

# *Azospirillum Actinidiae* sp. nov., a Nitrogen-Fixing Bacterium Isolated From The Roots of Kiwifruit Plants

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## Research Article

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# Abstract

A novel diazotrophic bacterium, designated CCTCC AB 2021101<sup>T</sup>, was isolated from fresh roots of kiwifruit. Cells of strain CCTCC AB 2021101<sup>T</sup> were Gram-negative, aerobic and rod-shaped, with motility provided by peritrichous flagella. The 16S rRNA analysis showed that strain CCTCC AB 2021101<sup>T</sup> belongs to the genus *Azospirillum* and is closely related to *Azospirillum melinis* (98.32%), *Azospirillum oryzae* (97.73%), *Azospirillum lipoferum* (96.98%), *Azospirillum humicireducens* (96.49%) and *Azospirillum largimobile* (96.01%) and lower sequence similarity (<96.0 %) to all other species of the genus *Azospirillum*. Strain CCTCC AB 2021101<sup>T</sup> was able to grow well at 35–40°C and pH 6.0–7.0, and tolerated up to 3.0 % (w/v) NaCl. The major saturated fatty acids are C<sub>14:0</sub>, C<sub>16:0</sub> and C<sub>18:0</sub>. C<sub>18:1</sub> ω7c and C<sub>16:0</sub> 3-OH were the major unsaturated and hydroxylated fatty acid. The G+C content was 67.8 mol%. Strain CCTCC AB 2021101<sup>T</sup> gave positive amplification for dinitrogen reductase (*nifH* gene). Highest *nifH* gene sequence similarities were obtained with *Azospirillum brasilense* AWB14<sup>T</sup> (95.9%), *Azospirillum zea* Gr24<sup>T</sup> (95.56%), *Azospirillum picis* DSM 19922<sup>T</sup> (96.79%), *Azospirillum lipoferum* B22<sup>T</sup> (94.88%) and *Azospirillum oryzae* COC8<sup>T</sup> (94.88%). The activity of the nitrogenase of the strain was further confirmed by acetylene-reduction assay, which was recorded as 81 nmol ethylene h<sup>-1</sup>. Based on these data, strain CCTCC AB 2021101<sup>T</sup> is considered to represent a novel endophytic diazotrophs species in the genus *Azospirillum*, for which the name *Azospirillum actinidiae* sp. nov. is proposed. The type strain is CCTCC AB 2021101<sup>T</sup>.

## Introduction

Plant Growth-Promoting Bacteria (PGPB) were a large number of probiotic communities widely distributed in soil (Glick, 2012). They were free-living around the roots of plants or colonized some or a portion of a plant's interior tissues to form specific symbiotic relationships with plants (e.g., *Rhizobia* spp. and *Frankia* spp.) (Gtari et al., 2020; Liu et al., 2019). PGPB promote plant growth directly usually by facilitating resource acquisition or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various biotic and abiotic stresses (Glick, 1995). For instance, the diazotrophic bacteria are able to fix nitrogen and provide it to plants (Fox et al., 2016; Geddes et al., 2015; Ren et al., 2019). Some soil bacteria were described to promote solubilization and bioavailability of inorganic or organic phosphorus by synthesizing low molecular weight organic acids or various phosphatases (Zeng et al., 2017; Rodríguez&Fraga, 1999; Rodriguez et al., 2004). And some other bacteria were shown to synthesize low-molecular mass siderophores, molecules with an exceptionally high affinity for Fe<sup>3+</sup> to facilitate iron uptake by plants (Jin et al., 2006; Sharma et al., 2003; Siebner-Freibach et al., 2003). Even bacterial volatile was produced to protect plants from drought stress (Song et al., 2008).

*Azospirillum* is one of the most important PGPB and considered as biofertiliser due to its plant-growth-promoting activities (Cassán&Diaz-Zorita, 2016; Cassán et al., 2009). Two original species including *Azospirillum lipoferum* and *Azospirillum brasilense* from the genus *Azospirillum* were first described (Tarrand et al., 1978). Currently, the genus comprises 23 species validly published and recorded in the

LPSN website (Euzéby's nomenclature list, <http://www.bacterio.net/azospirillum.html>). Members including *Azospirillum lipoferum*, *Azospirillum halopraeferens*, *Azospirillum doebereineriae*, *Azospirillum oryzae* and *Azospirillum melinis* have been isolated from the roots of various wild grasses or cultivated crops (Eckert et al., 2001; Peng et al., 2006; Reinhold et al., 1987; Tarrand et al., 1978; Xie&Yokota, 2005). Although the nitrogen fixation in planta has not yet been shown unequivocally in *Azospirillum*, the contribution of *Azospirillum* to plant growth is significant. It is generally accepted that members of the genus *Azospirillum* can enhance the growth of plants by the production of phytohormones, which are signal molecules that interfere with plant metabolism (Bashan&Holguin, 1997). For example, IAA was shown to be biosynthesized by *Azospirillum brasilense* to modify root morphology of wheat (Dobbelaere et al., 1999).

Kiwifruit is often referred as the king of fruits owing to its remarkably high vitamin C content and abundant minerals (Ferguson&Ferguson, 2013; Richardson et al., 2018). Its plant is dioecious and belongs to the genus *Actinidia*, which contains 54 species and 75 taxa (Li, 2007). Recent years, important studies about kiwifruit focus on genome sequencing, sex determination, nutrient biosynthesis and plant disease (Huang et al., 2013; Akagi et al., 2018; Li et al., 2018; Tang et al., 2019; Wang et al., 2019; Yue et al., 2020). Probiotics in the roots or rhizosphere soil of kiwifruit plants has not yet been reported. In the present study, a novel diazotrophic bacterium belonging to genus *Azospirillum* was isolated from the roots of kiwifruit plants, for which the name *Azospirillum actinidiae* was proposed. And the phylogenetic position and physiological properties were investigated. The nitrogen-fixing capability was determined by acetylene-reduction assay using a gas chromatograph system.

## Materials And Methods

### Isolation

Roots of F1 seedlings from a cross between *Actinidia chinensis* and *Actinidia eriantha* were collected from orchard of Anhui agricultural university in Anhui province, China. They were washed with sterile water to remove the rhizosphere soil on the surface. Then the roots were sterilized with 75% (w/v) alcohol and 8% (w/v) sodium hypochlorite for 2 mins respectively and washed using sterilized deionized water four times. Solid NFB medium supplemented with 0.01g/L KNO<sub>3</sub> were inoculated with serial dilutions of crushed roots. The composition of the NFB medium is as follows (L<sup>-1</sup>): malate (5.0 g); K<sub>2</sub>HPO<sub>4</sub> (0.5 g); MgSO<sub>4</sub>·7H<sub>2</sub>O (0.2 g); NaCl (0.1 g); CaCl<sub>2</sub>·2H<sub>2</sub>O (0.02 g); bromothymol blue 0.5% in 0.2 M KOH (2 ml); sterile, filtered vitamin solution (1 ml); sterile, filtered micronutrient solution (2 ml); 1.64% FeEDTA solution (4 ml); KOH (4.5 g). The pH was adjusted to 6.8 and 1.75 g/L agar was added. The vitamin solution contains, in 100 ml, biotin (10 mg) and pyridoxol-HCl (20 mg), dissolved at 100 °C in a water bath. The micronutrient solution consists of the following (L<sup>-1</sup>): CuSO<sub>4</sub>·5H<sub>2</sub>O (40 mg); ZnSO<sub>4</sub>·7H<sub>2</sub>O (0.12 g); H<sub>3</sub>BO<sub>3</sub> (1.4 g); Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O (1.0 g); MnSO<sub>4</sub>·H<sub>2</sub>O (1.175 g). After 24h incubation at 37 °C, one loop of pellicle-forming culture was transferred into fresh broth medium. Further purification was done on NFB agar plates. The purified strain was preserved at -80°C as a glycerol suspensions.

## Phylogenetic analysis

To establish the phylogenetic position of strain CCTCC AB 2021101<sup>T</sup>, the 16S rRNA gene sequence was determined in this study and subjected to comparative analysis. Genomic DNA from cells was extracted using a commercial genomic DNA extraction kit (Aidlab Biotechnologie). The primer pair 27F (5'-AGAGTTTGATCCT GGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') was used for amplification of the 16S rRNA gene. The PCR product was gel purified using Gel Extraction kit D2500-01 (Omega Biotek) and then cloned into a plasmid vector using a TA cloning kit (TaKaRa). The 16S rRNA gene cloned in the plasmid vector was sequenced by Sangon (Shanghai, China) using a downstream vector primer M13R (5'-CAGGAAACAGCTATGACC-3') and an upstream vector primer M13F 5'-GTAAAACGACGGCCAGT-3') as the sequencing primers. Identification of phylogenetic neighbours and the calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon-e server (<http://eztaxon-e.ezbiocloud.net/>; (Kim et al., 2012)). Multiple alignments were performed using the CLUSTAL X program (Thompson, 1997). Phylogenetic trees with 1200 bootstrap replications were reconstructed using the MEGA 6.0 program with the maximum composite likelihood model (Tamura et al., 2013). Clustering was performed with the neighbourjoining method (Saitou&Nei, 1987).

## Morphological examination and the optimum growth condition

Cell morphology was studied by scanning electron microscope (S-4800, Hitachi) after bacterial were immersed in 2.5% (v/v) glutaraldehyde fixative solution at 4°C for 12 hours and by transmission electron microscopy (JEM-1400; JEOL) after staining with 0.2 % uranyl acetate as well as by light microscopy (model A3000; Zeiss). Gram-staining was performed as described by Beveridge et al. (2009). Growth was tested using nutrient broth of NMS, NFB, TY and LB at 30-45°C (5°C increments) and pH 5–9 (1 pH unit increments).

## Measurement of cellular fatty acid and G+C content

Cellular fatty acid profiles of isolate *A. actinidiae* CCTCC AB 2021101<sup>T</sup>, *A. humicireducens* SgZ-5<sup>T</sup>, *A. lipoferum* ATCC 29707<sup>T</sup> and *A. oryzae* COC8<sup>T</sup> were determined with a gas chromatograph, using the Sherlock Microbial Identification System (MIDI), according to a standard protocol (Garcia et al., 1993). For the analysis of DNA G+C content, DNA samples were prepared and degraded enzymically into nucleosides as described by Mesbah et al. (1989).

## *nifH* PCR amplification

To estimate nitrogen-fixing ability of the isolate, a 320-base fragment of the *nifH* gene (encoding dinitrogenase reductase) was amplified from extracted DNA using two pairs of primers, FGPH19 (5'-TACGGCAARGGTGGNATHG-3') plus POLR (5'-ATSGCCATCATYTCCCGGA-3') and AQER (5'-GACGATGTAGATYTCC TG-3') plus POLF (5'-TGCGAYCCSAARGCGBACTC-3'). The PCR condition was described by Poly et al. and this product was purified and sequenced (Poly et al., 2001). The resulting sequence was compared with *nifH* sequences retrieved from NCBI using BLAST.

## Acetylene-reduction assay

To further confirm the nitrogen-fixing capability of the isolate, the acetylene-reduction assay was performed using the described procedure (Hardy et al., 1973). 50 ml vials containing 15 ml NFB medium were inoculated with strain CCTCC AB 2021101<sup>T</sup>, sealed with rubber septa and incubated at 30°C in the dark. When the OD was 0.8, 10 % (v/v) of the air phase was replaced with acetylene (Koch&Evans, 1966) and the vials were reincubated. The amount of ethylene was measured over 48h by using a gas chromatograph system (7820A, Agilent Technology).

## Results And Discussion

### Phylogenetic relationship

The 16S rRNA sequence analysis revealed that our isolated strain is a member of the genus *Azospirillum*, clustering with other *Azospirillum* species (Fig. 1). The closest relatives to strain CCTCC AB 2021101<sup>T</sup> are *A. melinis* LMG 24250<sup>T</sup>, *A. oryzae* COC8<sup>T</sup>, *A. lipoferum* ATCC 29707<sup>T</sup>, *A. humicireducens* SgZ-5<sup>T</sup> and *A. largimobile* ACM 2041<sup>T</sup> with 98.32%, 97.73%, 96.98%, 96.49% and 96.01% sequence similarity, respectively. Other species showed lower levels of similarity (<96.0 %).

### Description of *Azospirillum actinidiae* sp. nov.

Cells are Gram-negative, aerobic, rod-shaped (3.1-3.3 µm in length and 1.1-1.3 µm in width), motile with peritrichous flagella and mesophilic (Fig. 2 and 3). Colonies are white, circular and raised after 48 hours of incubation on NFB at 30°C. Colony size is about 1–2 mm in diameter. Four mediums (NMS, NFB, TY and LB) were tested for optimum growth. The bacterial strain can grow well in NFB and TY medium (Fig. 4). The optimum temperature for growth was tested using 30, 35, 40 and 45°C, respectively. The best growth was observed at 35 and 40°C and no growth occurred at 45°C (Fig.5). Of the range of pH values tested (5.0–9.0), the best growth occurred at pH 6.0–7.0 (Fig.6). Growth did not occur in the presence of 3% NaCl (Fig.7). The major saturated fatty acids were C<sub>14:0</sub>, C<sub>16:0</sub> and C<sub>18:0</sub>. C<sub>18:1</sub> ω7c and C<sub>16:0</sub> 3-OH were the major unsaturated and hydroxylated fatty acids (Table 1). The G+C content of the genomic DNA of strain CCTCC AB 2021101<sup>T</sup> was determined to be 67.8±0.2 mol%, which is within the range for similarity with the genus *Azospirillum*.

### Nitrogen-fixing ability

Nitrogenase gene *nifH* involved in activation of the Fe protein, iron molybdenum cofactor biosynthesis and electron donation was required for nitrogen fixation. To verify nitrogen-fixing ability of strain CCTCC AB 2021101<sup>T</sup>, the *nifH* gene was amplified and the expected 320 bp amplification product was obtained (Fig. 8). This PCR product was purified and sequenced. The sequence was deposited in GenBank (OL958547). Comparison of the results through an NCBI BLAST search revealed that highest sequence

similarities with the *nifH* gene of CCTCC AB 2021101<sup>T</sup> were *A. brasilense* AWB14<sup>T</sup>(95.9%), *A. zeae* Gr24<sup>T</sup>(95.56%), *A. picis* DSM 19922<sup>T</sup>(96.79%), *A. lipoferum* B22<sup>T</sup>(94.88%) and *A. oryzae* COC8<sup>T</sup>(94.88%).

To confirm the activity of the nitrogenase, acetylene-reduction assay was performed by a gas chromatograph system in NFB medium. The acetylene and ethylene standard gas had a strong signal at the retention time of 4.708 min and 4.219 min, respectively (Fig.9 a and b). In the culture medium without inoculation, there were a CO<sub>2</sub> signal and an acetylene peak at the retention time of 2.909 min and 4.651 min, respectively (Fig.9 c). The ethylene peaks were detected at the retention time of 4.222 min and 4.217 min in the culture medium inoculated by positive control *Sinorhizobium meliloti* (Rm1021) and *Azospirillum actinidiae* (Fig. 9 d and e), indicating reduction process of acetylene to ethylene. The activity was recorded as 81 nmol ethylene per hour at 30 °C. This amount is comparable with the values of other *Azospirillum* species, namely, *A. rugosum* IMMIB AFH-6<sup>T</sup>, *A. picis* IMMIB TAR-3<sup>T</sup>, *A. formosense* CC-Nfb-7<sup>T</sup> and *A. humicireducens* SgZ-5<sup>T</sup>, whose activity of the nitrogenase were 18, 93, 25 and 105 nmol ethylene h<sup>-1</sup>, respectively (Lin et al., 2012; Zhou et al., 2013; Lin et al., 2009; Young et al., 2008).

## Declarations

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### Competing interests

The authors have no relevant financial or non-financial interests to disclose.

## References

1. Akagi T et al (2018) A Y-encoded suppressor of feminization arose via lineage-specific duplication of a cytokinin response regulator in kiwifruit. *Plant Cell* 30:780–795. <https://doi.org/10.1105/tpc.17.00787>
2. Bashan Y, Holguin G (1997) *Azospirillum*–plant relationships: environmental and physiological advances (1990–1996). *Can J Microbiol* 43:103–121. <https://doi.org/10.1139/m97-015>
3. Beveridge TJ et al (2009) Use of the gram stain in microbiology. *Biotech Histochem* 76:111–118 <https://doi.org/10.1080/bih.76.3.111.118>
4. Cassán F, Diaz-Zorita M (2016) *Azospirillum* sp. in current agriculture: From the laboratory to the field. *Soil Biol Biochem* 103:117–130. <https://doi.org/10.1016/j.soilbio.2016.08.020>

5. Cassán F et al (2009) Cadaverine production by *Azospirillum brasilense* and its possible role in plant growth promotion and osmotic stress mitigation. *Eur J Soil Biol* 45:12–19.  
<https://doi.org/10.1016/j.ejsobi.2008.08.003>
6. Dobbelaere S et al (1999) Phytostimulatory effect of *Azospirillum brasilense* wild type and mutant strains altered in IAA production on wheat. *Plant Soil* 212:153–162.  
<https://doi.org/10.1023/A:1004658000815>
7. Eckert B et al (2001) *Azospirillum doebereineriae* sp. nov., a nitrogen-fixing bacterium associated with the C4-grass *Miscanthus*. *Int J Syst Evol Micr* 51:17–26. <https://doi.org/10.1099/00207713-51-1-17>
8. Ferguson AR, Ferguson LR (2013) Are kiwifruit really good for you? *Acta Hort* 131–138.  
<https://doi.org/10.17660/ActaHortic.2003.610.16>
9. Fox AR et al (2016) Major cereal crops benefit from biological nitrogen fixation when inoculated with the nitrogen-fixing bacterium *Pseudomonas protegens* Pf-5 X940. *Environ Microbiol* 18:3522–3534.  
<https://doi.org/10.1111/1462-2920.13376>
10. Garcia BM et al (1993) Gas chromatographic whole-cell fatty acid analysis as an aid for the identification of mixed mycobacterial cultures. *J Chromatography B: Biomedical Sciences and Applications* 2:299–303. [https://doi.org/10.1016/0378-4347\(93\)80502-U](https://doi.org/10.1016/0378-4347(93)80502-U)
11. Geddes BA et al (2015) Use of plant colonizing bacteria as chassis for transfer of N<sub>2</sub>-fixation to cereals. *Curr Opin Biotech* 32:216–222. <https://doi.org/10.1016/j.copbio.2015.01.004>
12. Glick BR (1995) The enhancement of plant growth by free-living bacteria. *Can J Microbiol* 41:109–117. <https://doi.org/10.1139/m95-015>
13. Glick BR (2012) Plant Growth-Promoting Bacteria: mechanisms and applications. *Scientifica*, 2012, 1-15. <https://doi.org/10.6064/2012/963401>
14. Gtari M et al (2020) *Frankia soli* sp. nov., an actinobacterium isolated from soil beneath *Ceanothus jepsonii*. *Int J Syst Evol Micr* 70:1203–1209. <https://doi.org/10.1099/ijsem.0.003899>
15. Hardy R et al (1973) Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. *Soil Biol Biochem* 5:47–81. [https://doi.org/10.1016/0038-0717\(73\)90093-X](https://doi.org/10.1016/0038-0717(73)90093-X)
16. Huang S et al (2013) Draft genome of the kiwifruit *Actinidia chinensis*. *Nat Commun* 4:2640–2648.  
<https://doi.org/10.1038/ncomms3640>
17. Jin CW et al (2006) Mechanisms of microbially enhanced Fe acquisition in red clover (*Trifolium pratense* L.). *Plant Cell Environ* 29:888–897. <https://doi.org/10.1111/j.1365-3040.2005.01468.x>
18. Kim O et al (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Micr* 62:716–721.  
<https://doi.org/10.1099/ijs.0.038075-0>
19. Koch B, Evans HJ (1966) Reduction of acetylene to ethylene by soybean root nodules. *Plant Physiol* 41:1748–1750. <https://doi.org/10.1104/pp.41.10.1748>
20. Li H et al (2018) First report of *diaporthe passiflorae* and *diaporthe nobilis* causing a postharvest kiwifruit rot in Sichuan province, China. *Plant Dis* 103:771. <https://doi.org/10.1094/PDIS-07-18-1220->

21. Li J et al (2007) In: Wu Z (ed) Flora of China,. In: vol 12. Science Press, Beijing, pp 334–362
22. Lin S et al (2012) *Azospirillum formosense* sp. nov., a diazotroph from agricultural soil. *Int J Syst Evol Micr* 62:1185–1190. <https://doi.org/10.1099/ijs.0.030585-0>
23. Lin SY et al (2009) *Azospirillum picis* sp. nov., isolated from discarded tar. *Int J Syst Evol Micr* 59:761–765. <https://doi.org/10.1099/ijs.0.65837-0>
24. Liu C et al (2019) A protein complex required for polar growth of rhizobial infection threads. *Nat Commun* 10:2848. <https://doi.org/10.1038/s41467-019-10029-y>
25. Mesbah M et al (1989) Precise measurement of the G+C content of deoxyribonucleic acid by high performance liquid chromatography. *Int J Syst Bacteriol* 39:159–167. <https://doi.org/10.1099/00207713-39-2-159>
26. Peng G et al (2006) *Azospirillum melinis* sp. nov., a group of diazotrophs isolated from tropical molasses grass. *Int J Syst Evol Micr* 56:1263–1271. <https://doi.org/10.1099/ijs.0.64025-0>
27. Poly F et al (2001) Improvement in the RFLP procedure for studying the diversity of *nifH* genes in communities of nitrogen fixers in soil. *Res Microbiol* 152:95–103. [https://doi.org/10.1016/S0923-2508\(00\)01172-4](https://doi.org/10.1016/S0923-2508(00)01172-4)
28. Reinhold B et al (1987) *Azospirillum halopraeferens* sp. nov., a nitrogen-fixing organism associated with roots of Kallar Grass (*Leptochloa fusca* (L.) Kunth). *Int J Syst Bact* 37:43–51. <https://doi.org/10.1099/00207713-37-1-43>
29. Ren B et al (2019) Rhizobial tRNA-derived small RNAs are signal molecules regulating plant nodulation. *Science* 365:919–922. <https://doi.org/10.1126/science.aav8907>
30. Richardson DP et al (2018) The nutritional and health attributes of kiwifruit: a review. *Eur J Nutr* 57:2659–2676. <https://doi.org/10.1007/s00394-018-1627-z>
31. Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol Adv* 17:319–339. [https://doi.org/10.1016/S0734-9750\(99\)00014-2](https://doi.org/10.1016/S0734-9750(99)00014-2)
32. Rodriguez H et al (2004) Gluconic acid production and phosphate solubilization by the plant growth-promoting bacterium *Azospirillum* spp. *Sci Nat-Heidelberg* 91:552–555. <https://doi.org/10.1007/s00114-004-0566-0>
33. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425. <https://doi.org/10.1093/oxfordjournals.molbev.a040454>
34. Sharma A et al (2003) Plant growth-promoting bacterium *Pseudomonas* sp. strain GRP3 influences iron acquisition in mung bean (*Vigna radiata* L. Wilzeck). *Soil Biol Biochem* 35:887–894. [https://doi.org/10.1016/S0038-0717\(03\)00119-6](https://doi.org/10.1016/S0038-0717(03)00119-6)
35. Siebner-Freibach H et al (2003) Siderophores sorbed on Ca-montmorillonite as an iron source for plants. *Plant soil* 251:115–124. <https://doi.org/10.1023/A:1022984431626>
36. Song MC et al (2008) 2R,3R-butanediol, a bacterial volatile produced by *Pseudomonas chlororaphis* O6, is involved in induction of systemic tolerance to drought in *Arabidopsis thaliana*. *Mol Plant*

- Microbe In 21:1067–1075. <https://doi.org/10.1016/j.exppara.2007.12.013>
37. Tamura K et al (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30:2725–2729. <https://doi.org/10.1093/molbev/mst197>
38. Tang W et al (2019) Chromosome-scale genome assembly of kiwifruit *Actinidia eriantha* with single-molecule sequencing and chromatin interaction mapping. *Gigascience* 8:1–10. <https://doi.org/10.1093/gigascience/giz027>
39. Tarrand JJ et al (1978) A taxonomic study of the *Spirillum lipoferum* group, with descriptions of a new genus, *Azospirillum* gen. nov. and two species, *Azospirillum lipoferum* (Beijerinck) comb. nov. and *Azospirillum brasilense* sp. nov. *Can J Microbiol* 24:967–980. <https://doi.org/10.1139/m78-160>
40. Thompson J (1997) The CLUSTAL\_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25:4876–4882. <https://doi.org/10.1093/nar/25.24.4876>
41. Wang L et al (2019) A MYB/bHLH complex regulates tissue-specific anthocyanin biosynthesis in the inner pericarp of red-centered kiwifruit *Actinidia chinensis* cv. Hongyang. *Plant J* 99:359–378. <https://doi.org/10.1111/tpj.14330>
42. Xie C, Yokota A (2005) *Azospirillum oryzae* sp. nov., a nitrogen-fixing bacterium isolated from the roots of the rice plant *Oryza sativa*. *Int J Syst Evol Micr* 55:1435–1438. <https://doi.org/10.1177/1524838008319633>
43. Young CC et al (2008) *Azospirillum rugosum* sp. nov., isolated from oil-contaminated soil. *Int J Syst Evol Micr* 58:959–963. <https://doi.org/10.1099/ijs.0.65065-0>
44. Yue J et al (2020) Kiwifruit Genome Database (KGD): a comprehensive resource for kiwifruit genomics. *Hortic Res.* 7 <https://doi.org/10.1038/s41438-020-0338-9>
45. Zeng Q et al (2017) Phosphate solubilization and gene expression of phosphate-solubilizing bacterium *Burkholderia multivorans* WS-FJ9 under different levels of soluble phosphate. *J Microbiol Biotechnol* 27:844–855. <https://doi.org/10.4014/jmb.1611.11057>
46. Zhou S et al (2013) *Azospirillum humicireducens* sp. nov., a nitrogen-fixing bacterium isolated from a microbial fuel cell. *Int J Syst Evol Micr* 63:2618–2624. <https://doi.org/10.1099/ijs.0.046813-0>

## Tables

Table 1. Comparison of the cellular fatty acid contents (%) and G+C content of strain CCTCC AB 2021101<sup>T</sup> and closely related species.

Fatty acid (%)	1	2	3	4
<b>Saturated</b>				
C <sub>14:0</sub>	2.32	0.85	0.81	0.41
C <sub>16:0</sub>	9.66	9.47	—	7.32
C <sub>17:0</sub>	0.01	0.26	1.28	0.18
C <sub>18:0</sub>	2.96	2.06	3.64	1.96
<b>Unsaturated</b>				
C <sub>16:1</sub> $\omega$ 9c	0.28	0.58	—	0.69
C <sub>17:1</sub> $\omega$ 6c	0.68	0.65	2.71	0.56
C <sub>17:1</sub> $\omega$ 8c	0.55	0.47	1.64	0.45
C <sub>18:1</sub> $\omega$ 7c	50.6	62.3	53.4	57.2
C <sub>18:1</sub> $\omega$ 9c	1.27	1.58	2.27	1.55
<b>Hydroxy</b>				
C <sub>16:0</sub> 3-OH	2.02	2.23	2.07	2.64
C <sub>18:1</sub> 2-OH	0.52	4.84	0.3	5.35
G+C content	67.8	67.7	69.0	66.8

Strains: 1, *A. actinidiae* CCTCC AB 2021101<sup>T</sup>; 2, *A. humicireducens* SgZ-5<sup>T</sup>; 3, *A. lipoferum* ATCC 29707<sup>T</sup>; 4, *A. oryzae* COC8<sup>T</sup>. Values are percentages of the total fatty acids and G+C content; —, not detected.

## Figures

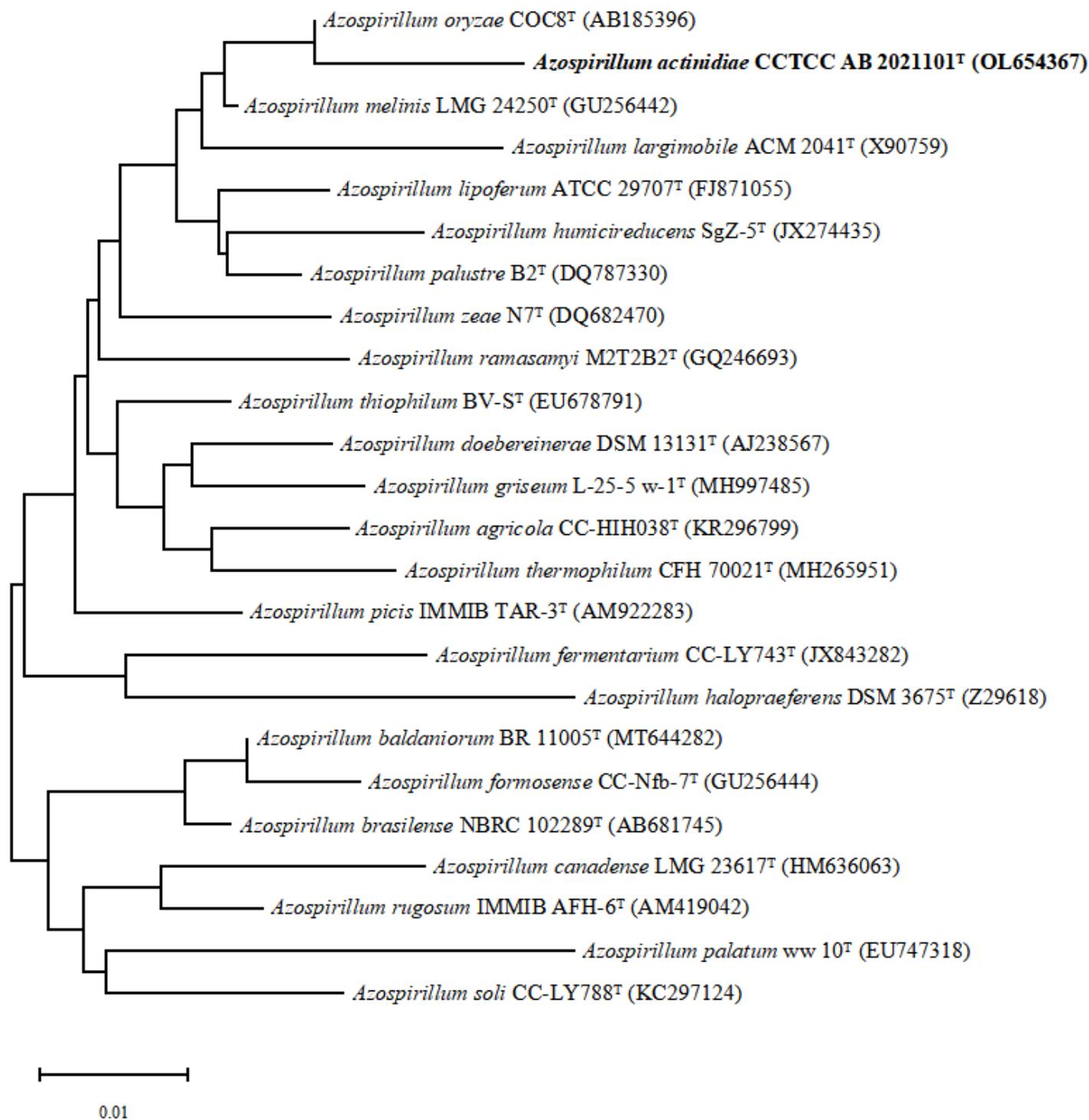


Figure 1

Phylogenetic analysis of strain CCTCC AB 2021101<sup>T</sup> based on 16S rRNA gene sequences. Distances and clustering were determined by using the neighbour-joining method with the software package MEGA version 6. Bar, 0.01 changes per nucleotide position.



Figure 2

Gram-Staining of strain CCTCC AB 2021101<sup>T</sup>.

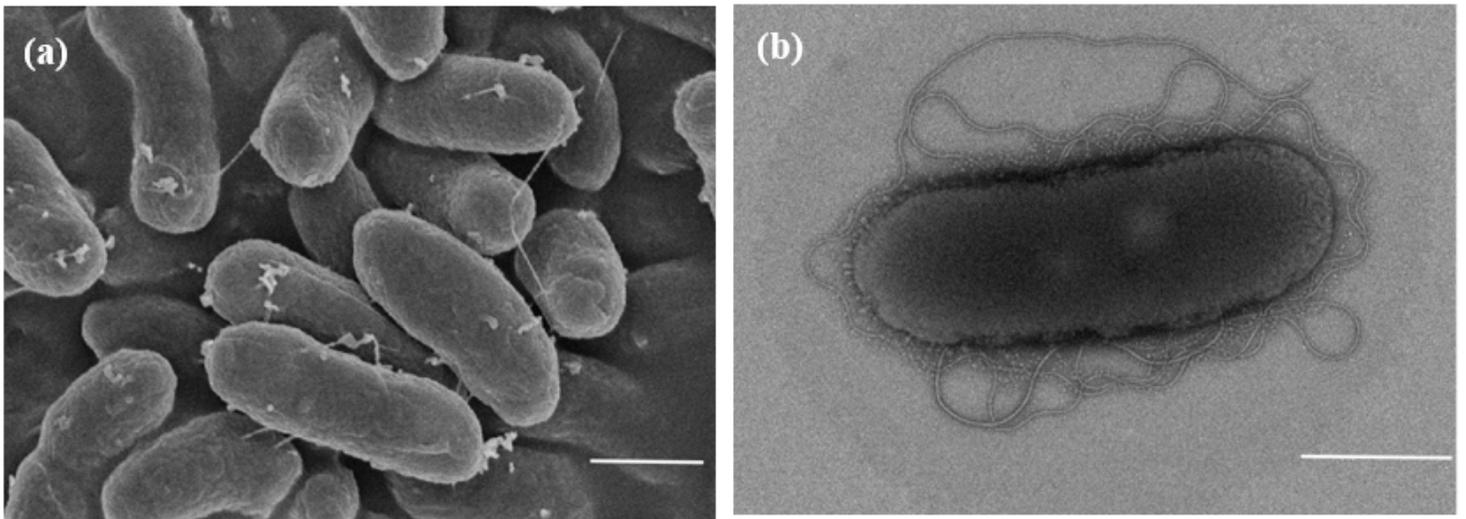


Figure 3

Cell morphology of strain CCTCC AB 2021101<sup>T</sup> observed under scanning electron microscope (a) and transmission electron microscope (b). Bar, 1 μm (a and b) , respectively.

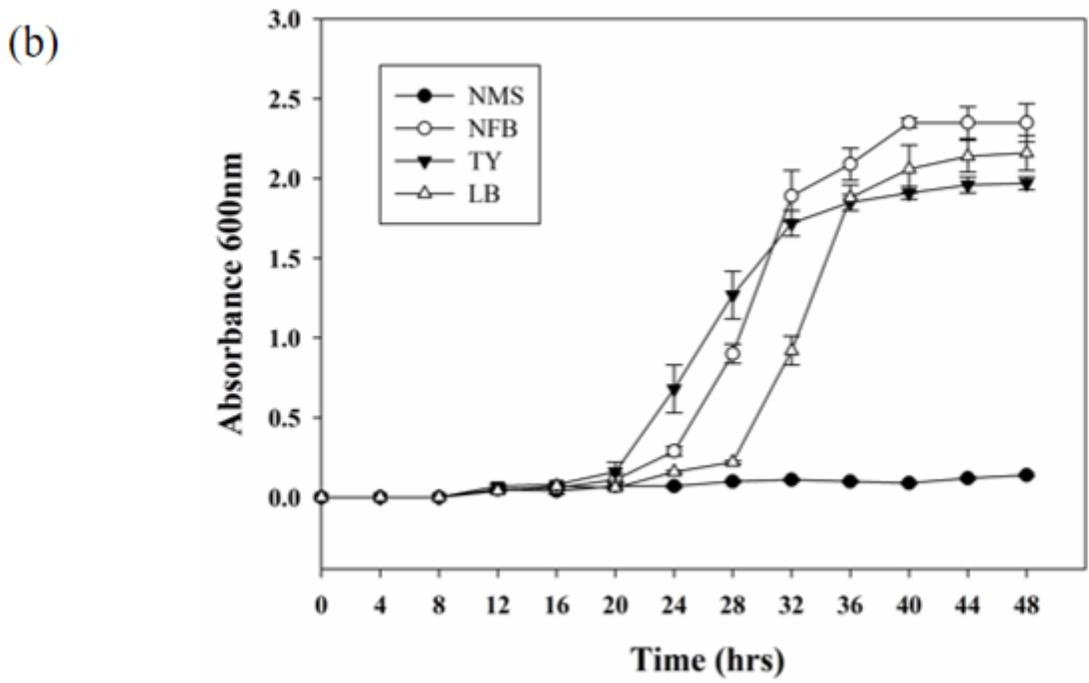
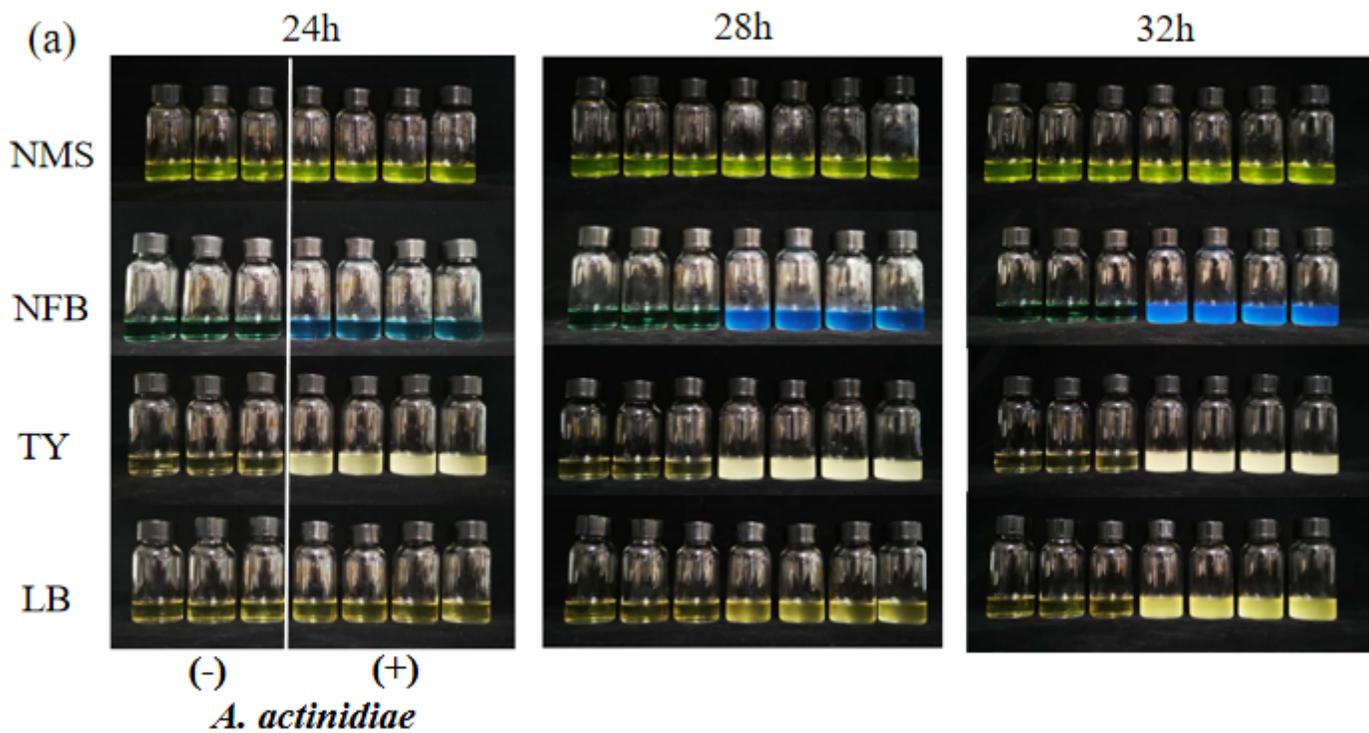


Figure 4

The growth response (a) and curve (b) of strain CCTCC AB 2021101<sup>T</sup> to four culture mediums, NMS, NFB supplemented with 0.01g/L KNO<sub>3</sub>, TY and LB.

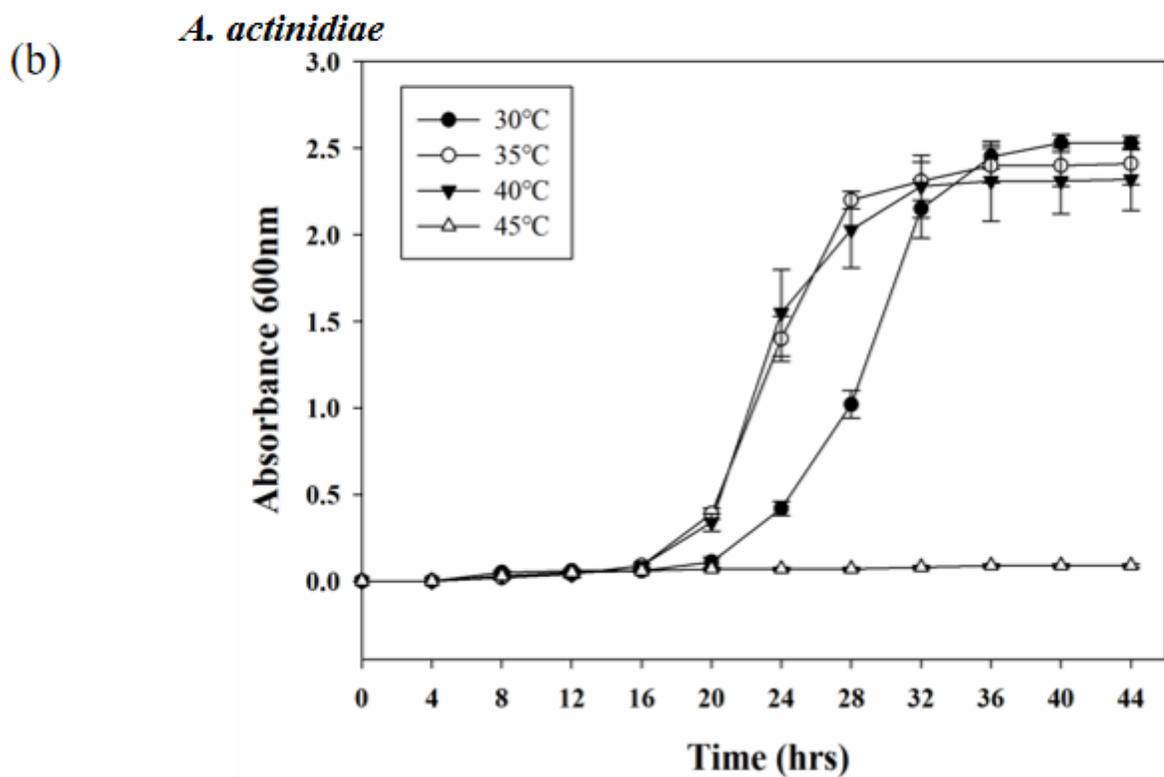
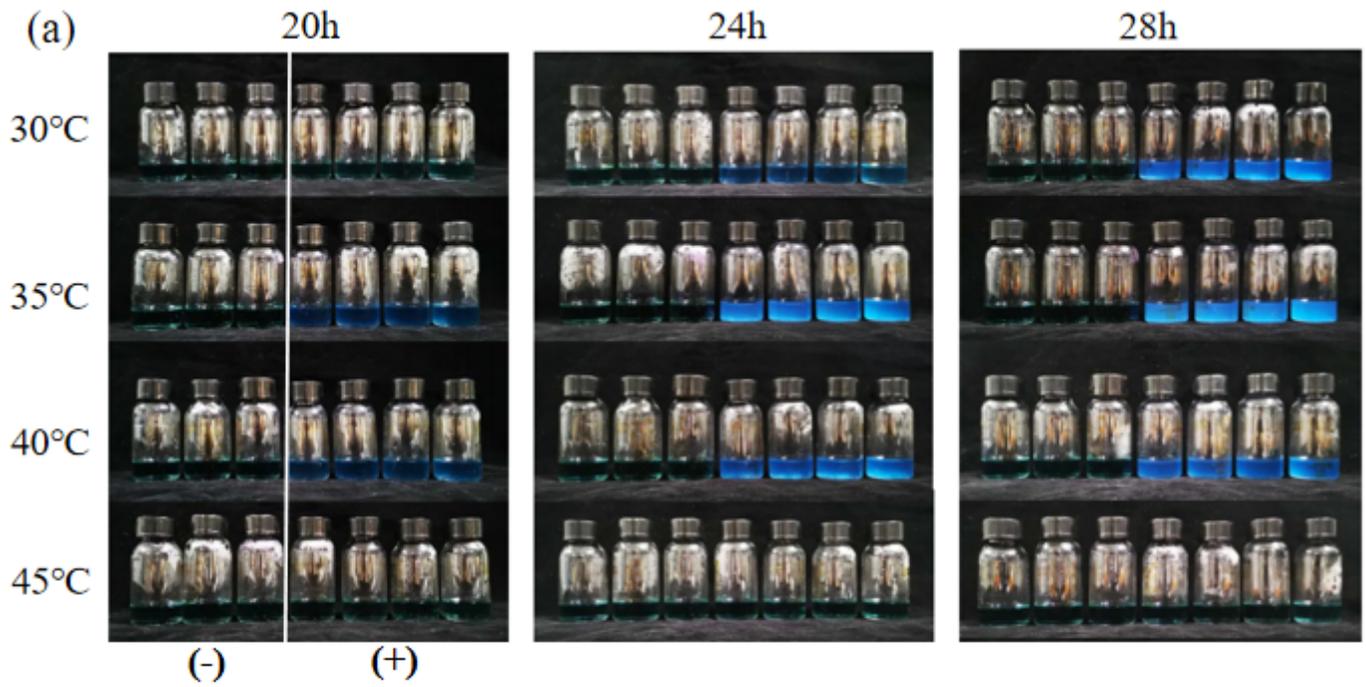


Figure 5

The growth response (a) and curve (b) of strain CCTCC AB 2021101<sup>T</sup> to different temperature. The bacterial cells were grown in pH 7.0 NFB medium supplemented with 0.01g/L KNO<sub>3</sub>.

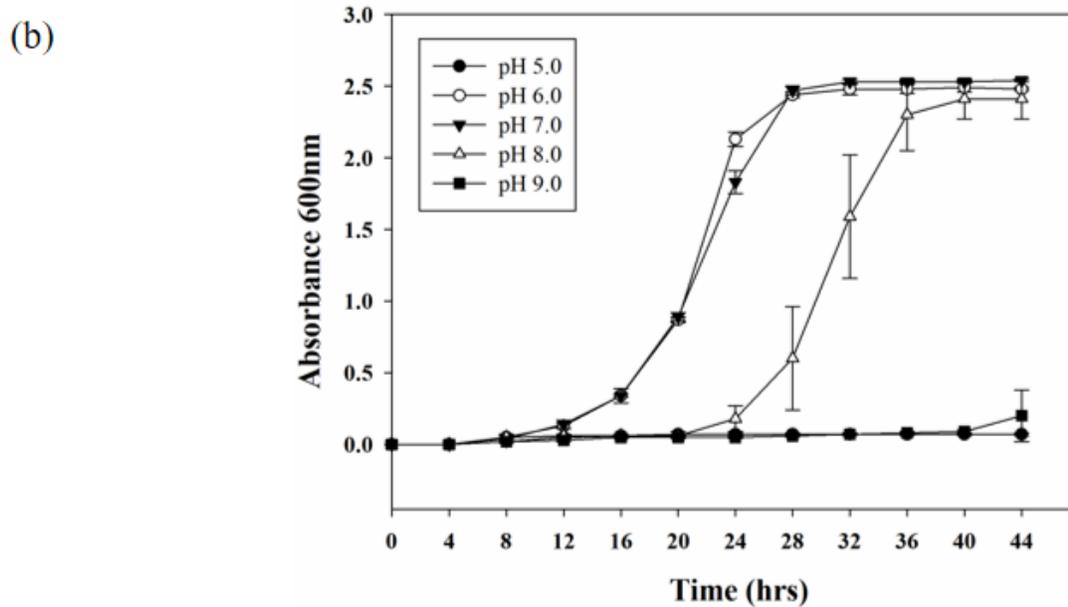
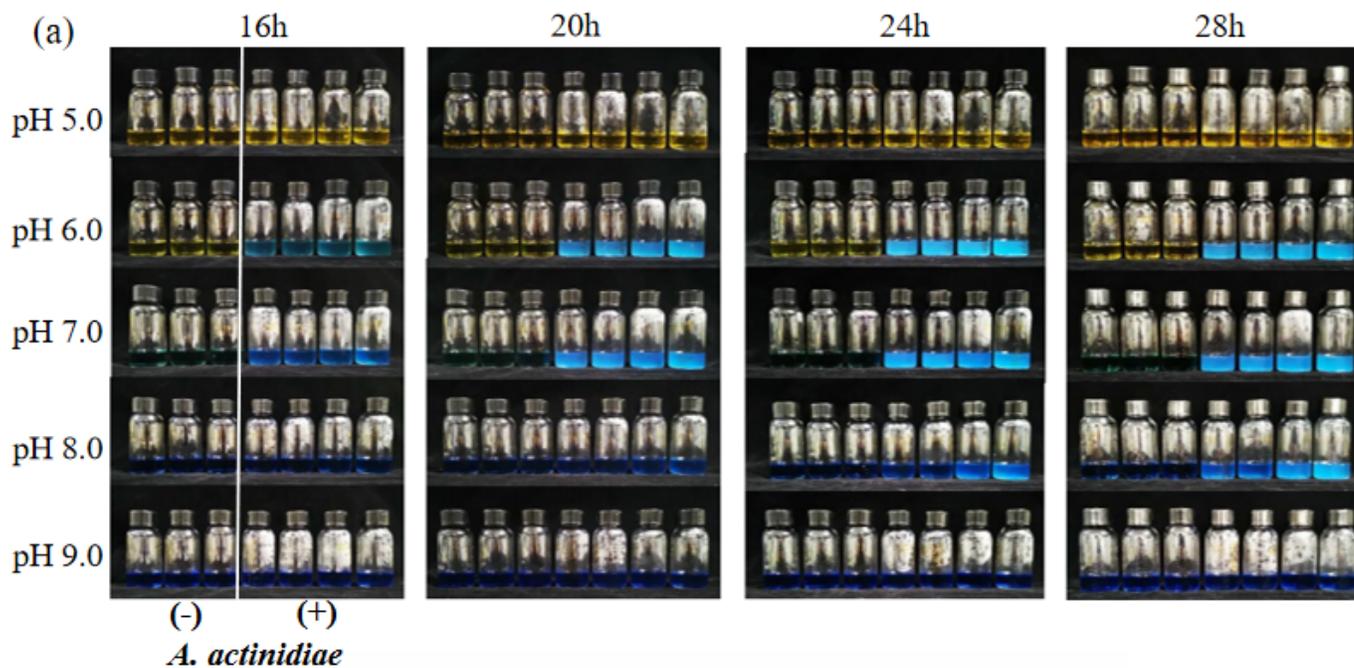


Figure 6

The growth response (a) and curve (b) of strain CCTCC AB 2021101<sup>T</sup> to different pH. The bacterial cells were grown in 35°C NFB medium supplemented with 0.01g/L KNO<sub>3</sub>.

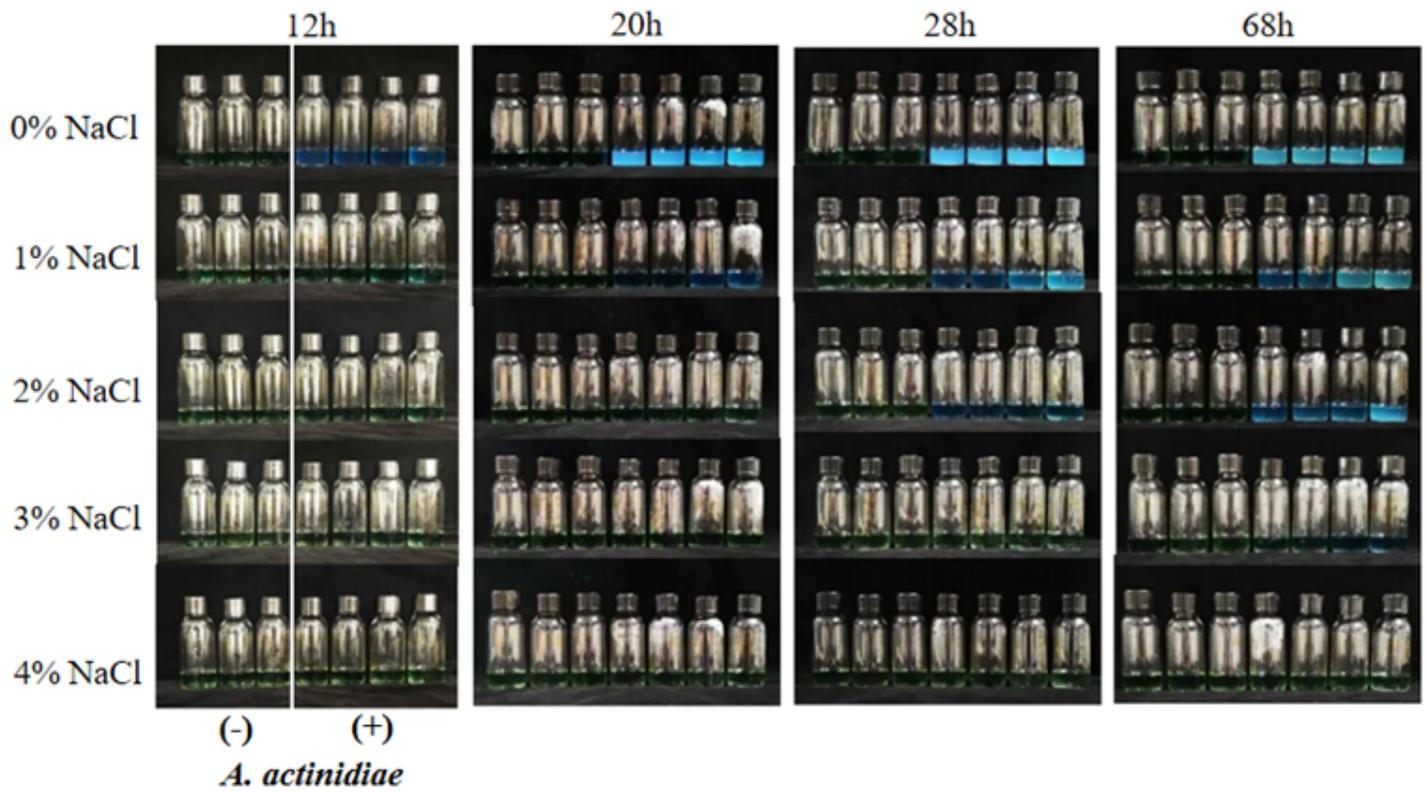


Figure 7

NaCl tolerance of strain CCTCC AB 2021101<sup>T</sup>. The bacterial cells were grown in 35°C NFB medium supplemented with 0.01g/L KNO<sub>3</sub> at pH 7.0.

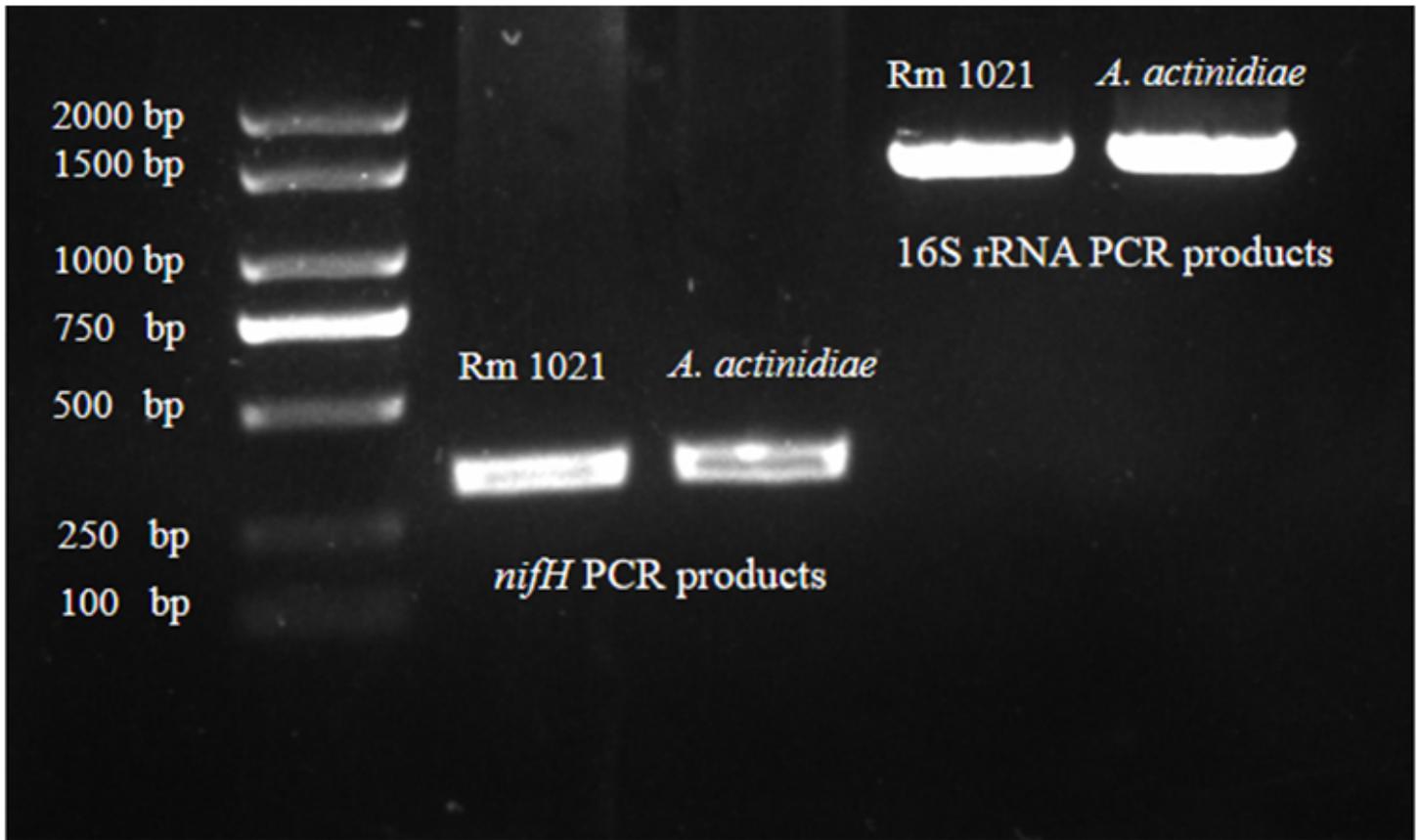


Figure 8

PCR products derived from specific amplification of the *nifH* gene and 16S rRNA gene. The PCR products of *Sinorhizobium meliloti* (Rm1021) was as a control.

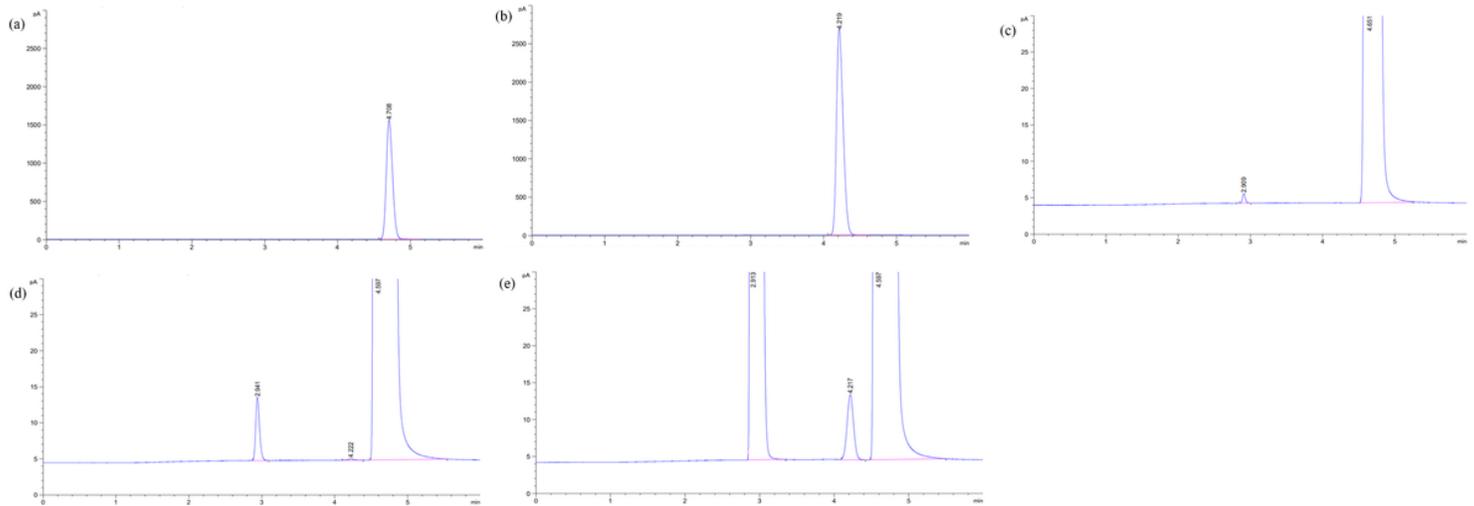


Figure 9

Estimation of nitrogen-fixing ability of strain CCTCC AB 2021101<sup>T</sup> (e) using acetylene-reduction assay. Acetylene calibration curve (a), ethylene calibration curve (b), culture medium of germ-free (c) and

nitrogen-fixing ability of *Sinorhizobium meliloti* (Rm1021) (d) were determined as control by a gas chromatography system (7820A, Agilent Technology), respectively.