Supplementary materials

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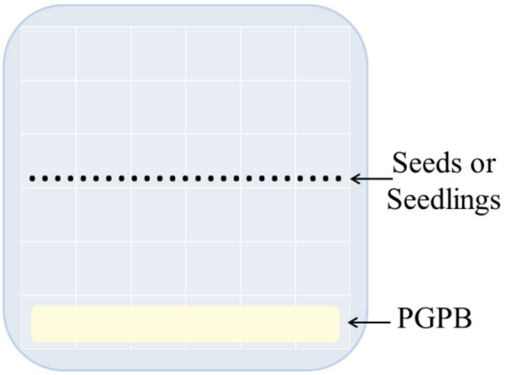

Fig. S1 Schematic diagram of the flat plate experiment.

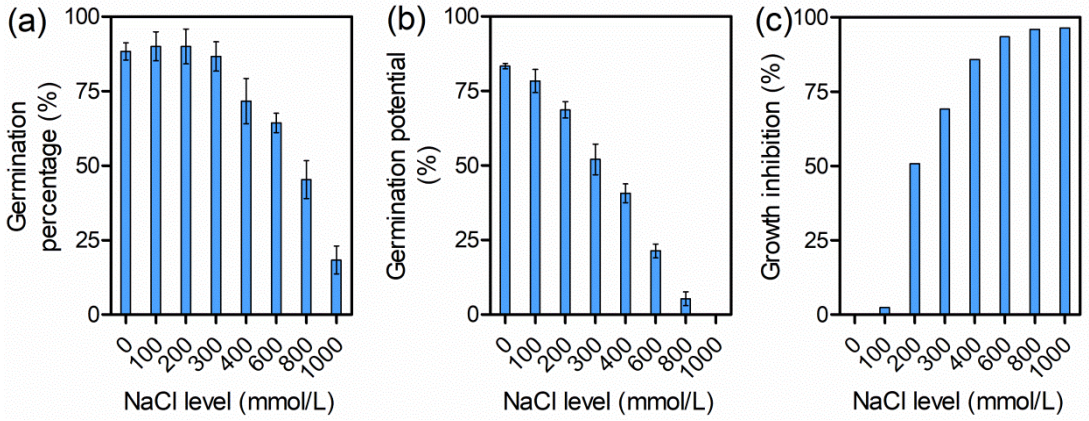

Fig. S2 Seed germination of *A. pictum* exposed to salt stress. (a) Germination percentage; (b) germination potential; (c) seedling growth inhibition (means of the inhibition ratio of seedling length and fresh weight) after germination for 14 days.

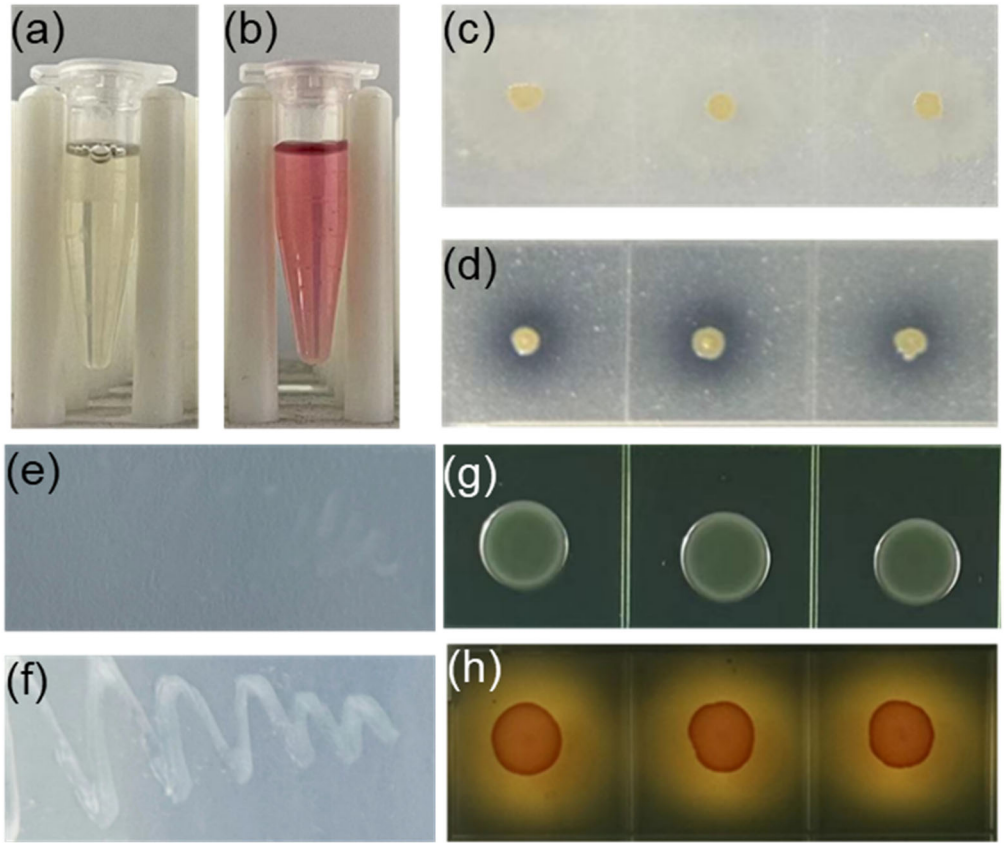

Fig. S3 Phenotypes of plant growth-promoting properties. (a) Negative for IAA production; (b) positive for IAA production; (c) negative for phosphate solubility; (d) positive for phosphate solubility; (e) negative for nitrogen fixation; (f) positive for nitrogen fixation; (g) negative for siderophore production; (h) positive for siderophore production.

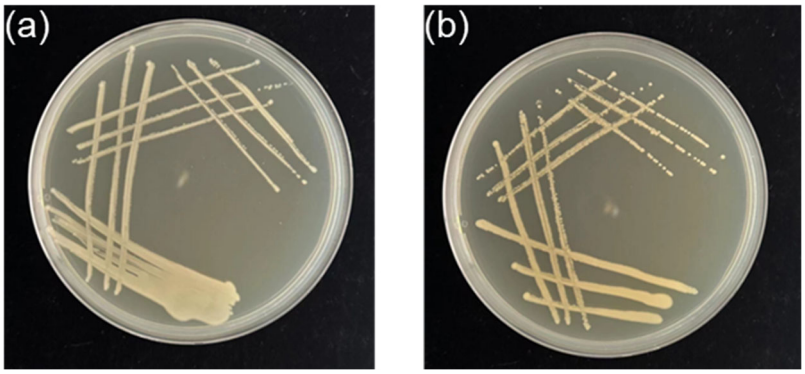

Fig. S4 Strain surface morphology. (a) Av16; (b) Av23.

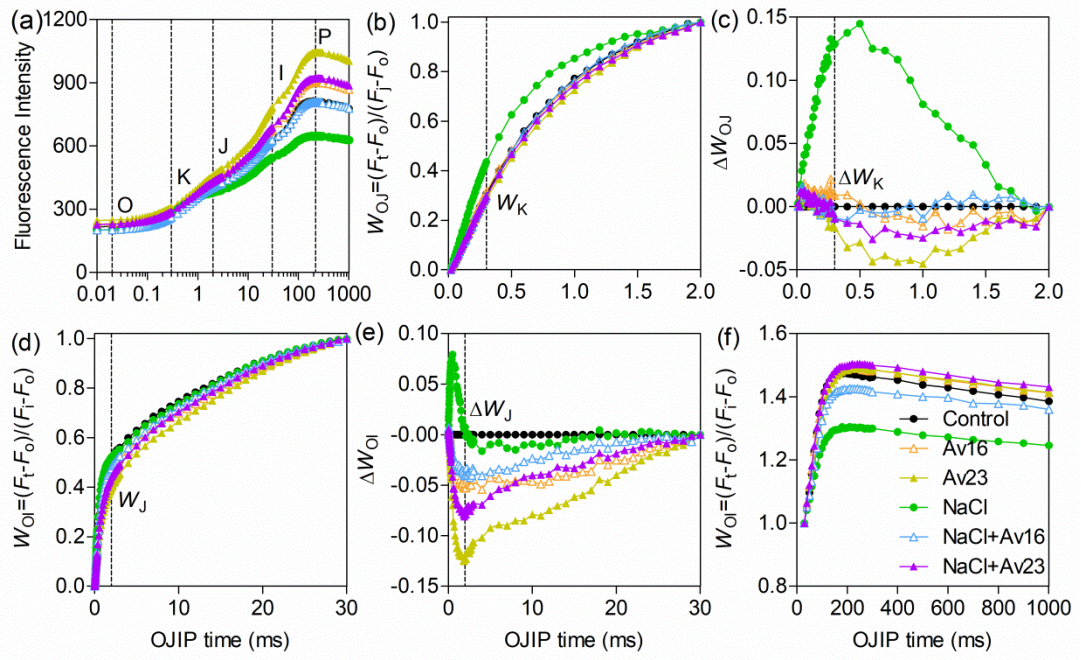

Fig. S5 Chlorophyll *a* fluorescence curve of *A. pictum* under salt and/or PGPB treatment. Maximum fluorescence (F_m) was induced by applying a 1000-millisecond (ms) pulse of saturating light. The labels O, K, J, I and P represent specific parameters at 0.02, 0.3, 2, 30, and 220 ms, respectively. (a) OJIP curve of the fluorescence intensity; (b) W_{OJ}; (c) $\Delta W_{OJ} = W_{OJ(treatment)} - W_{OJ(control)}$; (d) W_{OI} ($W_{OI} \leq 1$); (e) $\Delta W_{OI} = W_{OI(treatment)} - W_{OI(control)}$; (f) W_{OI} ($W_{OI} \geq 1$), the data are means of four replicates.

Table S1 Treatments in seed germination experiment

NaCl treatments (mmol/L)	PGPB
0	−PGPB, without PGPB
	Av16, with *Enterobacter* sp. Av16
	Av23, with *Acinetobacter* sp. Av23
200	−PGPB, without PGPB
	Av16, with *Enterobacter* sp. Av16
	Av23, with *Acinetobacter* sp. Av23
400	−PGPB, without PGPB
	Av16, with *Enterobacter* sp. Av16
	Av23, with *Acinetobacter* sp. Av23

Table S2 The components and concentrations of 1/4 Hoagland solution

	Concentrations		Concentrations
KNO_3	1.5 mmol/L	$MnSO_4$	2.0 μmol/L
$Ca(NO_3)_2$	1.0 mmol/L	$ZnSO_4$	2.0 μmol/L
$NH_4H_2PO_4$	0.5 mmol/L	$CuSO_4$	0.5 μmol/L
$MgSO_4$	0.25 mmol/L	H_2MoO_4	0.5 μmol/L
KCl	50 μmol/L	NaFeEDTA	25 μmol/L
H_3BO_3	25 μmol/L		

Table S3 Treatments in seedling experiments

NaCl treatments (mmol/L)	PGPB
0	−PGPB, without PGPB
	Av16, with *Enterobacter* sp. Av16
	Av23, with *Acinetobacter* sp. Av23
150	−PGPB, without PGPB
	Av16, with *Enterobacter* sp. Av16
	Av23, with *Acinetobacter* sp. Av23

Table S4 Description of OJIP-test parameters (Tsimilli-Michael, 2020; Guo et al., 2023)

Parameters	Description
F_o	Minimum fluorescence
F_m	Maximum fluorescence
M_o	Initial slope of fluorescence transient (O-J)
V_j	Relative variable fluorescence at 2 ms
V_i	Relative variable fluorescence at 30 ms
ΦP_o	Maximum quantum yield of primary PSII photochemistry
ΦE_o	Quantum yield for electron transport ($t = 0$)
ΦD_o	Quantum yield for energy dissipation ($t = 0$)
ψO	Probability that a trapped exciton moves an electron into the electron transport chain beyond Quinone A (Q_A^-) at $t = 0$
δR_o	Efficiency with which an electron from PQH_2 is transferred to final PSI acceptors
ΦR_o	Efficiency with which a PSII trapped electron is transferred to final PSI acceptors
TR_o/CS_m	Trapped energy fux cross-section (CS) at $t = t_{Fm}$
ET_o/CS_m	Electron transport fux per CS at $t = t_{Fm}$
DI_o/CS_m	Dissipated energy flux per active RC at $t = t_{Fm}$
RC/CS_m	Amount of PSII RCs per CS at $t = t_{Fm}$
PI_{abs}	Performance index on absorption basis
PI_{total}	Performance index of overall photochemistry

Table S5 Plant growth-promoting properties of different strains

Strains	IAA secretion (μg/mL) (n = 3)	Phosphate solubility index (D/d) (n = 4)	Nitrogen fixation (n = 9)	Siderophone production (n = 9)
Av01[#]	−	−	−	−
Av02	−	−	−	−
Av03	−	−	−	−
Av04	−	−	−	−
Av05	**67.5 ± 1.80 bc***	**1.65 ± 0.03 bcde***	**−**	**+**
Av06	**59.8 ± 2.74 c**	**1.83 ± 0.19 bcd**	**−**	**+**
Av07	−	−	−	−
Av08	30.0 ± 2.17 e	−	−	−
Av09	−	−	−	−
Av10	**30.8 ± 1.43 e**	**−**	**+**	**+**
Av11	−	−	−	−
Av12	−	−	+	−
Av13	**109 ± 6.77 a**	**1.42 ± 0.20 cde**	**+**	**+**
Av14	−	−	−	−
Av15	21.0 ± 1.28 fg	−	−	−
Av16	**70.0 ± 2.70 b**	**1.63 ± 0.04 cde**	**+**	**+**
Av17	**51.3 ± 1.80 d**	**1.94 ± 0.13 bc**	**+**	**+**
Av18	**64.7 ± 2.01 bc**	**−**	**+**	**+**
Av19	**20.2 ± 1.51 fg**	**2.23 ± 0.22 b**	**+**	**+**
Av20	**68.0 ± 4.40 b**	**−**	**+**	**+**
Av21	13.9 ± 1.80 g	−	+	−
Av22	48.6 ± 2.35 d	−	−	−
Av23	**45.7 ± 2.37 d**	**3.75 ± 0.32 a**	**+**	**+**
Av24	22.9 ± 2.11 ef	−	+	−
Av25	**23.3 ± 1.35 ef**	**3.17 ± 0.60 a**	**+**	**−**
Av26	15.8 ± 1.26 fg	1.61 ± 0.10 cde	−	−
Av27	−	1.22 ± 0.06 e	−	−
Av28	18.7 ± 0.72 fg	1.48 ± 0.21 cde	−	−
Av29	16.2 ± 1.66 fg	1.65 ± 0.03 bcde	−	−
Av30	−	1.73 ± 0.18 bcde	+	−
Av31	−	1.79 ± 0.25 bcde	−	−
Av32	13.9 ± 2.1 g	1.34 ± 0.14 de	−	−

Note: [#], Av01-Av16, Av17-Av24 and Av25-Av32 were isolated from rhizospheric soil, plant shoots, and roots, respectively;

*, Different letters indicate significant differences between strains;

+, Positive; −, Negative.

Table S6 Gram stain and morphological characteristics of two strains

Strains	Gram stain	Morphological characteristics of strains					
		Bacterium shape	Colony surface	Colony shape	Colony color	Opacity	Uplift
Av16	−	Rod	Moist	Circular	Faint yellow	Opaque	Raised
Av23	+	Rod	Moist	Circular	Faint yellow	Opaque	Flat

Note: +, Positive; −, Negative.

Materials and methods

Isolation of bacterial strains from rhizospheric soil and plant tissues

Bacterial strains in soil and plant tissues were isolated according to the methods described by Bharathi et al. (2004) and Tian et al. (2022), with some modifications. To isolate bacterial strains from rhizospheric soil, 100 mg of soil and 1 mL of distilled water (dH_2O) were thoroughly mixed in a 2 mL Eppendorf tube. The mixture was then diluted serially. To isolate bacterial strains from plant tissues, the surface of 0.2 g shoot and root samples were sterilized with 75% (v/v) ethanol and 2.5% (w/v) sodium hypochlorite, and then thoroughly washed with dH_2O. The plant samples were ground in 2 mL dH_2O, and the homogenates were centrifuged at 4 ℃ and 1000 *g* for 5 min. The supernatants were then diluted serially.

100 mL of each dilution of 10^{-3}, 10^{-4}, and 10^{-5} concentrations were plated onto solid Luria-Bertani (LB) medium containing 10 g/L tryptone, 5 g/L Yeast extract, 10 g/L NaCl and 15 g/L agar, respectively. The plates were then incubated at 28 ℃ for 24 to 36 h. Colonies with different morphological traits were selected, purified, and preserved. All instruments and dH_2O used were sterilized, and all procedures were performed under sterile conditions.

Analysis of plant growth-promoting traits of bacterial strains

Four plant growth-promoting traits of bacterial isolates were analyzed. The IAA secretion was determined using Sckowski's reagent (Gordon and Weber, 1951). Phosphate solubilization ability was quantified using the phosphate solubility index, calculated as the ratio of the transparent ring diameter (D) to the colony diameter (d) (D/d), after bacterial strains were grown on National Botanical Research Institute's phosphate growth medium (NBRIP) (Nautiyal, 1999). Siderophore production was assessed following the method described by Schwyn and Neilands (1987), in which a color change of solid LB medium containing chrome azurol S from blue to orange or yellow around bacterial colonies indicates siderophore production. Nitrogen fixation capacity was evaluated by bacteria growth on nitrogen-free Ashby solid medium (Pramanik et al., 2017). Bacterial isolates exhibiting three or more plant growth-promoting traits were selected to assess their ability to enhance the growth of *A. pictum* seedlings under salt stress.

Preparation of bacterial suspension

To prepare the bacterial suspension, the activated bacterial strain was inoculated in liquid LB medium at 28 ℃ with a rotation speed of 180 r/min for 12 h. It was then centrifuged at 4 ℃ and 5000 r/min for 15 min. The precipitate was washed twice with sterilized dH_2O, and then resuspended and diluted with sterilized dH_2O to achieve an optical density at 600 nm (OD_{600}) of 0.3 or 1.0 with sterilized dH_2O (Tian et al., 2022). The bacterial suspension was prepared immediately before use.

Identification of bacterial strains

The bacterial strains Av16 and Av23 were identified based on colony morphology, Gram staining and 16S rDNA gene sequencing followed by NCBI BLAST analysis.

Colony morphology and Gram staining: The activated bacterial strain was inoculated onto solid LB medium and incubated at 28 ℃ for 12 h to observe the morphology of individual colonies. Gram staining was performed according to the method described by Richard (2001), and observed using microscopy with oil immersion.

16S rDNA gene sequencing: Total DNA of the selected PGPBs was extracted according to the method described by Wilson (2001). PCR amplification was performed using the bacterial universal primer 27F/1492R (Frank et al., 2008), and the 16S rDNA was sequenced by Sangon Biotech (Shanghai, China). The complete sequencing results were used for BLAST analysis on NCBI. To estimate the phylogenetic position of the bacterial strains**,** a phylogenetic evolutionary tree was constructed using MEGA X software.

Calculation of germination percentage, germination potential, and germination index

Germination percentage (%) = (G/N) × 100 (G indicates the number of germinated seeds; N indicates total seeds to be tested);

Germination potential (%) = (n/N) × 100 (n indicates the total seeds germinated to day 3, N indicates total seeds to be tested);

Germination index = $\sum G_t/D_t$ (G_t indicates the number of germinated seeds at day t, D_t indicates day t).

References

Bharathi R, Vivekananthan R, Harish S, et al., 2004. Rhizobacteria-based bio-formulations for the management of fruit rot infection in chillies. *Crop Prot*, 23:835-843. https://doi.org/10.1016/j.cropro.2004.01.007

Frank JA, Reich CI, Sharma S, et al., 2008. Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Appl Environ Microbiol*, 74:2461–2470. https://doi.org/10.1128/AEM.02272-07

Gordon SA, Weber RP, 1951. Colorimetric estimation of indoleacetic acid. *Plant Physiol*, 26:192. https://doi.org/10.1104/pp.26.1.192

Guo H, Dong Q, Li S, et al., 2023. Effects of exogenous calcium on growth, chlorophyll fluorescence characteristics and antioxidant system of *Fraxinus malacophylla* seedlings. *Plant Physiol Biochem*, 201:107860. https://doi.org/10.1016/j.plaphy.2023.107860

Nautiyal CS, 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiol Lett*, 170:265-270. http://doi.org/10.1111/j.1574-6968.1999.tb13383.x

Pramanik K, Mitra S, Sarkar A, et al., 2017. Characterization of cadmium-resistant *Klebsiella pneumoniae* MCC 3091 promoted rice seedling growth by alleviating phytotoxicity of cadmium. *Environ Sci Pollut Res*, 24:24419-24437. http://doi.org/10.1007/s11356-017-0033-z

Richard C, 2001. Gram staining. *Curr Protoc Immunol*, Appendix 3: A.3O.1-A.3O.2. https://doi.org/10.1002/0471142735.ima03os23

Schwyn B and Neilands J, 1987. Universal chemical assay for the detection and determination of siderophores. *Anal Biochem*, 160:47-56. http://doi.org/10.1016/0003-2697(87)90612-9

Tian W, Li L, Xiao X, et al., 2022. Identification of a plant endophytic growth‐promoting bacteria capable of inhibiting cadmium uptake in rice. *J Appl Microbiol*, 132:520-531. http://doi.org/10.1111/jam.15201

Tsimilli-Michael M, 2020. Revisiting JIP-test: An educative review on concepts, assumptions, approximations, definitions and terminology. *Photosynthetica*, 58(SI):275–292. https://doi.org/10.32615/ps.2019.150

Wilson K, 2001. Preparation of genomic DNA from bacteria. *Curr Protoc Mol Biol*, 56: 2.4.1–2.4.5. https://doi.org/10.1002/0471142727.mb0204s56.