Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supporting Information

α-Fe_2O_3 nanomaterials strengthened the growth promoting effect of *Pseudomonas aurantiaca* strain JD37 on alfalfa via enhancing the nutrient interaction of plant-rhizobacteria symbiont

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Supporting Information consists of 8 pages, including 2 text, 4 tables and 6 figures.

Supporting Text

Text S1 Inorganic salt solution formulations

2.8 g/L Na_2HPO_4, 1.0 g/L KH_2PO_4, 0.5 g/L $(NH_4)_2SO_4$, 0.001 mg/L $CuCl_2 \cdot 2H_2O$, 0.5 mg/L Na_2EDTA, 0.03 mg/L H_3BO_3, 0.02 mg/L $CoCl_2 \cdot 6H_2O$, 0.2 mg/L $FeSO_4 \cdot 7H_2O$, 0.01 mg/L $ZnSO_4 \cdot 7H_2O$, 0.003 mg/L $MnCl_2 \cdot 4H_2O$, 0.003 mg/L $Na_2MoO_4 \cdot 2H_2O$, 0.002 mg/L $NiCl_2 \cdot 6H_2O$, 0.113 μg/L $MgCl_2 \cdot 6H_2O$, 0.05 mg/L $Ca(NO_3)_2 \cdot 4H_2O$. The chemicals were purchased from Beijing Coolaber technology Co., China.

Text S2 Genetical evidence for the conformation of JD37 in the rhizosphere

JD37 (*Pseudomonas chlororaphis* subsp. *aurantiaca* JD37) is a plant growth-promoting rhizobacterium, a Gram-negative bacterium isolated from potato rhizosphere soil (Shanghai, China)[1]. To identify the colonization of JD37 in the rhizophere, the total DNA in soil was extracted with the E.Z.N.A soil DNA Kit (Omega Biotek, USA) by Majorbio. Inc., China. Then the DNA concentration and purity were evaluated by a UV-vis spectrophotometer (NanoDrop 2000, Thermo Scientific, USA), and the DNA quality was assessed by agarose gel electrophoresis. According to the information of JD37[1,2], a pair of primers (F: ACCACCTGGACTGATACTGA, R: AAGGGCCATGATGACTTG; NCBI Accession number: OM698823; Product size: 480 bp.) was designed. qPCR reactions were performed using a real-time PCR system (Applied Biosystems StepOnePlus, CA) to amplify the genes of JD37[3]. The program setup was as these three stages below: 95 °C for 10 min; 35 cycles of 95 °C for 10 s, 50 °C for 10 s, and then 72 °C for 20 s; followed by a melt curve for specificity verification. Amplification reactions were performed with a final volume of 23.8 μL including 10 μL of SYBR Green ChemoHS qPCR Mix (Monad, China), 0.4 μL of forward primer, 0.4 μL of reverse primer and 5 μL of DNA template. The integrities and forms of soil DNA were analyzed with electrophoresis on a 1.0% agarose gel stained with 2% DuRed 10000× (Bridgen, China). Bands were visualized by a gel imaging system (Syngene, U.K.). Finally, the band brightness was quantified using image J software with 3 replicates.

Supporting Tables

Table S1 Resulting table of McFarland method

Tube number (McFarland)	0.5	1	2	3	4
1% $BaCl_2$ (mL)	0.05	0.1	0.2	0.3	0.4
1% H_2SO_4 (mL)	9.95	9.9	9.8	9.7	9.6
the approximate bacteria concentration ($\times 10^8$ CFU/mL)	1.5	3	6	9	12
OD_{600}*	0.1006	0.1446	0.2375	0.3342	0.4037

*The average of measured OD_{600}, n=3.

Table S2 Original soil minerals contents

Minerals	Contents
SiO_2	68.28 ± 5.81%
Al_2O_3	17.42 ± 2.60%
Fe_2O_3	4.96 ± 0.74%
K_2O	2.41 ± 0.92%
MgO	1.36 ± 1.37%
CaO	1.09 ± 0.63%
Na_2O	1.02 ± 1.94%
TiO_2	0.66 ± 0.91%
P_2O_5	0.15 ± 0.32%

Table S3 Metabolic pathways with significant differences caused by treatments related to 1.5×10^9 CFU/plant JD37

Treatment	Number of differential metabolic pathways	Metabolic pathways
9F vs CKN	8	(1)Fatty acid biosynthesis; (2)Pyrimidine metabolism; (3)Pentose and glucuronate interconversions; (4)Butanoate metabolism; (5)Glycerolipid metabolism; (6)Galactose metabolism; (7)Starch and sucrose metabolism; (8)Alanine, aspartate and glutamate metabolism
9F vs 9N	4	(1)Fatty acid biosynthesis; (2)Pyrimidine metabolism; (3)Pentose and glucuronate interconversions; (4)Glycerolipid metabolism
CKF vs CKN	4	(1)Pyrimidine metabolism; (2)Pentose and glucuronate interconversions; (3)Ascorbate and aldarate metabolism; (4)Inositol phosphate metabolism;
9F vs CKF	3	(1)Fatty acid biosynthesis; (2)Pyrimidine metabolism; (3)Pentose and glucuronate interconversions
9N vs CKN	3	(1)Pyrimidine metabolism; (2)Pentose and glucuronate interconversions; (3)Butanoate metabolism

Table S4 Metabolic pathways with significant differences caused by treatments related to 1.5 × 10^8 CFU/plantJD37

Treatment	Number of differential metabolic pathways	Metabolic pathways
8F vs CKN	7	(1)Fatty acid biosynthesis; (2)Inositol phosphate metabolism; (3)Pentose and glucuronate interconversions; (4)Pyrimidine metabolism; (5)Ascorbate and aldarate metabolism;(6)Butanoate metabolism; (7)Alanine, aspartate and glutamate metabolism
8F vs 8N	2	(1)Pentose and glucuronate interconversions; (2)Pyrimidine metabolism
CKF vs CKN	4	(1)Pyrimidine metabolism; (2)Pentose and glucuronate interconversions; (3)Inositol phosphate metabolism; (4)Ascorbate and aldarate metabolism
8F vs CKF	2	(1)Ascorbate and aldarate metabolism; (2)Inositol phosphate metabolism
8N vs CKN	2	(1)Butanoate metabolism; (2)Alanine, aspartate and glutamate metabolism

Supporting Figures

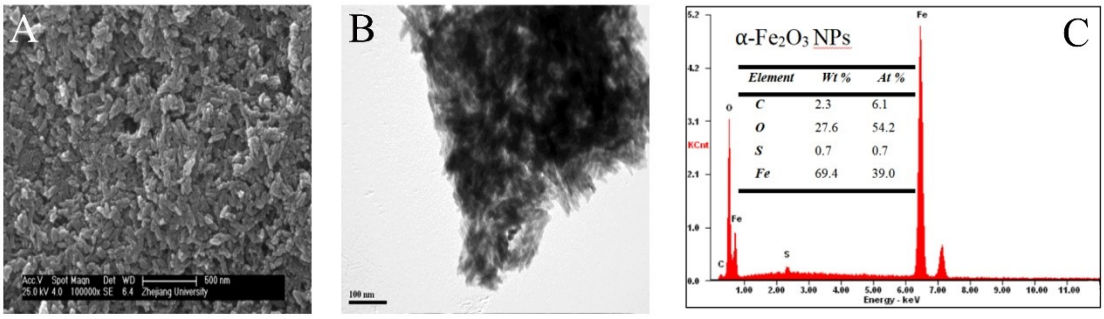

Fig S1. SEM (A), TEM (B) images and EDS analyses (C) of α-Fe_2O_3 (The data was adopted from our previous study[4]).

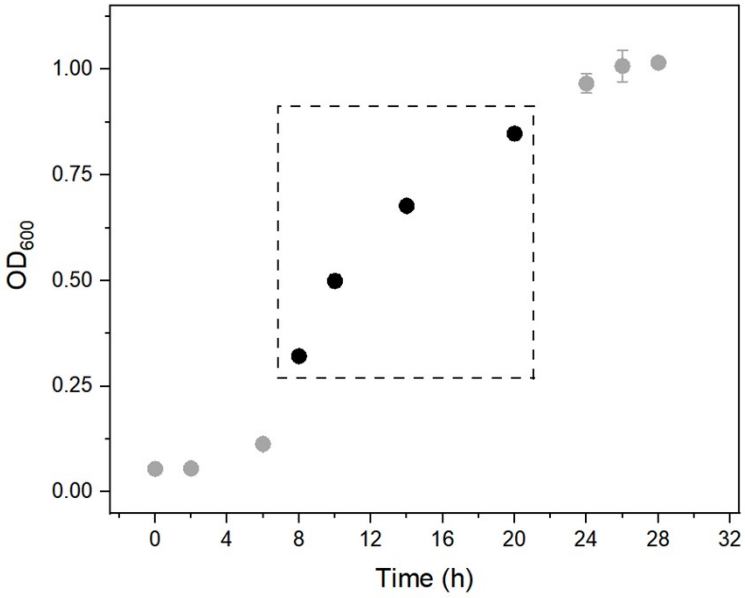

Fig. S2 JD37 growth kinetics within 32 h.

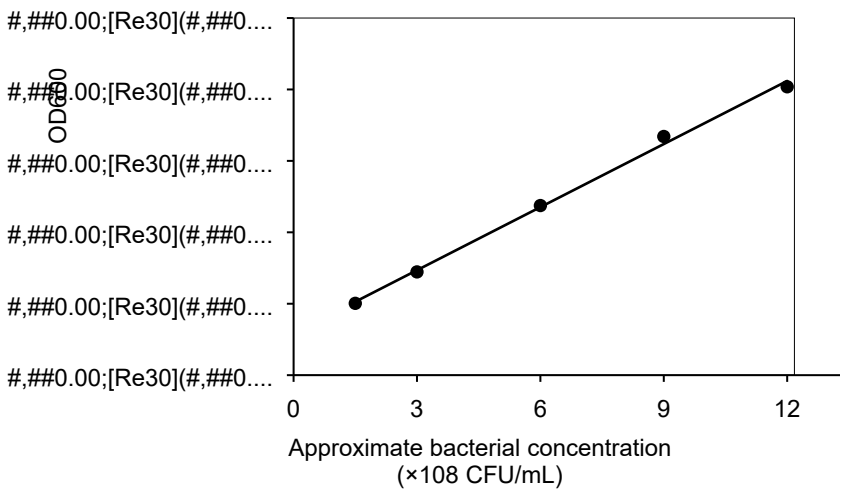

Fig. S3 Linear relationship between the turbidity and the concentration of bacteria.

Fig. S4 Overall physical appearance of alfalfa plants in soil treated by JD37 with or without α-Fe_2O_3 NMs after 65 d. Abbreviations for different treatments: (1) CKF: 1000 mg/kg α-Fe_2O_3 NMs, (2) 7F: 1000 mg/kg α-Fe_2O_3 NMs and 1.5×10^7 CFU/plant JD37, (3) 8F: 1000 mg/kg α-Fe_2O_3 NMs and 1.5×10^8 CFU/plant JD37,

(4) 9F: 1000 mg/kg α-Fe_2O_3 NMs and 1.5×10^9 CFU/plant JD37, (5) CKN: The control group without NMs or PGPR; (6) 7N: 1.5×10^7 CFU/plant JD37, (7) 8N: 1.5×10^8 CFU/plant JD37, (8) 9N: 1.5×10^9 CFU/plant JD37.

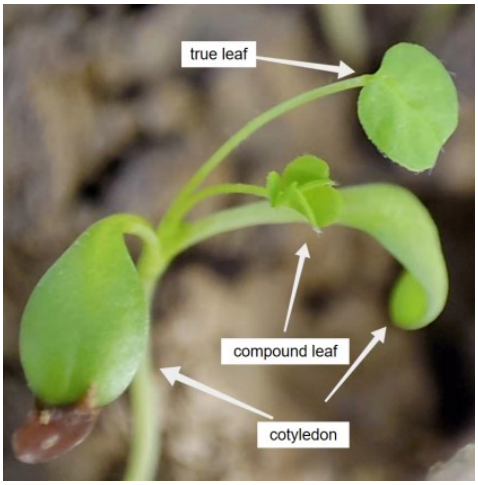

Fig. S5 The cotyledons, true leaf and compound leaf of alfalfa.

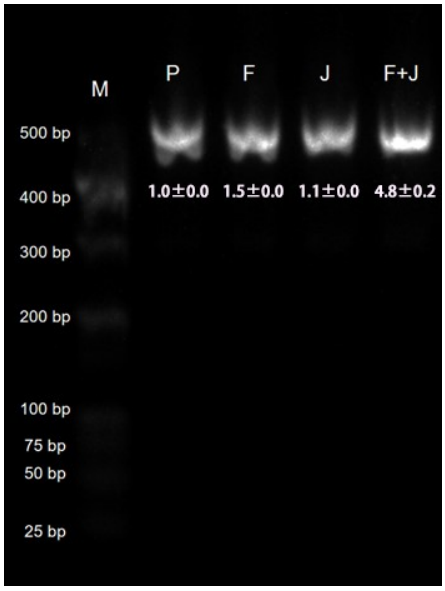

Fig. S6 Agarose gel electrophoresis of JD37 16s rRNA from the rhizosphere soil. P: control, F: treatment applied with 1000 mg/kg α-Fe_2O_3 NMs, J: treatment applied with 1.5×10^9 CFU/plant JD37, F+J: treatment applied with 1000 mg/kg α-Fe_2O_3 NMs and 1.5×10^9 CFU/plant JD37). The numbers below the bands represent the relative value of light intensity measured by the image J software.

References

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