Full Length Article

Distribution of Culturable Myxobacteria in Central Inner Mongolia and their Activity against *Phytophthora infestans*

Zhi Hua Wu[1,2,3], Xue Han Wang[1], Qiang Ma[1], Yi Xiu Ding[1], Pu Yu Zhao[1] and Hui Rong Liu[1*]

[1]*College of Life Sciences, Inner Mongolia Agricultural University, Hohhot 010010, P. R. China*
[2]*Baotou Teachers' College, Baotou 014030, P. R. China*
[3]*The Second Affiliated Hospital of Baotou Medical College, Inner Mongolia University of Science and Technology, Baotou 014030, P. R. China*
[*]*For correspondence: huirong_liu@imau.edu.cn; wuzhihuaqueen@163.com*

Abstract

Myxobacteria can produce rich and varied biological active substances against bacteria, fungi and viruses, which have great development and research value in medicine and agriculture. In this study, the diversity of culturable myxobacteria in central Inner Mongolia in China was studied and the effects of soil utilization mode, soil type and environmental parameters on the distribution of myxobacteria in this region were analyzed. Furthermore, the activities of myxobacteria against potato late blight pathogen were tested. The results showed that *Myxococcus*, *Corallococcus*, *Pyxidicoccus*, *Cystobacter*, *Archangium* and *Mellittangium* were the dominant genera of myxobacteria in this region. Soil utilization mode and soil types have obvious influence on the distribution of myxobacteria. The populations of myxobacteria were abundant in grassland and cultivated land samples, but few in woodland and unused land samples. The diversity of myxobacteria in the soil samples from fuvo-aquic soils, grey-cinnamon soils, castanozems, and bog soils was relatively rich, while the richness of myxobacteria in aeolian soils, solonetzs, skeletol soils and castano-cinnamon soils was poor. There was no significant correlation between myxobacteria distribution and soil environmental parameters (including the water content, pH value, content of organic matter, content of available phosphorus, content of hydrolytic nitrogen and content of available potassium). Most of the myxobacterial strains isolated in this area (83%) showed the activity against *P. infestans*, among which the proportion of the disease-resistant strains belonging to *Myxococcus* and *Corallococcus* was high, the proportion of the strains belonging to *Cystobacter* and *Mellittangium* in the medium, and the proportion of the strains belonging to *Pyxidicoccus* and *Archangium* low. The completion of this work will enrich the myxobacteria resource bank in Inner Mongolia and lay a foundation for the further study on myxobacteria and the development of biological pesticide against potato late blight. © 2021 Friends Science Publishers

Introduction

Myxobacteria, a group of gram-negative bacteria (Kim *et al.* 2016), with social behavior, complex multicellular behavior and morphogenesis (Sun *et al.* 2016), are considered to be higher prokaryotes and known for their predatory lifestyle (Livingstone *et al.* 2018). A typical feature of myxobacteria is that the cells move by gliding or creeping, often presenting a thin, translucent film on a solid plate, forming many concentric radial lines (Reichenbach 2001). The vegetative cells are rod-shaped, and the cell membrane is elastic (Zhang 2005). They are propagated in a binary fission manner and grow slowly. Under a variety of adverse conditions, such as nutrient deficiency, myxobacteria form visible fruiting bodies through a complex multicellular behavior. Fruiting bodies are usually of various shapes and sizes, with bright colors such as bright yellow, red, orange,

brown, or black (Anqel *et al.* 2017). Myxobacteria are good natural drug screening resources and have become a large group of microbial groups with great potential for research and development (Diez *et al.* 2012). Bioactive substances produced by myxobacteria have antibacterial, antifungal, anticancer, thrombolytic, antitumor, hypoglycemic, hypotensive activities and *etc.* (Zhang *et al.* 2011; Okanya *et al.* 2012). Myxobacteria have been actively screened for natural products for several decades, with more than 100 core structures and over 500 derivatives now having been published (Herrmann *et al.* 2017). Moreover, myxobacteria have great potential for biosynthesis and 50% ~ 100% of myxobacteria can synthesize secondary metabolites with biological activity (Wenzel and Müller 2009). In prokaryotes, myxobacteria rank just behind Actinomyces and Bacillus in the number of biologically active substances (Wang and Ma 2010). However, the ratio of positive

bacteria with bacteriostatic activity is higher than that of Actinomycetes. Because myxobacteria can produce many bioactive substances with novel structure and unique mechanism of action, they have become the "micro-factory" of secondary metabolites with biological activity (Weissman and Müller 2009). Therefore, more and more attention has been paid to the research of myxobacteria.

Potato is the fourth largest food crop in the world after rice, wheat, and corn. The most serious disease of potato is potato late bright, which is caused by *Phytophthora infestans* (Cui 2018). *P. infestans* is a devastating pathogen that causes death of potato stems and tuber mainly by invading the leaves, stems, and tubers of the plant (Haas *et al.* 2009). Due to the limitation of cognitive level on some scientific problems such as the pathogenic mechanism and the reproductive regulation mechanism of *P. infestans*, there is still no way to effectively prevent and control potato late blight, which has seriously hindered the production and industrialization of potato (Ren *et al.* 2016).

Baotou, Hohhot, Wulanchabu and Xilinguole are distributed in the central part of Inner Mongolia with special geographical conditions (Xu *et al.* 2001; Chen and Gong 2005). In this area, the terrain is dominated by plateaus and there are many types of soils. The precipitation gradually decreases from east to west under the influence of the topography and the distance from the ocean. The area has a typical mid-temperate zone monsoon climate with obvious climate change in the four seasons and large annual and daily temperature differences. Under these special geographical conditions, there are bound to be rich and diverse resources of myxobacteria. However, so far, there have been few reports on myxobacteria resources and their biological activities in central region of Inner Mongolia. In this study, myxobacteria were isolated from 139 of soil samples from the central region of Inner Mongolia and the correlation of diversity of culturable myxobacteria with environmental factors in this region was analyzed. Furthermore, the activities of the myxobacterial strains against *P. infestans* were determined.

Materials and Methods

Soil sample collection

Soil samples were collected from the four regions in the central Inner Mongolia Autonomous Region, Baotou, Hohhot, Wulanchabu and Xilinguole. A total of 139 soil samples, including 15 soil types and four utilization modes, were collected from the upper layer of the soil (5–20 cm depth) (Table 1).

Determination of soil parameters

The moisture content of the samples was determined by drying method (Ye and Zhang 1984). The pH value was determined according to the Ecology Common Experimental Research Methods and Techniques (Zhang 2006). $NaHCO_3$ leaching - molybdenum antimony and colorimetric method was used to determine the available phosphorus of soil samples (Xing *et al.* 2011). Soil available potassium was determined by tetraphenylborate nephelometry (Li *et al.* 1982). The alkaline hydrolysis diffusion method was used to determine the content of hydrolyzable nitrogen (Li 2010) and the potassium dichromate-volumetric method was used to determine the organic matter contents in the soils (Ji 2005).

Isolation of myxobacteria

Pretreatment of soil samples: The soil samples that had been naturally air-dried and screened at 60 mesh were baked at 58°C for 1 h to remove the poor heat resistance of miscellaneous bacteria. Approximately 30 g of the soil samples were placed in a 90 mm Petri dish, soaked overnight at room temperature with cycloheximide solution at a final concentration of 100 μg mL^{-1} to remove a portion of the mold and yeast.

Rabbit dung pellets inducing method: The pretreated soil samples were poured into the ST21CX solid culture dish and paved. Three sterilized rabbit dung pellets were embedded in half and the culture dishes were incubated at 30°C for 6 days. Continue to observe the formation of myxobacteria fruiting bodies (Liu *et al.* 2011).

Escherichia coli **inducing method:** *Escherichia coli* DH-5α bacteria solution (20 μL) was added to 200 mL of LB liquid medium (Wu 2018) and the cells were shaken overnight. The next day, *Escherichia coli* DH-5α bacteria solution was centrifuged and the cell precipitate was rowed three uniform and thick parallel lines on WCX medium. Pretreated soil samples with soybean size were picked and placed on the end of three parallel lines, and then the culture dishes were incubated at 30°C (Sood *et al.* 2014).

Filter paper inducing method: A sterilized filter paper was placed on the ST21CX medium as the only carbon source and energy source. Pretreated soil samples with soybean size were placed in different positions on the filter paper, and the culture dishes were incubated at 30°C (Gaspari *et al.* 2005).

Purification of myxobacteria

Directly pick the fruiting body: The myxobacteria were identified and the border of characteristic colonies or the head of fruiting bodies was picked directly on fresh VY/2 medium with a sterile inoculation needle to remove a portion of the larger and immobile bacteria (Guo *et al.* 2007). Fruiting body of myxobacteria on the filter paper or the corroded filter paper was transferred to fresh ST21CX medium with sterilized filter paper by a sterile toothpick at 30°C constant temperature culture. This method requires repeated transfer, until there is no other bacterial growth in the medium.

Table 1: Details of the soil samples

Different types of soil samples	Cultivated land	Grassland	Woodland	Unused land	Total
Fluvo-aquic soil	5	8		2	15
Grey-cinnamon	8	10	2	2	22
Castanozems	13	19	1	1	34
Litho soil	-	2	-	1	3
Saline	3	3	1	1	8
Alluvial soil	1	1	1	-	3
Brown pedocals	3	9	1	-	13
Gray forest soil	2	3	2	-	7
Bog soil	2	3	1	-	6
Aeolian soil	1	2	1	-	4
Meadow soil	5	6	-	-	11
Solonetzs	-	1	-	-	1
Chernozem	3	4	-	-	7
Skeletol soil	-	1	-	-	1
Castano-cinnamon soil	2	2	-	-	4
Total	48	74	10	7	139

-: The number of samples was 0

Secondary inducing method: Bacteriolytic myxobacteria that cannot be directly purified were transferred into fresh WCX medium with three uniform and thick *E. coli* lines and incubated at 30°C. Then the fruiting bodies were picked from the edges of the expanded colonies to the VY/2 medium to further culture.

Examination and preservation of myxobacteria

The fruiting bodies of the strains were incubated in the CAS liquid medium and shaken culture for 36 h, then to observe whether there was turbidity in the CAS liquid medium. As the myxobacteria grow slowly in nutrient-rich medium, while the other miscellaneous bacteria grow fast relatively, so the clarification of CAS liquid culture medium can be considered that the strain has been pure. The purified myxobacteria strains were stored in 20% sterilized glycerol at -80°C for a long time.

Identification of myxobacteria

Morphological observation: The Nikon SMZ745 stereomicroscope and Sony digital camera were used to observe and photograph the colony morphology and fruiting bodies of myxobacteria on the VY/2 medium and ST21CX medium.

Molecular identification: The total chromosomal DNA was extracted as described by Zhou *et al.* (2004). The first set of primers (forward 27F: 5′-AGAGTTTGATCCTGGCTCAG-3′, and reverse 1495R: 5′-CTACGGCTACCTTGTTACGA-3′) was designed to amplify the partial 16S rDNA of myxobacteria (Zhang *et al.* 2010). The reaction started with denaturation at 94°C for 30 s, followed by primer annealing at 55°C for 45 s and primer extension at 72°C for 90 s. After 30 cycles of reaction, an extension step was followed at 72°C for 5 min. The PCR products were sent to Beijing Liuhe Huada Gene Technology Co., Ltd. for sequencing. The 16S rDNA sequences of the tested strains were compared with the known sequences in the GenBank database by BLAST in NCBI.

Activity analysis of myxobacteria against *P. infestans*

The activity of the purified myxobacterial strains against *P. infestans* was detected by the plate confrontation culture method (Li *et al.* 2011). The phytopathogenic oomycete *P. infestans* was cultured on a rye medium in a 9-cm dish at 18°C for 3 days in the dark until the colony grew to approximately 1 cm in diameter. Agar block of purified strains was placed 1 cm away from the front of the colony. After incubating at 18°C for 8 days, the distance between the edge of the colony and the agar block (control: 0 mm) was measured. The resistance of each strain to *P. infestans* was tested three times.

Data processing

The preliminary processing and mapping of the data was done using Excel 2010 and the correlation analysis was done using SPSS V. 24.0.

Results

The determination of soil environmental parameters

The parameters of 139 soil samples were measured and compared with the soil nutrient grading standards of the second national soil survey in China (Table 2). The results showed that about 38.85% of the soil samples were alkaline. 49.64% of the soil samples were neutral and 11.51% of the soil samples were acidic. The water content of 87.77% soil samples was lower than 16%, which was in the state of light drought to severe drought. For the content of organic matter, 56.12% of soil samples were at a low to very low level. The content of available phosphorus was distributed evenly, with a certain proportion in each grade. Of which, 43.17% of soil samples were at medium level, 36.70% at low to extremely low level and 20.15% at high to very high level. As for the content of available potassium, 31.65% of soil samples were at medium level and 46.76% at low to extremely low level.

Table 2: Nutrient grade distribution of soil samples from the central region of Inner Mongolia

Environmental parameter levels of soil samples	Percentage of environmental parameters of soil samples at different levels（%）					
	Water content	pH value	Content of organic matter	Content of available phosphorus	Content of hydrolyzed nitrogen	Content of available potassium
I		3.60	10.07	5.76	5.76	6.47
II	7.19	35.25	10.79	14.39	3.60	15.11
III	5.04	49.64	23.02	43.17	11.51	31.65
IV	15.83	10.07	37.41	28.06	23.74	28.06
V	27.34	1.44	18.71	2.88	31.56	10.79
VI	44.60	0	0	5.76	23.74	7.91

I: Strong alkaline or very rich. II: Slightly alkaline or rich. III: Neutral or suitable. IV: Slightly acidic or low. V: Highly acidic or very low. VI: Extremely acidic or extremely low

Table 3: Number of myxobacterial strains isolated and purified by different methods

| Methods of isolation	No. of isolated strains	No. of purified strains	Purification rate of strains (r/%)
Rabbit dung pellets inducing method	158	153	96.83
Escherichia coli inducing method	245	231	94.29
Filter paper inducing method	283	0	0
Total number	686	384	55.98

However, the content of hydrolyzed nitrogen was generally in a state of deficiency, with 79.04% of soil samples at a low to extremely low level. These results indicated that soil fertility was generally poor in central Inner Mongolia.

Isolation and identification of myxobacteria

The myxobacteria were isolated and identified from 139 soil samples collected from the central region of Inner Mongolia. A total of 686 strains with morphological characteristics of myxobacteria were isolated and 384 strains of which were purified and identified, with an average purification rate of 55.98% (Table 3). Among them, the number of strains isolated by filter paper inducing method was the most, while the number of strains isolated by rabbit dung pellets inducing method was the least. However, the strains isolated by rabbit dung pellets inducing method were the easiest to be purified, and the purification rate of this method was the highest. The purification rate of filter paper inducing method was the lowest.

According to the classification standards of Bergey's Manual of Systemaic Bacteriology (Boone and Castenholz 2004), from the characteristics of the color and morphology of colonies and fruiting bodies of the purified strains and their gliding movement on the plate, these strains can be preliminarily classified into six genera, including *Myxococcus*, *Corallococcus*, *Pyxidicoccus*, *Cystobacter*, *Archangium* and *Melittangium* (Fig. 1 and 2).

The purified 384 strains were identified further by 16S rRNA gene sequences. The results showed that the similarities between the 16S rRNA gene sequences of all strains and the sequences reported in the GenBank database were more than 97%. Phylogenetic tree analysis with Neighbor-Joining Tree Algorithm showed that all strains could be divided into 2 large branches and 4 small branches. *Myxococcus*、*Corallococcus* and *Pyxidicoccus* were on the
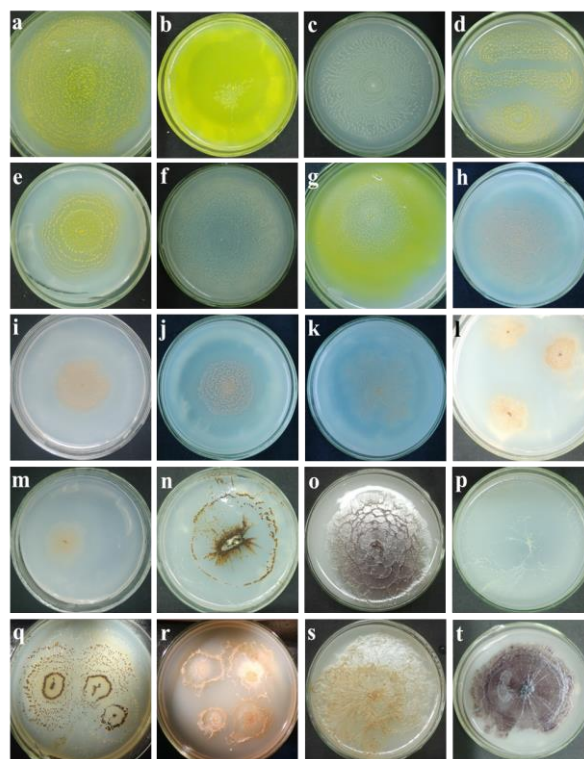
Fig. 1: Colony morphology of myxobacteria isolated by the rabbit dung pellets inducing method and *E. coli* inducing method
(**a**) *Myxococcus fulvus* (**b**) *Myxococcus virescens* (**c**) *Myxococcus coralloides* (**d**) *Myxococcus xanthus* (**e**) *Myxococcus favescens* (**f**) *Myxococcus stipitatus* (**g**) *Myxococcus macrosporus* (**h**) *Corallococcus coralloides* (**i**) *Corallococcus exiguus* (**j**) *Corallococcus macrosporus* (**k**) *Corallococcus* spp. (**l**) *Pyxidicoccus fallax* (**m**) *Pyxidicoccus* spp. (**n**) *Cystobacter badius* (**o**) *Cystobacter violaceus* (**p-q**) *Cystobacter* spp. (**r**) *Archangium gephyra* (**s**) *Archangium* spp. (**t**) *Melittangium* spp.

same large branch, belonging to Myxococcaceae. The other large branch included *Archangium*, *Cystobacter* and *Melittangium*, all belonging to Cystobacteraceae. According to the morphological classification of myxobacteria, both Myxococcaceae and Cystobacteraceae belonged to the Cystobacterineae (Fig. 3).

Through morphological observation and 16S rRNA gene sequences analysis, all of 348 strains belonged to Cystobacterineae, including 278 strains of Myxococcaceae and 70 strains of Cystobacteraceae. These strains were classified into six genera, including *Myxococcus*, *Corallococcus*, *Pyxidicoccus*, *Cystobacter*, *Archangium* and
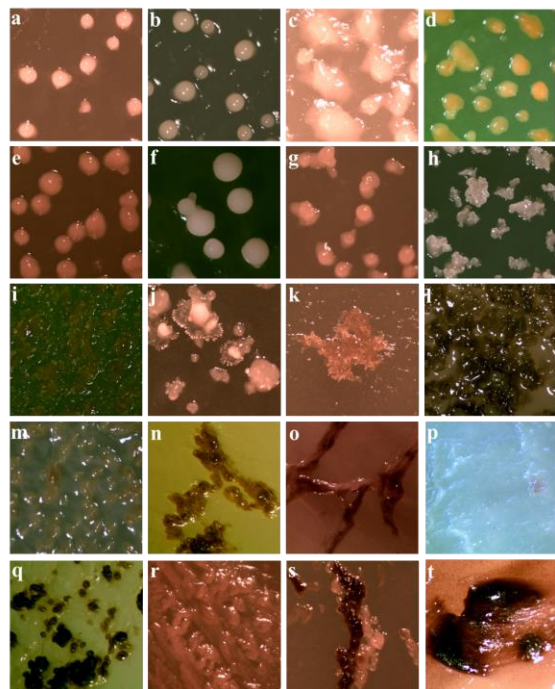
Fig. 2: Fruiting body morphology of myxobacteria isolated from rabbit dung pellets inducing method and *E. coli* inducing method (30×)

(**a**) *Myxococcus fulvus,* (**b**) *Myxococcus virescens,* (**c**) *Myxococcus coralloides,* (**d**) *Myxococcus xanthus,* (**e**) *Myxococcus favescens,* (**f**) *Myxococcus stipitatus,* (**g**) *Myxococcus macrosporus,* (**h**) *Corallococcus coralloides,* (**i**) *Corallococcus exiguous,* (**j**) *Corallococcus macrosporus,* (**k**) *Corallococcus* spp., (**l**) *Pyxidicoccus fallax,* (**m**) *Pyxidicoccus* spp., (**n**) *Cystobacter badius,* (**o**) *Cystobacter violaceus,* (**p-q**) *Cystobacter* spp., (**r**) *Archangium gephyra,* (**s**) *Archangium* spp. and (**t**) *Melittangium* spp.

Melittangium. Among them, 193 strains belonged to *Myxococcus* (*Myxococcus fulvus* 67 strains, *Myxococcus virescens* 41 strains, *Myxococcus coralloides* 6 strains, *Myxococcus xanthus* 45 strains, *Myxococcus favescens* 19 strains, *Myxococcus stipitatus* 10 strains, *Myxococcus macrosporus* 5 strains). 76 strains belonged to *Corallococcus* (*Corallococcus coralloides* 39 strains, *Corallococcus exiguous* 27 strains, *Corallococcus macrosporus* 8 strains, *Corallococcus* spp. 2 strains). 9 strains belonged to *Pyxidicoccus* (*Pyxidicoccus fallax* 8 strains, *Pyxidicoccus* spp. 1 strain).45 strains belonged to *Cystobacter* (*Cystobacter violaceus* 25 strains, *Cystobacter badius* 16 strains, *Cystobacter* spp. 4 strains).10 strains belonged to *Archangium* (*Archangium gephyra* 8 strains, *Archangium* spp. 2 strains). 15 strains belonged to *Mellittangium* (*Mellittangium* spp. 15 strains).

Distribution of myxobacteria in soil samples of different utilization types

It could be clearly seen from Table 4 that the average number of the myxobacterial strains isolated and purified from cultivated land soil samples was the highest, repectively 5.02 and 2.69, followed by that of grassland and woodland soil samples. The average number of the myxobacteria strains isolated and purified from the unused land soil samples was the lowest.

The myxobacteria strains belonging to Myxococcaceae and Cystobacteraceae were all obtained from soil samples of four different utilization types. Of which, six genara of myxobacteria, including *Myxococcus*, *Corallococcus*, *Pyxidicoccus*, *Cystobacter*, *Archangium* and *Melittangium*, were purified from the soil samples of cultivated land, grassland and woodland, while only three genera of myxobacteria were purified from the samples of unused land, including *Myxococcus*, *Corallococcus* and *Cystobacter*. The average number of the strains belonging to *Myxococcus*, *Cystobacter* and *Corallococcus* was the highest in the cultivated land soil samples, but the lowest in the unused land soil samples. The strains belonging to *Pyxidicoccus*, *Archangium* and *Mellittangium* were all not purified from the unused land soil samples. The average number of the strains belonging to *Mellittangium* was also the highest in the cultivated land soil samples, while the average number of the strains belonging to *Pyxidicoccus* and *Archangium* was the highest in the woodland soil samples. In soil samples of four utilization types, the average number of the strains belonging to *Myxococcus* ranked first, followed by the average number of the strains belonging to Corallococcus, and the average number of the strains belonging to Cystobacter ranked third. The average number of the strains belonging to *Pyxidicoccus*, *Archangium*, and *Mellittangium* was significantly lower than the average number of the strains belonging to the other three genera, and in the unused land soil samples, the three genera were not even isolated.

Distribution of myxobacteria in soil samples of different types

It could be clearly seen from Table 5 that the average number of strains isolated from solonetzs and alluvial soils was the highest and the average number of strains purified from solonetzs was the highest, while the average number of strains isolated from chernozems was the lowest and the average number of strains purified from gray-cinnamon soils was the lowest.

Myxococcaceae and Cystobacteraceae were all purified from 15 different soil types, with differences in the varieties and quantities of the strains. Only three genera of myxobacteria were purified from aeolian soils, solonetzs, skeletol soils and castano-cinnamon soils. Four genera of myxobacteria were purified from litho soils, alluvial soils and gray forest soils. Five genera of myxobacteria were purified from saline soils, brown pedocals, meadow soils and chernozems. Six genera of myxobacteria were purified from fluvo-aquic soils, grey-cinnamon soils, castanozems, and bog soils, showing a rich diversity of myxobacteria. Of the 6 genera of myxobacteria isolated, only *Myxococcus* was isolated from all soil types. The average number of the
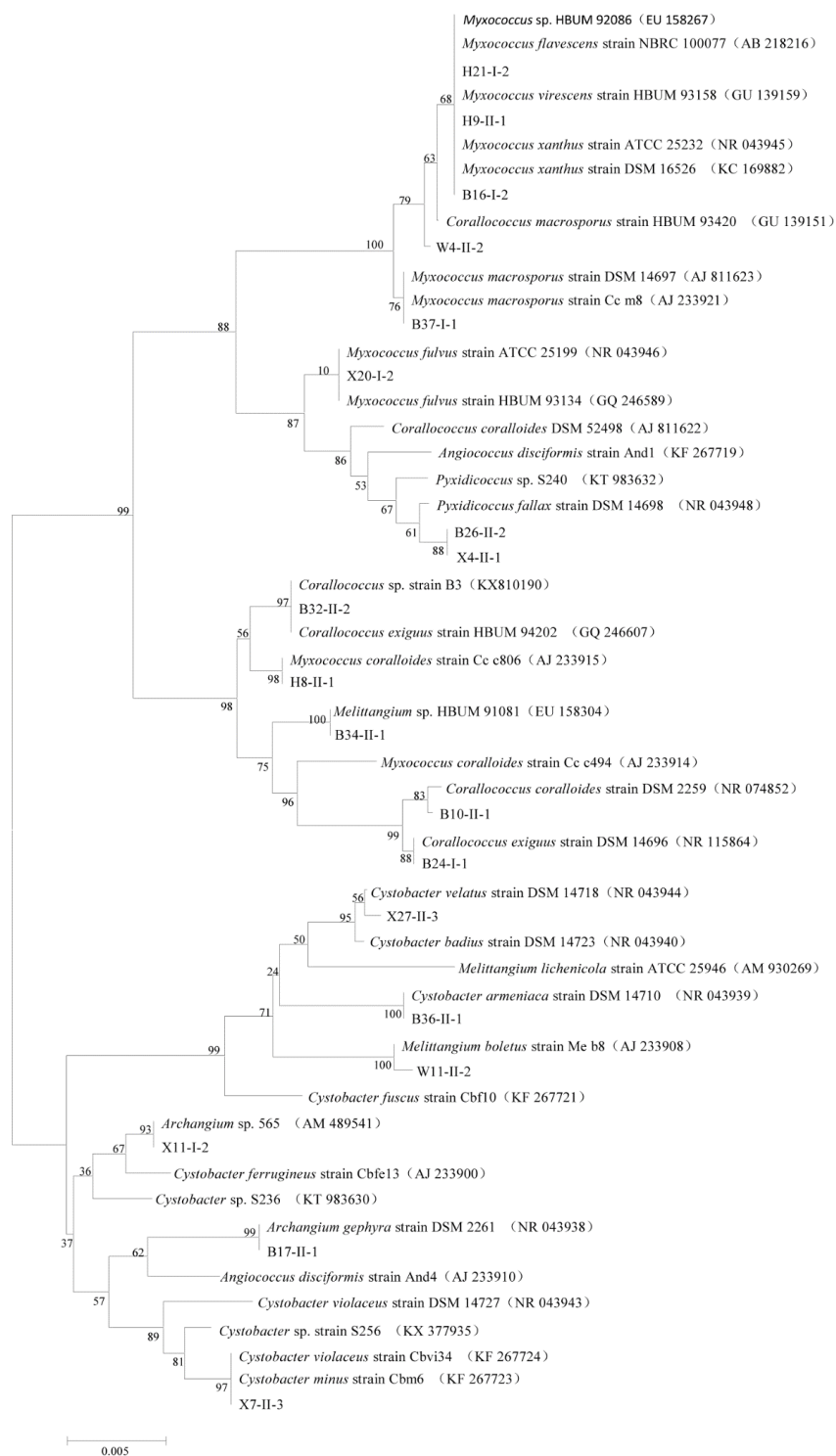
Fig. 3: Phylogenetic tree for the myxobacterial strains based on 16S rRNA gene sequences

strains belonging to *Myxococcus* isolated from alluvial soils and solonetzs was the highest, while the average number of the strains belonging to *Myxococcus* isolated from chernozems was the lowest. The strains belonging to

Corallococcus were isolated from all soil types except skeletol soils, in which the average number of strains belonging to *Corallococcus* was the highest in solonetzs and the lowest in bog soils. The strains belonging to *Cystobacter*

Table 4: Distribution of myxobacteria in soil samples of different utilization types

Utilization types of soil samples	Total number of the isolated strains	Average number of the isolated strains/soil sample	Total number of the purified strains	Average number of the purified strains/soil sample	*Myxococcus*		*Corallococcus*		*Pyxidicoccus*		*Cystobacter*		*Archangium*		*Mellittangium*	
					Total number	Average number	Total number	Average number	Total number	Average number	Total number	Average number	Total number	Average number	Total number	Average number
Cultivated land	241	5.02	129	2.69	70	1.46	30	0.63	3	0.06	16	0.33	4	0.08	6	0.13
Grassland	338	4.57	185	2.50	106	1.43	37	0.50	5	0.07	24	0.32	5	0.07	8	0.11
Woodland	45	4.50	23	2.30	11	1.10	6	0.60	1	0.10	3	0.30	1	0.10	1	0.10
Unused land	26	3.71	11	1.57	5	0.71	3	0.43	0	0	2	0.29	0	0	0	0
Total	650	4.68	348	2.50	193	1.39	76	0.55	9	0.06	45	0.32	10	0.07	15	0.11

Table 5: Distribution of myxobacteria in different types of soil samples

Different types of soil samples	Total number of the isolated strains	Average number of the isolated strains /soil sample	Total number of the purified strains	Average number of the purified strains / soil sample	*Myxococcus*		*Corallococcus*		*Pyxidicoccus*		*Cystobacter*		*Archangium*		*Mellittangium*	
					Total number	Average number	Total number	Average number	Total number	Average number	Total number	Average number	Total number	Average number	Total number	Average number
Fluvo-aquic soil	72	4.80	38	2.53	21	1.40	8	0.53	1	0.07	5	0.33	1	0.07	2	0.13
Grey-cinnamon	101	4.59	56	2.55	28	1.27	14	0.64	1	0.05	9	0.41	1	0.05	3	0.14
Castanozems	154	4.53	84	2.47	49	1.44	19	0.56	1	0.03	10	0.29	2	0.06	3	0.09
Litho soil	14	4.67	8	2.67	4	1.33	2	0.67	0	0	1	0.33	1	0.33	0	0
Saline	40	5.00	22	2.75	12	1.50	5	0.63	2	0.25	2	0.25	0	0	1	0.13
Alluvial soil	18	6.00	10	3.33	6	2.00	2	0.67	0	0	1	0.33	1	0.33	0	0
Brown pedocals	60	4.62	33	2.54	22	1.69	7	0.54	1	0.08	2	0.15	0	0	1	0.08
Gray forest soil	30	4.29	13	1.86	7	1.00	3	0.43	0	0	2	0.29	0	0	1	0.14
Bog soil	32	5.33	18	3.00	9	1.50	2	0.33	1	0.17	3	0.50	1	0.17	2	0.33
Aeolian soil	20	5.00	11	2.75	6	1.50	3	0.75	0	0	2	0.50	0	0	0	0
Meadow soil	50	4.55	25	2.27	14	1.27	5	0.45	1	0.09	4	0.36	1	0.09	0	0
Solonetzs	6	6.00	4	4.00	2	2.00	1	1.00	1	1.00	0	0	0	0	0	0
Chernozem	29	4.14	14	2.00	6	0.86	3	0.43	0	0	3	0.43	1	0.14	1	0.14
Skeletol soil	5	5.00	3	3.00	1	1.00	0	0	0	0	1	1.00	0	0	1	1.00
Castano-cinnamon soil	19	4.75	9	2.25	6	1.50	2	0.50	0	0	0	0	1	0.25	0	0
Total	650	4.68	348	2.50	193	1.39	76	0.55	9	0.06	45	0.32	10	0.07	15	0.11

were isolated from all soil types except solonetzs and castano-cinnamon soils. The average number of strains belonging to *Cystobacter* was the highest in skeletol soils and the lowest in brown pedocals. The strains belonging to *Pyxidicoccus* were isolated from all soil types except litho soils, alluvial soils, gray forest soils, aeolian soils, chernozems, skeletol soil and castano-cinnamon soils. The average number of strains belonging to *Pyxidicoccus* was the highest in solonetzs and the lowest in castanozems. The strains belonging to *Archangium* were isolated from all soil types except saline soils, brown pedocals, gray forest soils, aeolian soils, solonetzs and skeletol soils. The average number of strains belonging to *Archangium* was the highest in litho soils and alluvial soils and the lowest in grey-cinnamon soils. The strains belonging to *Mellittangium* were isolated from all soil types except litho soils, alluvial soils, aeolian soils, meadow soils, solonetzs and castano-cinnamon soils. The average number of strains belonging to *Mellittangium* was the highest in skeletol soils and the lowest in brown pedocals. In most soil types, the average strain number of *Myxococcus* ranked first, that of *Corallococcus* followed and the average strain number of *Cystobacter* ranked third. The average strain number of myxobacteria from the other three genera was significantly lower than that from the above three genera and in some soil types, myxobacteria from some genera were not even isolated.

Correlation analysis between the distribution of culturable myxobacteria and environmental parameters

The Pearson correlation coefficient was shown in Table 6.

The number of bacteriolytic myxobacteria and the total number of strains were weak positively correlated with water content, pH value, content of organic matter, content of hydrolytic nitrogen and content of available potassium, and weak negatively correlated with the content of available phosphorus. The number of celluloytic myxobacteria were weak positively correlated with water content and content of organic matter, and weak negatively correlated with the content of available phosphorus and content of available potassium, and no significant correlation with pH value and content of hydrolytic nitrogen. It could also be seen from the table that there was no significant correlation between the environmental parameters and the number of the bacteriolytic myxobacteria, the number of the celluloytic myxobacteria and the total number of strains. This may be due to the isolation and purification process of myxobacteria was cumbersome and difficult, resulting in the loss of some strains. On the other hand, it may be due to the addition of antibiotics in the culture medium damaged some myxobacteria, resulting in the varieties of isolated myxobacteria less. Therefore, the number of strains currently isolated did not very objectively represent the number of strains actually contained in the sample.

Activity analysis of myxobacteria against *P. infestans*

Among 348 strains of myxobacteria, 289 strains showed different degrees of inhibition on the growth of *P. infestans*, accounting for 83% of the total myxobacteria. Among them, 24 strains showed strong antagonistic activity with a

diameter of inhibition zone more than 20 mm. The inhibition zone diameter of 124 strains was 15~20 mm. The inhibition zone diameter of 148 strains was 10~15 mm. The inhibition zone diameter of only one strain was less than 10 mm (Fig. 4).

As shown in Table 7, the statistical analysis on the antagonistic activity of different species and genera of myxobacteria showed that *Myxococcus* had the highest percentage of resistant strains, 90.67%. *Corallococcus* was next with a percentage of 86.84% for resistant strains. The percentage of resistant strains of *Cystobacter* and *Mellittangium* was 66.67%. The percentage of the strains belonging to *Pyxidicoccus* with the activity against *P. infestans* was 44.44%. The percentage of resistant strains of *Archangium* was 40%.

Discussion

According to the analysis and statistics of soil environmental parameters in central Inner Mongolia, it was found that the soil samples in this area were basically neutral or alkaline, and the water content of soil samples was very low, generally in a dry state; the content of hydrolyzed nitrogen was in a state of deficiency; the content of organic matter and available potassium more than half of the soil samples were in a deficient state, while the content of available phosphorus was relatively high, only 36.70% of the soil samples were below the medium level, but the suitability of a single element cannot change the overall fertility status of this area. Ding *et al.* (2017) analyzed the physical and chemical properties of the soil in western Inner Mongolia and found that the soil in this area was basically neutral and alkaline, the water content was very low, and it was generally in a dry state. The average content of alkali nitrogen was very low. The average content of available potassium and organic matter were at a low level, while the average content of available phosphorus was high. This was basically the same as the physical and chemical properties of the soil in central Inner Mongolia. The analysis of soil nutrients in Guanzhong area of Shanxi Province by Zhao (2015) showed that the contents of organic matter, available nitrogen, available phosphorus and available potassium in the area are respectively in the fifth, fourth, third and second grades of soil nutrient grading standards. That is to say, except the organic matter in the state of deficiency, other environmental parameters were in the appropriate and above levels. Fu (2005) analyzed the soil resources in the vegetable base of Zhengzhou City of Henan Province and found that the soil fertility in the suburbs was medium. Apart from the low content of available phosphorus, the other nutrient contents were at a medium level. In comparison, it can be seen that the soil in the central part of Inner Mongolia is seriously arid and the soil fertility level is low, which can also explain a series of ecological environment deterioration problems such as severe sandstorm, desertification, and vegetation degradation in

Table 6: Person correlation coefficient of soil parameters and the distribution of myxobacteria

Pearson correlation coefficient (r)	Bacteriolytic myxobacteria	Celluloytic myxobacteria	Total No. of strains
Water content	0.093	0.012	0.072
pH value	0.089	0.000	0.063
Organic matter	0.013	0.005	0.012
Available phosphorus	-0.017	-0.009	-0.016
Hydrolytic nitrogen	0.076	0.000	0.054
Available potassium	0.098	-0.035	0.052

|r| < 0.2 is very low correlation. 0.2 ≤ |r| < 0.4 is low correlation. 0.4 ≤ |r| < 0.7 for moderate correlation. 0.7 ≤ |r| < 0.9 is highly correlated. 0.9 ≤ |r| < 1.0 is extremely high correlation
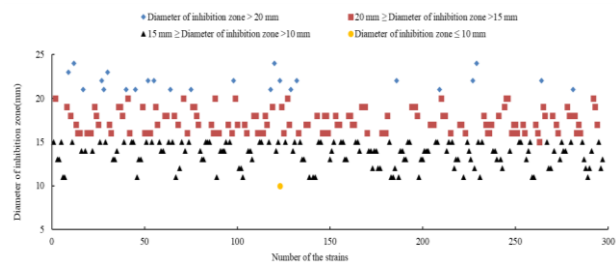

Fig. 4: Scatter diagram of inhibition zone diameter of myxobacteria strains against *P. infestans*

Table 7: Antibiotic activity of different species of myxobacteria against *P. infestans*

Species	Total No. of strains	No. of strains against *P. infestans*	Resistant strains (%)
Myxococcus fulvus	67	64	95.52
Myxococcus virescens	41	39	95.12
Myxococcus coralloides	6	5	83.33
Myxococcus xanthus	45	41	91.11
Myxococcus favescens	19	15	78.95
Myxococcus stipitatus	10	7	70.00
Myxococcus macrosporus	5	4	80.00
Corallococcus coralloides	39	35	89.74
Corallococcus exiguous	27	24	88.89
Corallococcus macrosporus	8	6	75.00
Corallococcus spp.	2	1	50.00
Pyxidicoccus fallax	8	4	50.00
Pyxidicoccus spp.	1	0	0.00
Cystobacter badius	16	11	68.75
Cystobacter violaceus	25	17	68.00
Cystobacter spp.	4	2	50.00
Archangium gephyra	8	4	50.00
Archangium spp.	2	0	0.00
Mellittangium spp.	15	10	66.67
Total	348	289	83.05

this area.

Morphological observation and 16S rRNA gene sequence analysis revealed that diversity of myxobacteria in the collected soil samples were rich. The isolated 348 strains of myxobacteria belonged to Cystobacterineae, including 278 strains of Myxococcaceae and 70 strains of Cystobacteraceae. Among them, there were 193 strains of *Myxococcus*, 76 strains of *Corallococcus*, 9 strains of *Pyxidicoccus*, 45 strains of *Cystobacter*, 10 strains of *Archangium* and 15 strains of *Melittangium*. The results

indicated that myxobacteria had superior viability in the poor soil environment. These bacteria were the dominant genus of myxobacteria in central Inner Mongolia. Scientists have also found myxobacteria in soil samples collected in the Antarctic, which did not survive in the laboratory conditions. *Myxococcus* and *Nannocystis* have been found in the environment of 6°C ~ 8°C in the Alps for the first time (Menne and Rückert 1988) and traces of *Myxococcus* have also been found in the hot desert soil of Arizona (Reichenbach 1970). These findings indicated that myxobacteria can survive in extreme environment, indicating their strong survival ability, which is consistent with the result of this study. Charousová *et al.* (2017) isolated a total of 79 strains of myxobacteria from 10 soil samples collected in Slovakia, belonging to *Myxococcus*, *Corallococcus*, *Sorangium*, and *Polyangium*. Li *et al.* (2014) used Semi-Nested PCR-DGGE technology to analyze 8 soils collected from Xinjiang and found 5 genera of myxobacteria, less than the genera found in this study. However, they also found the genera that were not present in this experiment, namely *Stigmatella* and *Anaeromyxobacter*. Wu *et al.* (2005) in Shandong University and Zhou (2013) used molecular hybridization and high-throughput sequencing technology to analyze the diversity of myxobacteria in the campus soil, and the results showed that myxobacteria in soil samples were rich in diversity. They covered almost all known families and a large number of unclassified myxobacteria. Therefore, we speculate that new myxobacterial resources may need to rely on new methods to isolate in the future.

According to the distribution of myxobacteria in the samples of different utilization types, 6 genus of myxobacteria were purified from the soil samples of cultivated land, grassland and woodland, while only 3 genus of myxobacteria were purified from the samples of unused land, namely *Myxococcus*, *Corallococcus* and *Cystobacter*. In terms of myxobacteria population, 19 species of myxobacteria were purified from grassland samples. Cultivated land samples were followed by 18 species. 13 species of myxobacteria were purified from the woodland samples, while only 8 species were purified from the unused land samples. It can be seen that the utilization types of soil had a significant impact on the species and genus distribution of myxobacteria. Among them, the myxobacteria in the grassland and cultivated land samples were abundant, while the myxobacteria in the woodland and unused land samples are less. Charousová *et al.* (2017) isolated myxobacteria from alpine soil, forest soil and farmland soil of Slovakia, and found that *Myxococcus* and *Corallococcus* were the most widely distributed and the best source for myxobacteria was farmland soil. However, Li *et al.* (2005) and Ding (2017) respectively found that myxobacteria were the most abundant in the forest samples in Hebei Province and Ordos plateau, slightly different from the results of this study. The reason may be that the difference in the sampling location and the physical and chemical properties of the samples resulted in a difference in the population of myxobacteria in the soil samples of the same utilization type. It can be seen from the distribution of myxobacteria in the soil samples of different types that six genera of myxobacteria were purified from Fluvo-aquic soils, Grey-cinnamon soils, Castanozems and Bog soils, showing a rich diversity of myxobacteria. Among various types of soil, Fluvo-aquic soils, Grey-cinnamon soils, Castanozems and Bog soils are the relatively fertile soil types, while Aeolian soils is the relatively poor soil type, proving that myxobacteria grow better in the nutrient-rich environment. Although some environmental parameters of Skeletol soil were at the appropriate level, there was only one sample of Skeletol soils and Solonetzs, so the general significance of results is poor. This result was consistent with the study of Li *et al.* (2005) on the distribution of myxobacteria in samples of different properties.

The results of correlation analysis between the distribution of myxobacteria and environmental parameters showed that there was no significant correlation between them. There may be several reasons for this: Firstly, due to the cumbersome and difficult isolation and purification process of myxobacteria, some strains were lost during the isolation and purification process. Second, due to some pretreatment measures of the soil samples before isolation, some of the myxospores in the samples were possibly damaged to be unable to geminate normally. For example, generally, the samples were treated at 58°C for 12 h before isolation of myxobacteira. The pretreatment time may be too long. Third, due to the addition of antibiotics in the culture medium, some myxobacteria could be inhibited, resulting in fewer types of myxobacteria isolated. Therefore, the currently isolated strains do not very objectively represent the species actually contained in the sample, resulting in biased results. Zhou (2013) found that the abundance of myxobacteria was less correlated with temperature, pH, content of organic carbon, carbon to nitrogen ratio, and had no correlation with precipitation and particle grading. The central region of Inner Mongolia has a vast territory and a large number of soil types. There are many reasons for the difference in the diversity of myxobacteria. In addition to the environmental factors studied in this paper, heavy metals, salinity, rainfall, topography and other factors may also affect the diversity of myxobacteria.

Several myxobacterial metabolites have been purified and shown to be bioactive and therefore myxobacteria represent an underexploited resource for bioactive discovery (Landwehr *et al.* 2016). In this study, the activity analysis of myxobacteria against *P. infestans* showed that 289 strains of 348 strains of myxobacteria showed different degrees of inhibition on the growth of *P. infestans*, and the percentage of resistant strains was as high as 83%. Due to the growing population, more and more pesticides are used to improve the production of food, but they are generally highly toxic, and pesticide

poisoning incidents occur every year. Potato is one of the world's four major food crops, after wheat, corn and rice, but its production is reduced annually due to tuber rot. Potato late blight is a devastating disease caused by *P. infestans*, which can cause the death of potato stem and leaves and rancid tuber (Kroon *et al.* 2011). Therefore, it is extremely urgent to develop effective and harmless pesticides to prevent and control potato late blight. In this study, most of the myxobacterial strains isolated from soil samples from central Inner Mongolia showed the activity in inhibiting the growth of potato late blight pathogen *P. infestans* providing a new idea for the research and development of effective pesticides for the prevention and control of potato late blight.

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Author Contributions

Wu ZH and Liu HR conceived the experiments, Wu ZH, Wang XH and Ma Q conducted the experiments, Wu ZH, Ding YX and Zhao PY analysed the results. All authors reviewed the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest, and manuscript is approved by all authors for publication. There is no conflict of interest among the institutions regarding the research when it has been conducted at the institutions other than authors institutions. If such a conflicting situation arises, the authors will be held responsible.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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