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## Supplementary Material

# Exploration of Endophytes from Alfalfa (*Medicago sativa* L.) as Biocontrol Agents

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### ABSTRACT

Endophytes are increasingly investigated as biocontrol agents for agricultural production. The identification of new endophytes with high effectiveness against plant disease is very important. A total of 362 strains of endophytes, including fungi, bacteria, and actinomycete, were isolated from alfalfa (*Medicago sativa* L.) collected in Hebei, Inner Mongolia and Ningxia provinces of China. The three strains of endophytic bacteria (NA NX51R-5, NA NX90R-8, and NA NM1S-1) with strong biocontrol capability with >50% effectiveness were screened against the common alfalfa root rot pathogen *Fusarium oxysporum* F. sp. *medicaginis* in alfalfa seedling germination experiments on MS medium and pot experiments. Using phylogenetic analysis, the isolates of NA NM1S-1 and NA NX51R-5 were identified as *Bacillus* spp. by 16S rDNA, while NA NX90R-8 was found to be *Pseudomonas* sp.

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## Appendix

16S rDNA sequences of endophytic bacteria (E1, E2, and E3):

> SAMN11658551-Endophytic bacteria-NA NM1S-1-E1-16S rDNA

CCTGCTGGCGCGTGCTATACATGCAGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGG  
 GTAACCTGCCATAAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAAGTGCATGGTTCGAAATTGAAAGGCG  
 GCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTACCAAGGCAACGATGCGTAGCCGACCTG  
 AGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTC  
 TGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTA AAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGC  
 ACCTTGACGGTACCTAACAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATT  
 GGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGA  
 GTGAGAAGAGGAAAGTGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTCTGGTC  
 TGTAACTGACACTGAGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCTGGTAGTCCACGCCGTAACGATGAGTGCTAAGTGTT  
 AGAGGGTTCCGCCCTTATAGTGTGAAGTTAACGCATTAAGCACTCCGCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGA  
 CGGGCCCCGCACAAGCGGTGGAGCATGTGGTTAATTCAAGCAACGCGAGACCTACCAGTCTTGACATCTCTGAAACTCTAGAGATAG  
 GGCTTCTTCCGGAGCAGAGTGACAGTGTGCATGTGTCGTCAGCTCGTGTGAGATGTGGGTAGTC

> SAMN11658552-Endophytic bacteria-NA NX51R-5-E2-16S rDNA

CTGCATGCGGGTGCTATACATGCAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTA  
 ACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGCTTGTGTTGAACCGCATGGTTCAAACATAAAAGGTGGCT  
 TCGGCTACCACTTACAGATGGACCCGCGCGCATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGA  
 GGGTGATCGGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGA  
 CGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACC  
 TTGACGGTACCTAACAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGG  
 CGTAAAGGGCTCGCAGCGGTTCTTAAGTCTGATGTGAAAGCCCCGGCTCAACCGGGAGGGTCAATGGAAACTGGGGAACCTGAGTG  
 CAGAAGAGGAGAGTGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGT  
 AACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCTGGTAGTCCACGCCGTAACGATGAGTGCTAAGTGTTAT  
 GGGGTTTCCGCCCTTATGCTGCAGCTAACGCATTAAGCACTCCGCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACG  
 GGGGCCCGACAAGCGGTGGAGCATGTGGTTAATTCAAGCAACGCGAGAACCTTACCAGGTCTTGACATCCTCTGACATCCTAGAGAT  
 AGGACGTCCCCTTCGGGCGAGAGTGACAGTGTGCATGTGTCGTCAGCTCGTGTGAGATGTGGGTAAGTCCGCAACGAGCGCAACCTT  
 TGATCTTAGTTGCCAGCATTCA

> SAMN11658553-Endophytic bacteria-NA NX90R-8-E3-16S rDNA

GACGATCGCAGCTACCATGCAAGTCGAGCGGTTGACGGGAGCTTGCTCCCTGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTG  
 CCTATTAGTGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACTGCTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCCTTGCCTAA  
 TAGATGAGCCTAGGTGGATTAGCTAGTTGGTGGGGTAAAGGCTCACCAAGGCGACGATCCGTAACCTGGTCTGAGAGGATGATCAGTCAC  
 ACTGGAAGTGAAGACAGGTCAGACTCCTACGGGAGGCAGCAGTGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGC  
 GTGTGTGAAGAAGGTCTTCGGATTGTAAGCACTTAAAGTTGGGAGGAAGGGTTGTAGATTAATACTCTGCAATTTTGACGTTACCGACAG  
 AATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGAAGCGTTAATCGGAATTAAGTGGGCGTAAAGCGCGCGTA  
 GGTGGTTTGTAAAGTTGGATGTGAAAAGCCCCGGGCTCAACCTGGGAACCTGCATCCAAAACCTGGCAAGCTAGAGTACGGTAGAGGGTGGT  
 GAATTTCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGT  
 GCGAAAAGCGTGGGGAGCAAACAGGATTAGATACCTGGTAGTCCACGCCGTAACGATGTCAACTAGCCGTTGGGTTCTTGAGAACTTA  
 GTGGCGCAGCTAACGCATTAAGTTGACCGCTGGGGAGTACGGCCGCAAGGTTAAACTCAAATGAATTGACGGGGGCCCGACAAGCG  
 GTGGAGCATGTGGTTAATTCAAGCAACGCGAAGAAACCTTACCTGGGCCCTGACATGCTGAGAAGTTCAGAGATGGATTGGTGCT  
 TCGGGAACCTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCTGTCT  
 TAGTACCAGCACGTTATGGTGGTCTCTAGGAGACTGCGTGACCAATCGCAGGAAGGTGGGAATGAACGTTCAAGGTCATTATGGGCC  
 TTACGGCCAAGGGACT

**Supplemental Table 1:** Data on collection sites and the vegetal condition of alfalfa.

Name	Collection time	Location	Soil	Ecosystem; plants	Evaluation of growth
HB1	2015.08	Langfang base of CAAS	loess	Experimental field; A variety of vegetable crops	good
HB2	2015.08	Langfang base of CAAS	loess	Experimental field; A variety of vegetable crops	good
HB3	2016.09	Yangerzhuang Town, Hebei Huanghua	loess saline-alkali land	Experimental field; Exiguous weeds	good
HB4	2016.09	Yangerzhuang Town, Hebei Huanghua	loess saline-alkali land	Experimental field; Exiguous weeds	good
HB5	2016.09	Yangerzhuang Town, Hebei Huanghua	loess saline-alkali land	Experimental field; Exiguous weeds	good
HB6	2016.09	Yangerzhuang Town, Hebei Huanghua	loess saline-alkali land	Experimental field; Exiguous weeds	good
HB7	2016.09	Yangerzhuang Town, Hebei Huanghua	loess saline-alkali land	Experimental field; Exiguous weeds	good
HB8	2016.09	Yangerzhuang Town, Hebei Huanghua	loess saline-alkali land	Experimental field; Exiguous weeds	good
HB9	2016.09	Yangerzhuang Town, Hebei Huanghua	loess saline-alkali land	Experimental field; Exiguous weeds	good
HB10	2016.09	Yangerzhuang Town, Hebei Huanghua	loess saline-alkali land	Experimental field; Exiguous weeds	good
HB11	2016.09	Yangerzhuang Town, Hebei Huanghua	loess saline-alkali land	Experimental field; Exiguous weeds	good
HB12	2016.09	Yangerzhuang Town, Hebei Huanghua	loess saline-alkali land	Experimental field; Exiguous weeds	good
HB13	2016.09	Yangerzhuang Town, Hebei Huanghua	loess saline-alkali land	Experimental field; Exiguous weeds	good
HB14	2016.09	Yangerzhuang Town, Hebei Huanghua	loess saline-alkali land	Experimental field; Exiguous weeds	good
NM1	2015.07	Inner Mongolia Xilin Gol League	sandy soil	Exiguous weeds	optimal
NM2	2015.07	Inner Mongolia Xilin Gol League	sandy soil	Exiguous weeds	optimal
NX1	2015.09	Military Racecourse, Xixia District, Ningxia	loess	Fragmentation cultivation; Corn	optimal
NX2	2015.09	Military Racecourse, Xixia District, Ningxia	loess	Fragmentation cultivation; Corn	optimal
NX3	2015.09	Military Racecourse, Xixia District, Ningxia	loess	Fragmentation cultivation; Corn	optimal
NX4	2015.09	Military Racecourse, Xixia District, Ningxia	loess	Fragmentation cultivation; Corn	optimal
NX5	2015.09	Military Racecourse, Xixia District, Ningxia	loess	Fragmentation cultivation; Corn	optimal
NX6	2015.09	Military Racecourse, Xixia District, Ningxia	loess	Fragmentation cultivation; Corn	optimal
NX7	2015.09	Military Racecourse, Xixia District, Ningxia	loess	Fragmentation cultivation; Corn	optimal
NX8	2015.09	Military Racecourse, Xixia District, Ningxia	loess	Fragmentation cultivation; Corn	optimal

NX9	2015.09	Military Racecourse, Xixia District, Ningxia	loess	Fragmentation cultivation; Corn	optimal
NX10	2015.09	Military Racecourse, Xixia District, Ningxia	loess	Fragmentation cultivation; Corn	optimal
NX11	2015.09	Military Racecourse, Xixia District, Ningxia	loess	Fragmentation cultivation; Corn	optimal
NX12	2015.09	Military Racecourse, Xixia District, Ningxia	loess	Fragmentation cultivation; Corn	optimal
NX13	2015.09	Military Racecourse, Xixia District, Ningxia	loess	Fragmentation cultivation; Corn	optimal
NX14	2015.09	Hot Spring Farm, Helan County, Ningxia	loess	Lowland rivers; Reed corn	optimal
NX15	2015.09	Hot Spring Farm, Helan County, Ningxia	loess	Lowland rivers; Reed corn	optimal
NX16	2015.09	Hot Spring Farm, Helan County, Ningxia	loess	Lowland rivers; Reed corn	optimal
NX17	2015.09	Hot Spring Farm, Helan County, Ningxia	loess	Lowland rivers; Reed corn	optimal
NX18	2015.09	Hot Spring Farm, Helan County, Ningxia	loess	Lowland rivers; Reed corn	optimal
NX19	2015.09	Hot Spring Farm, Helan County, Ningxia	loess	Lowland rivers; Reed corn	optimal
NX20	2015.09	Hot Spring Farm, Helan County, Ningxia	loess	Lowland rivers; Reed corn	optimal
NX21	2015.09	Hot Spring Farm, Helan County, Ningxia	loess	Lowland rivers; Reed corn	optimal
NX22	2015.09	Hot Spring Farm, Helan County, Ningxia	loess	Lowland rivers; Reed corn	optimal
NX23	2015.09	Hot Spring Farm, Helan County, Ningxia	loess	Lowland rivers; Reed corn	optimal
NX24	2015.09	Hot Spring Farm, Helan County, Ningxia	loess	Lowland rivers; Reed corn	optimal
NX25	2015.09	Hot Spring Farm, Helan County, Ningxia	loess	Lowland rivers; Reed corn	optimal
NX26	2015.09	Hot Spring Farm, Helan County, Ningxia	loess	Lowland rivers; Reed corn	optimal
NX27	2015.09	Jingquan Farm, Shizuishan, Ningxia 1	Sand contains a large amount of gravel	Near the Horan Mountains; Vegetation scarce	bad
NX28	2015.09	Jingquan Farm, Shizuishan, Ningxia 1	Sand contains a large amount of gravel	Near the Horan Mountains; Vegetation scarce	bad
NX29	2015.09	Jingquan Farm, Shizuishan, Ningxia 1	Sand contains a large amount of gravel	Near the Horan Mountains; Vegetation scarce	bad
NX30	2015.09	Jingquan Farm, Shizuishan, Ningxia 1	Sand contains a large amount of gravel	Near the Horan Mountains; Vegetation scarce	bad

NX31	2015.09	Jingquan Farm, Shizuishan, Ningxia 1	Sand contains a large amount of gravel	Near the Horan Mountains; Vegetation scarce	bad
NX32	2015.09	Jingquan Farm, Shizuishan, Ningxia 1	Sand contains a large amount of gravel	Near the Horan Mountains; Vegetation scarce	bad
NX33	2015.09	Jingquan Farm, Shizuishan, Ningxia 1	Sand contains a large amount of gravel	Near the Horan Mountains; Vegetation scarce	bad
NX34	2015.09	Jingquan Farm, Shizuishan, Ningxia 1	Sand contains a large amount of gravel	Near the Horan Mountains; Vegetation scarce	bad
NX35	2015.09	Jingquan Farm, Shizuishan, Ningxia 1	Sand contains a large amount of gravel	Near the Horan Mountains; Vegetation scarce	bad
NX36	2015.09	Jingquan Farm, Shizuishan, Ningxia 1	Sand contains a large amount of gravel	Near the Horan Mountains; Vegetation scarce	bad
NX37	2015.09	Jingquan Farm, Shizuishan, Ningxia 1	Sand contains a large amount of gravel	Near the Horan Mountains; Vegetation scarce	bad
NX38	2015.09	Jingquan Farm, Shizuishan, Ningxia 1	Sand contains a large amount of gravel	Near the Horan Mountains; Vegetation scarce	bad
NX39	2015.09	Jingquan Farm, Shizuishan, Ningxia 1	Sand contains a large amount of gravel	Near the Horan Mountains; Vegetation scarce	bad
NX40	2015.09	Jingquan Farm, Shizuishan, Ningxia 1	Sand contains a large amount of gravel	Near the Horan Mountains; Vegetation scarce	bad
NX41	2015.09	Jingquan Farm, Shizuishan, Ningxia 1	Sand contains a large amount of gravel	Near the Horan Mountains; Vegetation scarce	bad
NX42	2015.09	Jingquan Farm, Shizuishan, Ningxia 1	Sand contains a large amount of gravel	Near the Horan Mountains; Vegetation scarce	bad
NX43	2015.09	Jingquan Farm, Shizuishan, Ningxia 2	sandy soil	Lowland; More grass and Suaeda glauca	good
NX44	2015.09	Jingquan Farm, Shizuishan, Ningxia 2	sandy soil	Lowland; More grass and Suaeda glauca	good
NX45	2015.09	Jingquan Farm, Shizuishan, Ningxia 2	sandy soil	Lowland; More grass and Suaeda glauca	good
NX46	2015.09	Jingquan Farm, Shizuishan, Ningxia 2	sandy soil	Lowland; More grass and Suaeda glauca	good
NX47	2015.09	Jingquan Farm, Shizuishan, Ningxia 2	sandy soil	Lowland; More grass and Suaeda glauca	good
NX47	2015.09	Jingquan Farm, Shizuishan, Ningxia 2	sandy soil	Lowland; More grass and Suaeda glauca	good
NX48	2015.09	Jingquan Farm, Shizuishan, Ningxia 2	sandy soil	Lowland; More grass and Suaeda glauca	good
NX49	2015.09	Jingquan Farm, Shizuishan, Ningxia 2	sandy soil	Lowland; More grass and Suaeda glauca	good
NX50	2015.09	Jingquan Farm, Shizuishan, Ningxia 2	sandy soil	Lowland; More grass and Suaeda glauca	good

NX51	2015.09	Jingquan Farm, Shizuishan, Ningxia 2	sandy soil	Lowland; More grass and Suaeda glauca	good
NX52	2015.09	Jingquan Farm, Shizuishan, Ningxia 2	sandy soil	Lowland; More grass and Suaeda glauca	good
NX53	2015.09	Jingquan Farm, Shizuishan, Ningxia 2	sandy soil	Lowland; More grass and Suaeda glauca	good
NX54	2015.09	Jingquan Farm, Shizuishan, Ningxia 2	sandy soil	Lowland; More grass and Suaeda glauca	good
NX55	2015.09	Jingquan Farm, Shizuishan, Ningxia 3	sandy soil	Cultivated along the road; poplar forest, corn	good
NX56	2015.09	Jingquan Farm, Shizuishan, Ningxia 3	sandy soil	Cultivated along the road; poplar forest, corn	good
NX57	2015.09	Jingquan Farm, Shizuishan, Ningxia 3	sandy soil	Cultivated along the road; poplar forest, corn	good
NX58	2015.09	Jingquan Farm, Shizuishan, Ningxia 3	sandy soil	Cultivated along the road; poplar forest, corn	good
NX59	2015.09	Jingquan Farm, Shizuishan, Ningxia 3	sandy soil	Cultivated along the road; poplar forest, corn	good
NX60	2015.09	Jingquan Farm, Shizuishan, Ningxia 3	sandy soil	Cultivated along the road; poplar forest, corn	good
NX61	2015.09	Jingquan Farm, Shizuishan, Ningxia 3	sandy soil	Cultivated along the road; poplar forest, corn	good
NX62	2015.09	Jingquan Farm, Shizuishan, Ningxia 3	sandy soil	Cultivated along the road; poplar forest, corn	good
NX63	2015.09	Jingquan Farm, Shizuishan, Ningxia 3	sandy soil	Cultivated along the road; poplar forest, corn	good
NX64	2015.09	Jingquan Farm, Shizuishan, Ningxia 3	sandy soil	Cultivated along the road; poplar forest, corn	good
NX65	2015.09	Ningxia Jianquan Village	loess	Surrounding the countryside; corn	good
NX66	2015.09	Ningxia Jianquan Village	loess	Surrounding the countryside; corn	good
NX67	2015.09	Ningxia Jianquan Village	loess	Surrounding the countryside; corn	good
NX68	2015.09	Ningxia Jianquan Village	loess	Surrounding the countryside; corn	good
NX69	2015.09	Ningxia Jianquan Village	loess	Surrounding the countryside; corn	good
NX70	2015.09	Ningxia Jianquan Village	loess	Surrounding the countryside; corn	good
NX71	2015.09	Ningxia Jianquan Village	loess	Surrounding the countryside; corn	good
NX72	2015.09	Ningxia Jianquan Village	loess	Surrounding the countryside; corn	good
NX73	2015.09	Ningxia Jianquan Village	loess	Surrounding the countryside; corn	good
NX74	2015.09	Ningxia Jianquan Village	loess	Surrounding the countryside; corn	good
NX75	2015.09	Helanshan Farm, Xixia District, Ningxia	Sand contains a small amount of gravel	Near the Horan Mountains; More purslane, corn	optimal

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NX76	2015.09	Helanshan Farm, Xixia District, Ningxia	Sand contains a small amount of gravel	Near the Horan Mountains; More purslane, corn	optimal
NX77	2015.09	Helanshan Farm, Xixia District, Ningxia	Sand contains a small amount of gravel	Near the Horan Mountains; More purslane, corn	optimal
NX78	2015.09	Helanshan Farm, Xixia District, Ningxia	Sand contains a small amount of gravel	Near the Horan Mountains; More purslane, corn	optimal
NX79	2015.09	Helanshan Farm, Xixia District, Ningxia	Sand contains a small amount of gravel	Near the Horan Mountains; More purslane, corn	optimal
NX80	2015.09	Helanshan Farm, Xixia District, Ningxia	Sand contains a small amount of gravel	Near the Horan Mountains; More purslane, corn	optimal
NX81	2015.09	Helanshan Farm, Xixia District, Ningxia	Sand contains a small amount of gravel	Near the Horan Mountains; More purslane, corn	optimal
NX82	2015.09	Helanshan Farm, Xixia District, Ningxia	Sand contains a small amount of gravel	Near the Horan Mountains; More purslane, corn	optimal
NX83	2015.09	Helanshan Farm, Xixia District, Ningxia	Sand contains a small amount of gravel	Near the Horan Mountains; More purslane, corn	optimal
NX84	2015.09	Helanshan Farm, Xixia District, Ningxia	Sand contains a small amount of gravel	Near the Horan Mountains; More purslane, corn	optimal
NX85	2015.09	Helanshan Farm, Xixia District, Ningxia	Sand contains a small amount of gravel	Near the Horan Mountains; More purslane, corn	optimal
NX86	2015.09	Helanshan Farm, Xixia District, Ningxia	Sand contains a small amount of gravel	Near the Horan Mountains; More purslane, corn	optimal
NX87	2015.09	Helanshan Farm, Xixia District, Ningxia	Sand contains a small amount of gravel	Near the Horan Mountains; More purslane, corn	optimal
NX88	2015.09	Helanshan Farm, Xixia District, Ningxia	Sand contains a small amount of gravel	Near the Horan Mountains; More purslane, corn	optimal
NX89	2015.09	Helanshan Farm, Xixia District, Ningxia	Sand contains a small amount of gravel	Near the Horan Mountains; More purslane, corn	optimal
NX90	2015.09	Helanshan Farm, Xixia District, Ningxia	Sand contains a small amount of gravel	Near the Horan Mountains; More purslane, corn	optimal
NX91	2015.09	Helanshan Farm, Xixia District, Ningxia	Sand contains a small amount of gravel	Near the Horan Mountains; More purslane, corn	optimal
NX92	2015.09	Helanshan Farm, Xixia District, Ningxia	Sand contains a small amount of gravel	Near the Horan Mountains; More purslane, corn	optimal

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**Supplemental Table 2:** Cucumber seedling disease symptoms scoring scale based on the leaf appearance.

Disease scale	Disease symptoms
0	Healthy and uninfected plants (no external symptoms)
1	Initial signs of wilting (yellowing)
2	Up to 25% of the leaves and root with symptoms
3	Up to 50% of the leaves and root with symptoms
4	Up to 75% of the leaves and root with symptoms
5	Plants dead

**Supplemental Table 3:** Cucumber seedling disease symptoms scoring scale based on root appearance.

Disease scale	Disease symptoms
0	The roots are white and long and fill the whole test tube
1	The roots fill half the tube, few roots were the bottom of the tube, yellow white.
2	The roots are shorter, fill 1/3-1/2 of the test tube, slight yellow
3	The principal root is short, fibrous roots are few, and fill only 1/4-1/3 tube, slight yellow
4	The fibrous roots are few, and the principal root is yellow
5	The fibrous roots are few, and the principal roots is rotten

**Supplemental Table 4:** Alfalfa seedling disease symptoms scoring scale based on root appearance.

Disease scale	Disease symptoms
0	The roots are white
1	1/4 of the root length is brown; rest is yellowish
2	1/4 to 1/2 of the root length is brown; rest is slight yellow
3	1/2 to 3/4 of the root length is brown; rest is yellow
4	>3/4 of the root length is brown; rest is yellow brown
5	The plant is dead, or the principal root is rotten

**Supplemental Table 5:** Information on partial sequences of ITS sequences published in NCBI.

Accession number	Genus	Compared isolates on GenBank
KR868229.1	<i>Trichoderma</i>	<i>T. aureoviride</i> strain ZWPBG1
KY764845.1	<i>Trichoderma</i>	<i>T. afroharzianum</i> isolate CTCCSJ-G-JK40051-Ma
KU896356.1	<i>Trichoderma</i>	<i>T. harzianum</i> isolate CTCCSJ-ASD50452
KR868283.1	<i>Trichoderma</i>	<i>T. harzianum</i> strain ZWPUEB15
KU896359.1	<i>Trichoderma</i>	<i>T. harzianum</i> isolate CTCCSJ-AMH50459-Zhao
KU896332.1	<i>Trichoderma</i>	<i>T. harzianum</i> isolate CTCCSJ-AGS50340-Zhao
HF912167.2	<i>Rhizoctonia</i>	<i>R. solani</i> strain AG-5, isolate RCC5
KJ620369.1	<i>Fusarium</i>	<i>F. solani</i> strain T-ICA06NP
AY605713.1	<i>Trichoderma</i>	<i>T. harzianum</i> , strain: CBS 226.95 T

**Supplemental Table 6:** Information on type strain of partial sequences of 16S rDNA published in NCBI.

Accession number	Isolates
AB271745.1	<i>Bacillus cereus</i> , strain: NBRC 15305 T
AB592491.1	<i>Bacillus cereus</i> , strain: JCM 2152 T
AM747812.1	[ <i>Brevibacterium</i> ] <i>halotolerans</i> , strain: DSM 8802 T
X83409.1	<i>Arthrobacter sulfureus</i> , strain: DSM 20167 T
X55060.1	<i>Bacillus cereus</i> , strain: NCDO 1771 T
AB021181.1	<i>Bacillus atrophaeus</i> , strain: JCM 9070 T
AB271745.1	<i>Bacillus cereus</i> , strain: NBRC 15305 T
D16266.1	<i>Bacillus cereus</i> , strain: IAM 12605 T
AF290547.1	<i>Bacillus cereus</i> , strain: ATCC 14579 T
D16273.1	<i>Bacillus megaterium</i> , strain: IAM 13418 T
GU252112.1	<i>Bacillus megaterium</i> , strain: ATCC 14581 T
AB021191.1	<i>Bacillus mojavensis</i> , strain: IFO 15718 T



AB363735.1	<i>Bacillus mojavensis</i> , strain: NBRC 15718 T
AJ276351.1	<i>Bacillus subtilis</i> , strain: DSM10 T
AB680257.1	<i>Bacillus subtilis</i> , strain: NBRC 12210 T
FJ917386.1	<i>Bacillus subtilis</i> , strain: KCTC 3135 T
AB271744.1	<i>Bacillus subtilis subsp.</i> , strain: NBRC 13719 T
EF423592.1	<i>Bacillus subtilis subsp.</i> , strain: BCRC 10255 T
HQ223107.1	<i>Bacillus tequilensis</i> , strain: ATCC BAA-819 T
EU138487.1	<i>Bacillus tequilensis</i> , strain: NRRL B-41771 T
AJ312209.1	<i>Curtobacterium flaccumfaciens pv. flaccumfaciens</i> , strain: LMG 3645 T
AJ853910.1	<i>Microbacterium maritypicum</i> , strain: DSM 12512 T
AJ233423.1	<i>Pantoea agglomerans</i> , strain: DSM 3493 T
AB681812.1	<i>Pantoea agglomerans</i> , strain: NBRC 102470 T
AJ251466.1	<i>Pantoea agglomerans</i> , strain: NCTC9381T
AY691188.2	<i>Pseudomonas argentinensis</i> , strain: CH01 T
AY970952.1	<i>Pseudomonas moraviensis</i> , strain: CCM 7280 T
JQ344321.1	<i>Pseudomonas punonensis</i> , strain: LMT03 T
AJ575816.1	<i>Pseudomonas sp.</i> , strain: C36 T
AJ584833.1	<i>Psychrobacter aquatica</i> , strain: CMS 56 T

**Supplemental Table 7:** Accession numbers of partial sequences of 16S rDNA sequences published in NCBI for identification of E1 and E2.

Accession number	Isolates
NR_115531.1	<i>Sporolactobacillus terrae</i> strain M-116
KY777579.1	<i>Bacillus cereus</i> strain HD4
KU898281.1	<i>Bacillus toyonensis</i> strain J1
KF836520.1	<i>Bacillus anthracis</i> strain 262AG3
HQ917121.1	<i>Bacillus thuringiensis</i> strain X6
EF433410.1	<i>Bacillus licheniformis</i> strain BCRC 11702
DQ513325.1	<i>Paenibacillus polymyxa</i> strain JW-21
AY373357.1	<i>Bacillus mycoides</i> strain c2
KJ787122.1	<i>Bacillus axarquiensis</i> strain CHMS1B6
KU821696.1	<i>Bacillus subtilis</i> strain LLP-2

**Supplemental Table 8:** Accession numbers of partial sequences of 16S rDNA sequences published in NCBI for identification of E3.

Accession number	Isolates
JQ317790.1	<i>Pseudomonas argentinensis</i> strain W1AP5
GU935266.1	<i>Pseudomonas</i> spp. EK-I1
KY393316.1	<i>Pseudomonas argentinensis</i> strain IC32-08
FJ791163.1	<i>Pseudomonas</i> spp. enrichment culture clone C2
NR_109583.1	<i>Pseudomonas punonensis</i> strain LMT03

**Supplemental Table 9:** Antagonistic activity of endophytic bacteria selected against pathogenic fungi.

Isolates	Pathogens			
	<i>Fusarium oxysporum</i> f. sp. <i>medicaginis</i>		<i>Rhizoctonia solani</i>	
NA NM2R-6	+	-	-	+++
NA NM1S-1	++++	++	++++	+++
NA NX12S-2	++	-	+	++
NA NX16L-1	++++	-	-	++
NA NX1S-1	+++	-	+++	+++
NA NX21L-1	+++	-	++	++++
NA NX21R-3	+	-	+	+
NA NX26L-2	+	-	+	++
NA NX33L-1	++++	-	-	++

NA NX36L-1	++++	++++	++++	++++
NA NX36L-2	+++	++	+++	++++
NA NX36S-1	+	-	++	+
NA NX37S-2	++	-	-	-
NA NX37L-3	+	-	+++	++
NA NX54S-1	++	-	-	-
NA NX57R-2	++	-	++	+++
NA NX63L-2	++++	-	-	+
NA NX65S-3	++	-	++	++
NA NX67R-4	+++	-	+++	+
NA NX67S-3	++	++	-	++
NA NX73R-1	++	-	++	++
NA NX76S-1	+	-	-	+++
NA NX81R-1	++	-	++	+
NA NX40R-1	+++	-	+	+++
NA HB2S-3	++	-	++++	+
NA NX90R-1	-	-	+	+

Note: "-" indicates no bacteriostasis activity, no bacteriostasis band, T = 0; "+" indicates bacteriostasis activity, 0 < T < 1mm; "+" indicates strong bacteriostasis activity, 1mm < T < 2mm; "++" indicates strong bacteriostasis activity, 2mm < T < 5mm; "++++" indicates strong bacteriostasis activity, T < 5mm.

**Supplemental Table 10:** Inhibition of endophytic fungi against pathogenic fungi.

Isolates	Pathogens			
	<i>Fusarium oxysporum</i> f. sp. <i>medicaginis</i> (FOM)	<i>Rhizoctonia solani</i>	<i>Fusarium oxysporum</i> f. sp. <i>cucumerinum</i> (FOC)	<i>Phytophthora capsici</i>
PDA NM1S-4	86.22%	66.80%	83.61%	88.32%
PDA NX36S-1	79.56%	53.34%	71.56%	75.33%
PDA NX45L-1	71.35%	48%	76.15%	63.72%
PDA NX40S-1	74.05%	56%	67.95%	67.81%
PDA NX40L-7	72.81%	52.44%	76.35%	70.80%
PDA NX25L-1	58.03%	43.56%	77.99%	68.25%
PDA NX90S-2	89.33%	62.22%	80.74%	82.12%
PDA NX54R-1	67.29%	49.78%	75.57%	68.25%

Note: Bacteriostasis rate (%) = (colony radius of pathogenic bacteria cultured alone - colony growth radius of pathogenic fungi cultured confrontationally) / colony radius of pathogenic fungi cultured alone \*100%.

**Supplemental Table 11:** Antagonistic activity of endophytic actinomycetes against pathogenic fungi.

Isolates	Pathogens			
	<i>Fusarium oxysporum</i> f. sp. <i>medicaginis</i> (FOM)	<i>Rhizoctonia solani</i>	<i>Fusarium oxysporum</i> f. sp. <i>cucumerinum</i> (FOC)	<i>Phytophthora capsici</i>
G1 NX63L-1	++++	++++	++++	++++
G1 NX35L-1	-	-	-	-

Note: "-" indicates no bacteriostasis activity, no bacteriostasis band, T = 0; "+" indicates bacteriostasis activity, 0 < T < 1mm; "+" indicates strong bacteriostasis activity, 1mm < T < 2mm; "++" indicates strong bacteriostasis activity, 2mm < T < 5mm; "++++" indicates strong bacteriostasis activity, T < 5mm.

**Supplemental Table 12:** Control effects of the endophyte strains against Cucumber Fusarium wilt (leaf tissues).

Relative control effect (%)	Isolates		Fungi (Isolate)		Actinomycetes (Isolate)		Total (Isolate)	
	Bacteria (Isolate)	Rate (%)	Fungi (Isolate)	Rate (%)	Actinomycetes (Isolate)	Rate (%)	Total (Isolate)	Rate (%)
≤0	82	30.6%	73	79.3%	1	50%	156	43.1%
0-10	18	6.7%	9	9.8%	0	0	27	7.5%
10-20	22	8.2%	9	9.8%	0	0	31	8.6%

20-30	25	9.3%	1	1.1%	1	50%	26	7.2%
30-40	36	13.4%	0	0	0	0	36	9.9%
40-50	25	9.3%	0	0	0	0	25	6.9%
50-60	29	11.0%	0	0	0	0	29	8.0%
60-70	18	6.7%	0	0	0	0	18	5.0%
70-80	10	3.7%	0	0	0	0	10	2.8%
80-90	3	1.1%	0	0	0	0	3	0.8%
90-100	0	0	0	0	0	0	0	0
总计	268	100%	92	100%	2	100%	362	100%

Note: Relative control effect (%) = (Controlled disease index – Treatment index)×100/ Controlled disease index.

**Supplemental Table 13:** Control effects of the endophyte strains against Cucumber Fusarium wilt (root tissues).

Relative control effect (%)	Isolates Bacteria ( Isolate)	Rate (%)	Bacteria ( Isolate)	Rate (%)	Bacteria ( Isolate)	Rate (%)	Bacteria ( Isolate)	Rate (%)
≤0	79	29.5%	90	97.8%	1	50%	170	49.6%
0-10	21	7.8%	1	1.1%	0	0	22	6.1%
10-20	29	10.8%	1	1.1%	1	50%	30	8.3%
20-30	29	10.8%	0	0	0	0	29	8.0%
30-40	48	17.9%	0	0	0	0	48	13.3%
40-50	23	8.6%	0	0	0	0	23	6.4%
50-60	23	8.6%	0	0	0	0	23	6.4%
60-70	8	3.0%	0	0	0	0	8	2.2%
70-80	7	2.6%	0	0	0	0	7	1.9%
80-90	1	0.4%	0	0	0	0	1	0.3%
90-100	0	0	0	0	0	0	0	0
总计	268	100%	92	100%	2	100%	362	100%

Note: Relative control effect (%) = (Controlled disease index – Treatment index)×100/ Controlled disease index.

**Supplemental Table 14:** Screening for endophytes strains against Cucumber Fusarium wilt.

Isolate	Leaf/root	Relative effectivity (%)	Isolate	Leaf/root	Relative effectivity (%)
NA NX51R-5	leaf	84	NA NX51R-5	root	78.3
NA NX90R-8	leaf	80	NA NX90R-8	root	84
NA NM1S-1	leaf	84	NA NM1S-1	root	72
NA NX23L-2	leaf	72	NA NX33S-1	root	60
NA NX16L-3	leaf	72	NA NX35R-1	root	64
NA NX35R-1	leaf	68	NA NX17L-2	root	36
NA NX17L-2	leaf	64	NA NM1S-3	root	48
NA NM1S-3	leaf	80	NA NX55L-3	root	58
NA NX46L-3	leaf	64	NA NX52L-3	root	48

**Supplemental Table 15:** Information for partial sequences of ITS sequences published in NCBI and the similarity of eight endophytic fungi isolates with the compared isolates.

Isolates	Accession number	Genus	Compared GenBank	isolates on Similarity
PDA NM1S-4	KR868229.1	<i>Trichoderma</i>	<i>T. aureoviride</i> strain ZWPBG1	99%
PDA NX45L-1	KY764845.1	<i>Trichoderma</i>	<i>T. afroharzianum</i> isolate CTCCSJ-G-JK40051-Ma	99%
PDA NX40S-1	KU896356.1	<i>Trichoderma</i>	<i>T. harzianum</i> isolate CTCCSJ-ASD50452	99%
PDA NX40L-7	KR868283.1	<i>Trichoderma</i>	<i>T. harzianum</i> strain ZWPUEB15	99%

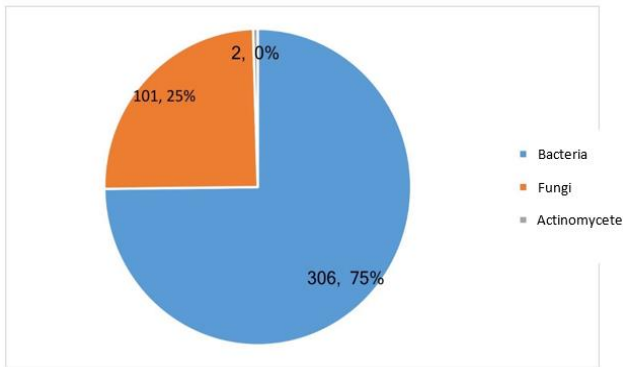
PDA NX90S-2	KU896359.1	<i>Trichoderma</i>	<i>T. harzianum</i> isolate CTCCSJ-AMH50459-Zhao	99%
PDA NX54R-1	KU896332.1	<i>Trichoderma</i>	<i>T. harzianum</i> isolate CTCCSJ-AGS50340-Zhao	99%
PDA NX25L-1	HF912167.2	<i>Rhizoctonia</i>	<i>R. solani</i> strain AG-5, isolate RCC5	99%
PDA NX36S-1	KJ620369.1	<i>Fusarium</i>	<i>F. solani</i> strain T-ICA06NP	99%

**Supplemental Table 16:** Information for partial sequences of 16S rDNA sequences published in NCBI and the similarity of eight endophytic bacteria isolates with the compared isolates.

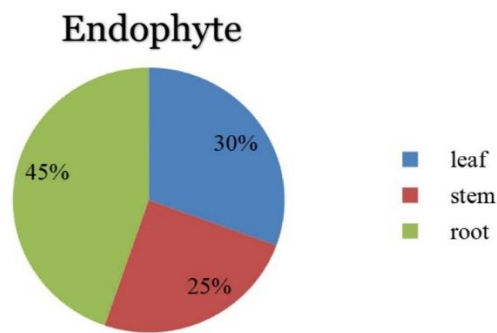
Name	Accession Number	Classification	Isolates	Identification
NA NX67S-3	GU354333.1	<i>Curtobacterium</i> spp.	Endophytic bacterium sh293 clone sh293	97%
NA NX1S-1	GU447235.1	<i>Psychrobacter</i> spp.	<i>P. aquaticus</i> strain A1	100%
NA NX21L-1	KF424661.1	<i>Microbacterium</i> spp.	<i>M. maritypicum</i> strain XH074	100%
NA NX57R-2	KT369868.1	<i>Arthrobacter</i> spp.	<i>A. sulfureus</i> strain XSH (1)	99%
NA NX36L-2	KP279888.1	<i>Curtobacterium</i> spp.	<i>C. spp.</i> DP122B	100%
NA NX37S-2	KP279888.1	<i>Curtobacterium</i> spp.	<i>C. spp.</i> DP122B	100%
NA NX73R-1	KX099273.1	<i>Brevibacterium</i> spp.	<i>B. sp.</i> strain M-14	99%
NA NX76S-1	KT765839.1	<i>Pantoea</i> spp.	<i>P. agglomerans</i> strain CZ-BHG003	99%
NA NX90R-1	KT369868.1	<i>Arthrobacter</i> spp.	<i>A. sulfureus</i> strain XSH (1)	99%
NA NX12S-2	KP343685.1	<i>Bacillus</i> spp.	<i>B. megaterium</i> strain BLZ01	99%
NA NX16L-1	KT183565.1	<i>Bacillus</i> spp.	<i>B. spp.</i> S3S555	100%
NA NX36S-1	KX891544.1	<i>Bacillus</i> spp.	<i>B. atrophaeus</i> strain 02	100%
NA NX37L-3	KJ528873.1	<i>Bacillus</i> spp.	<i>B. cereus</i> strain 6N	98%
NA NX36L-1	KF818640.1	<i>Bacillus</i> spp.	<i>B. mojavensis</i> strain 263XG8	98%
NA NX63L-2	KF818640.1	<i>Bacillus</i> spp.	<i>B. mojavensis</i> strain 263XG8	98%
NA NX65S-3	KR063197.1	<i>Bacillus</i> spp.	<i>B. megaterium</i> strain BS17	97%
NA NM1S-1	KU904283.1	<i>Bacillus</i> spp.	<i>B. subtilis</i> strain GX S-11	95%
NA NX81R-1	KC692194.1	<i>Bacillus</i> spp.	<i>B. subtilis</i> strain ML102B	99%
NA NX40R-1	KM117217.1	<i>Bacillus</i> spp.	<i>B. spp.</i> C26(2014)	99%
NA HB2S-3	KX269836.1	<i>Bacillus</i> spp.	<i>B. subtilis</i> strain CMH4X	99%
NA NM2R-6	KY440054.1	<i>Pseudomonas</i> spp.	<i>P. spp.</i> strain M6	99%
NA NX21R-3	KR189891.1	<i>Pseudomonas</i> spp.	<i>P. spp.</i> UIWRF0147	100%
NA NX26L-2	KT318820.1	<i>Pseudomonas</i> spp.	<i>P. spp.</i> RhizorgI	97%
NA NX33L-1	JQ317790.1	<i>Pseudomonas</i> spp.	<i>P. argentinensis</i> strain WIAP5	100%
NA NX54S-1	KF147126.1	<i>Pseudomonas</i> spp.	<i>P. spp.</i> R1SsM3P1C2	100%
NA NX67R-4	EU196754.1	<i>Pseudomonas</i> spp.	<i>P. spp.</i> XJ13	94%

**Supplemental Table 17:** Accession numbers of the E1, E2, and E3 endophytic bacteria based on partial sequences of 16S rDNA published in NCBI.

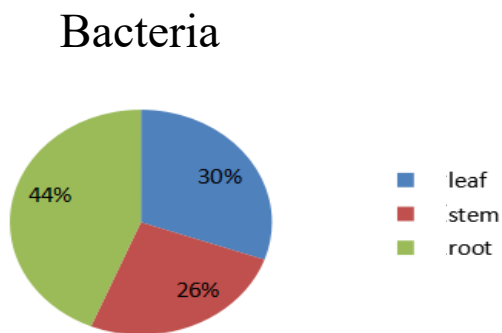
Name	Accession Number	Classification
NA NM1S-1 (E1)	SAMN11658551	<i>Bacillus</i> spp.
NA NX51R-5 (E2)	SAMN11658552	<i>Bacillus</i> spp.
NA NX90R-8 (E3)	SAMN11658553	<i>Microbacterium</i> spp.



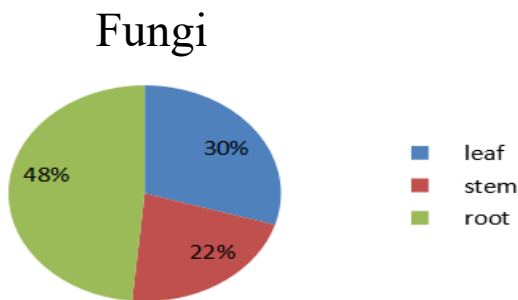
**Supplemental Figure 1:** Diversity of endophytes from collected alfalfa.



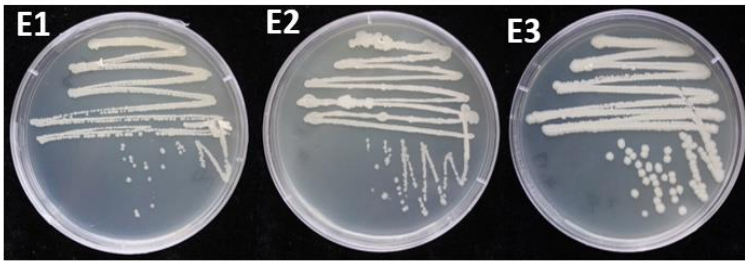
**Supplemental Figure 2:** Incidence of isolation of endophytes from alfalfa root, stem, and leaf tissues.



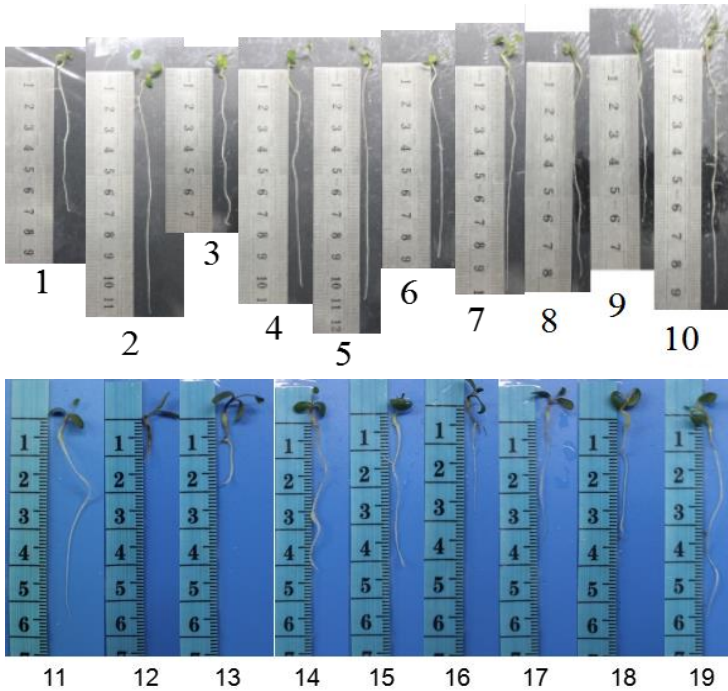
**Supplemental Figure 3:** Percentage of endophytic bacteria distributed in the root, stem, and leaf tissues of collected alfalfa.



**Supplemental Figure 4:** Percentage of endophytic fungi distributed in the root, stem, and leaf tissues of collected alfalfa.

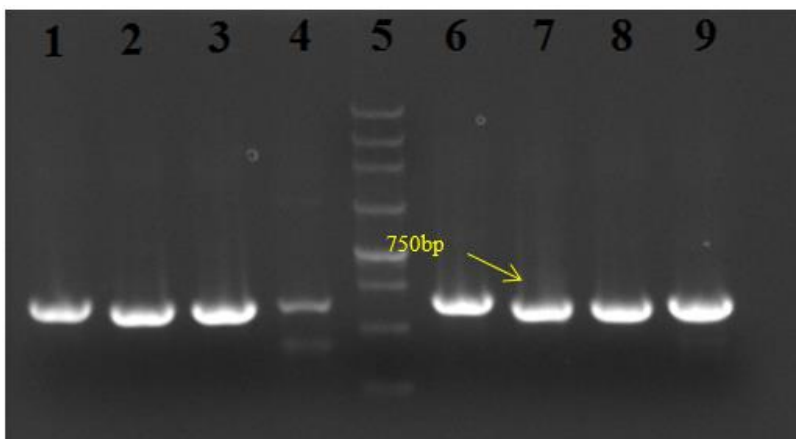


**Supplemental Figure 5:** Colonies of screened endophytic bacteria E1 (NA NM1S-1), E2 (NA NX51R-5), and E3 (NA NX90R-8).

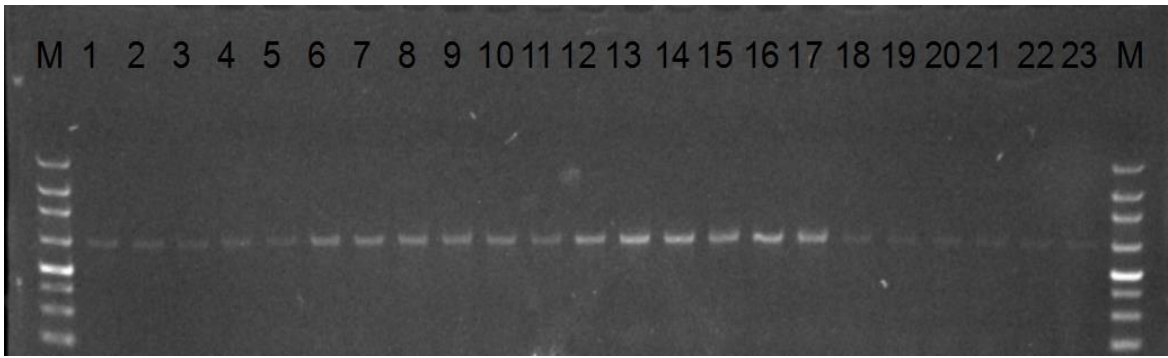


**Supplemental Figure 6:** Effect endophyte and pathogen treatments on the growth of alfalfa.

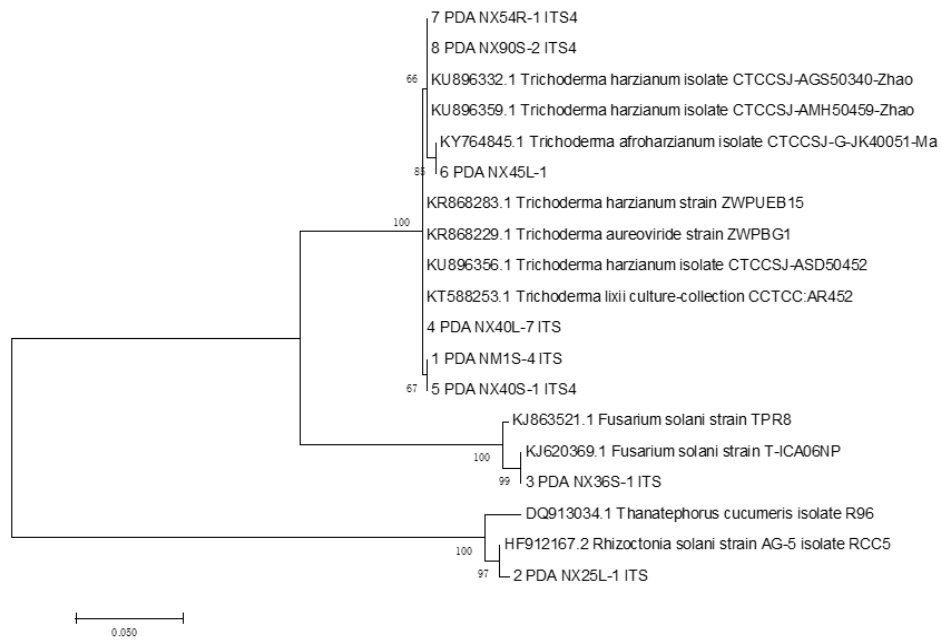
1: CK2; E1  $10^4$  CFU/ml; 3: E1  $10^6$  CFU/ml; 4: E1  $10^8$  CFU/ml; 5: E2  $10^4$  CFU/ml; 6: E2  $10^6$  CFU/ml; 7: E2  $10^8$  CFU/ml; 8: E3  $10^4$  CFU/ml; 9: E3  $10^6$  CFU/ml; 10: E3  $10^8$  CFU/ml; 11: CK; 12: F4 dilution 0X; 13: F4 dilution 10X; 14: F4 dilution 100X; 15: F4 dilution 1000X; 16: F5 dilution 0X; 17: F5 dilution 10X; 18: F5 dilution 100X; 19: F5 dilution 1000X (F4 dilution 0X OD<sub>600</sub>=2.13; F5 dilution 0X OD<sub>600</sub>=2.10).



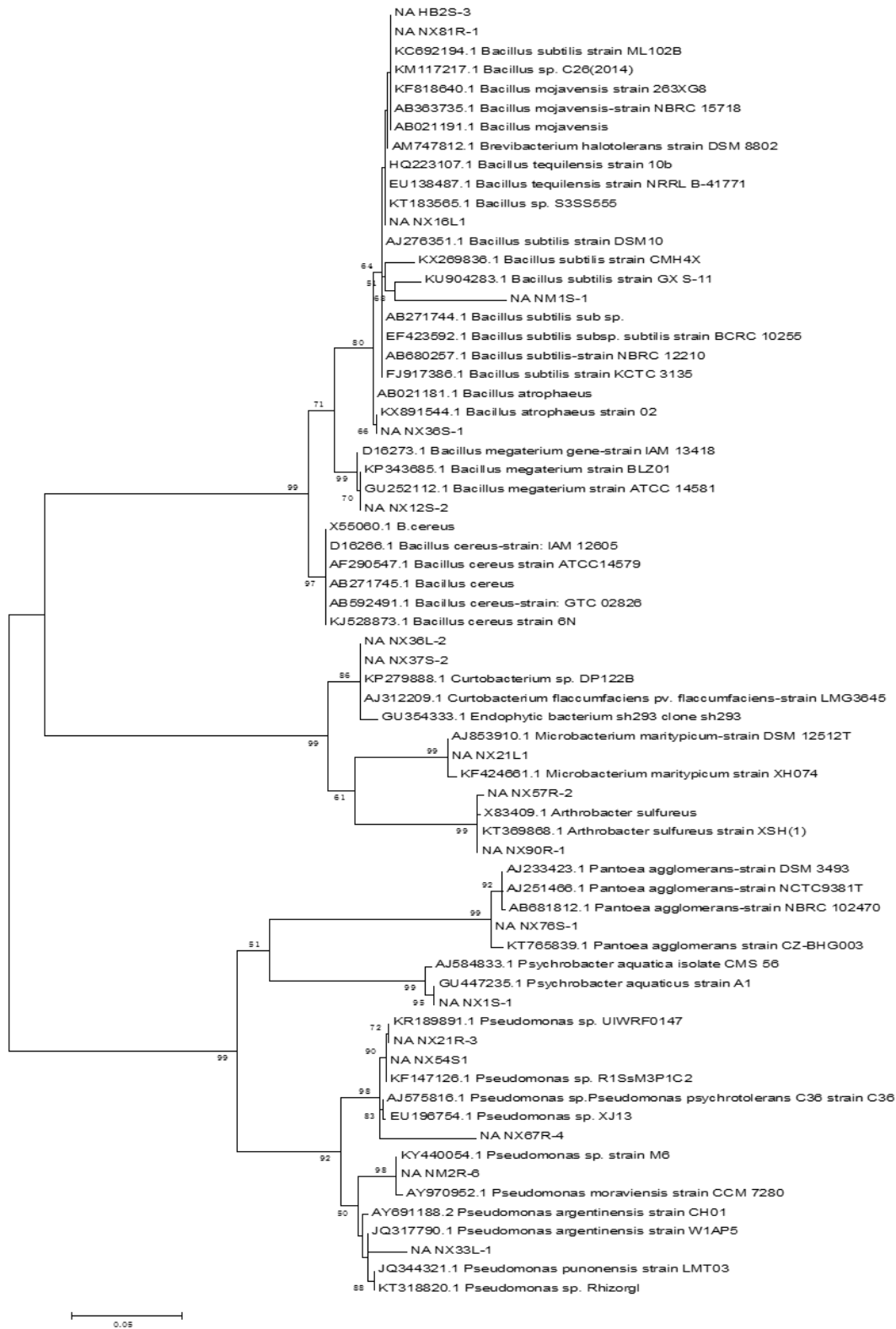
**Supplemental Figure 7:** Electrophoresis for PCR amplification of ITS sequence from endophytic fungi.



**Supplemental Figure 8:** Electrophoresis for PCR amplification of 16S rDNA sequence from endophytic bacteria.



**Supplemental Figure 9:** Phylogenetic tree of endophytic fungi.



Supplemental Figure 10: Phylogenetic tree of endophytic bacteria.