutants on PVC was very poor when studying the formation mechanism of *Pseudomonas aeruginosa* biofilm, indicating that flagella was indispensable in the process of biofilm formation, indicating that bacterial motility was positively correlated with its biofilm formation ability. Their research showed that there was a positive correlation between bacterial motility, biofilm formation ability and antibiotic resistance. It has also been reported that bacterial motility is negatively correlated with biofilm formation. Kim (2008) et al [50] explored the effect of flagellar mutations on the biofilm formation of *Y.enterocolitica*. It was found that for the virus, if the gene that is a positive regulator of flagellar expression mutates, the biofilm formation will decrease, while the gene mutation that encodes the negative regulator of flagellar expression will make the biofilm formation stronger. In this study, the resistance of *A.splendens* to sulfonamide antibiotics was stronger than that of other antibiotics. Among them, the number of *Bacillus* was the most resistant, followed by the bacteria of *Pseudomonas* sp., *Enterobacter* sp. and *Siccibacter* sp.. *Pseudomonas* sp. and *Bacillus* can enhance the ability of hosts to resist pathogens by producing bioactive substances [51]; there was a significant positive correlation between biofilm formation ability and aminoglycoside antibiotic resistance (Fig.6). The reason may be that the presence of biofilms can provide an advantage for bacteria to better exist in antibiotic-contaminated environments. Their co-occurrence and correlation indicate that they may have a common regulatory mechanism, which may also be the reason why bacteria initiate biofilm gene expression. There was no significant correlation between motility and biofilm formation ability, and there was no significant correlation with bacterial resistance. The reason may be that the number of variables is too small to make the data results deviate from the expected, or the number of repeated values of a single strain is too small to analyze it separately and the relationship between the two variables is not observed.

The bacterial diversity of *A.splendens* from different regions is different, but most strains have certain motility and biofilm formation ability, and can produce resistance to one or more antibiotics. The strains with excellent biological characteristics were screened out, which provided fungal resources for improving the tolerance of plants under different stresses.

5. Conclusions

(1) The culturable bacteria in the seed zone of *Achnatherum splendens* had species diversity. The isolated bacteria were identified to belong to 3 phyla and 14 genera, among which *Bacillus* was the dominant genus in the seed zone of *Achnatherum splendens* in different regions.

(2) Among the 41 strains isolated from *Achnatherum splendens*, 40 strains were motile and had a moving diameter of > 2.00 cm.

(3) Among the 41 strains, 16 strains were strong biofilm-forming bacteria, 14 strains were medium biofilm-forming bacteria, 10 strains were weak biofilm-forming bacteria, and 1 strain had no biofilm-forming ability.

(4) The bacteria carried by *Achnatherum splendens* had certain resistance to macrolides (erythromycin), cephalosporins (ceftazidime), penicillins (ampicillin), aminoglycosides (kanamycin, amikacin, gentamicin), sulfonamides (sulfadiazine, sulfamethoxazole), among which the resistance to sulfonamides was the strongest.

Author Contributions: Conceptualization: Zhenfen Zhang, Jie Yang. Data curation: Xuan Hou. Formal analysis: Jinjing Xie, Haiyan Chen, Jie Yang. Methodology: Zhenfen Zhang, Jinjing Xie, Jie Yang. Writing – original draft: Jie Yang. Writing – review & editing: Zhenfen Zhang.

Funding: This research was funded by the National Natural Science Foundation of China (32060396), Natural Science Foundation of Gansu Province, China (20JR10RA562) and Key Laboratory of Grassland Ecosystem of the Ministry of Education (KLGE202203).

Data Availability Statement: Data availability Data will be made available on request.

Conflicts of Interest: The authors declare no conflict of interest.

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