

Appendix

Method for plant growth promoting traits test

Based on the results of first round plant growth promoting (PGP) traits test, 10 promising isolates were further investigated for their PGP traits including solubilization of AlPO_4 and FePO_4 , as well as the 1-Aminocyclopropane-1-carboxylate deaminase (ACCD) activity and ammonia production.

Phosphate solubilization ability was investigated according to the method used by Liu et al. (Liu et al. 2014). The experiments were performed in 250-ml Erlenmeyer flasks containing 50 ml of liquid Pikovskaya medium (PVK) medium containing $\text{Ca}_3(\text{PO}_4)_2$ or FePO_4 as insoluble phosphate sources at a concentration of 5 g L^{-1} . Flasks were inoculated by adding half milliliter of *Trichoderma* spore suspensions ($\approx 10^8 \text{ CFU ml}^{-1}$) and cultured on a rotary shaker (200 rpm) for 5 days at 28°C . The liquid cultures were centrifuged at $10\,000\times g$ for 10 mins and the phosphate solubilization was determined quantitatively using colourimetric molybdate/antimony method (Murphy and Riley 1962). The experiment was repeated three times.

For determination of ACCD activity in *Trichoderma*, 100 μL spore suspension was inoculated in 10 ml synthetic medium (SM; (Yedidia et al. 1999)) and the incubated at 180 rpm for 48 h and 28°C . The mycelia were harvested washed twice with SM (without ammonium). The washed mycelia were then transferred to 5 mL of SM without ammonium but with 3 mM ACC and incubated for 48h. At the end of the induction period, ACCD activity was evaluated quantitatively by measuring the amount of α -ketobutyrate produced by the deamination of ACC (Penrose and Glick 2010). ACCD activity was expressed in mmol of α -ketobutyrate mg^{-1} protein h^{-1} . Protein concentrations were determined using the BCA assay kit purchased from Solarbio (Beijing, China). The experiment was repeated three times.

For the detection of ammonia production, *Trichoderma* isolates were grown in test tubes containing peptone water (10.0 g peptone; 5.0 g NaCl; 1000 ml distilled water); 7.0 pH (Dey et al. 2004). The tubes were inoculated with 100 μL spore suspension was inoculated in 10 ml peptone water and incubated for 3 days at 180 rpm and 28°C . The ammonia was detected by adding Nessler's reagent. A faint yellow color indicated a small amount of ammonia and deep yellow to brownish color indicated maximum production of ammonia.

References

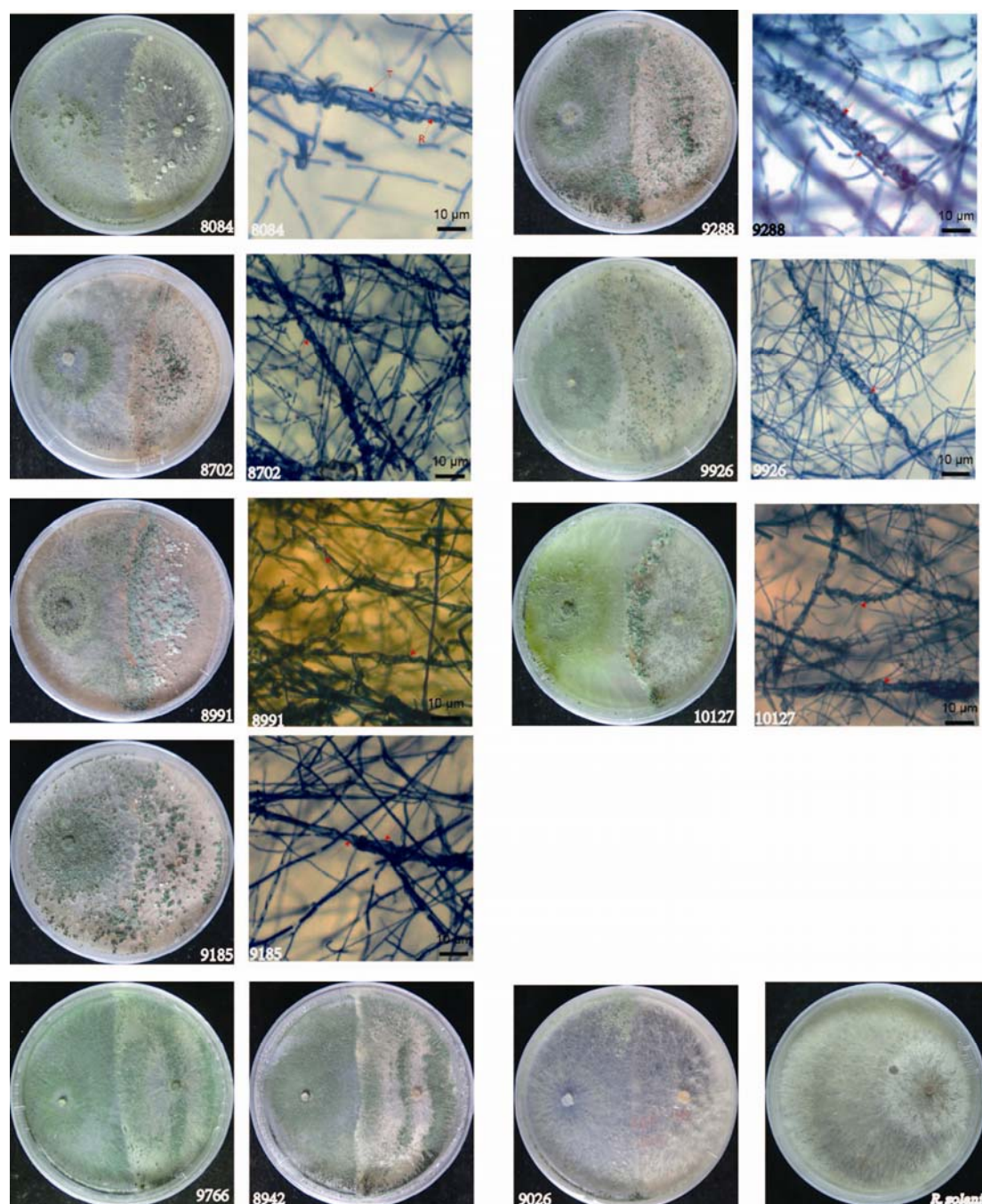
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Appendix A Inhibition of *R. solani* growth by *Trichoderma* isolates and hyphae interaction. Growth of *Trichoderma* (left) and *R. solani* (right) in direct confrontation assays. Red arrows indicate the hyphae of *Trichoderma*.

Appendix B Plant growth promoting traits of *Trichoderma*

Species	Isolate	AlPO ₄ (µg mL ⁻¹)	FePO ₄ (µg mL ⁻¹)	ACCd mM mg ⁻¹ h ⁻¹	NH ₄ ⁺
<i>T. atrobrunneum</i>	9926	14.9±4.1a	29.2±5.3bc	40.6±15.6ab	+
<i>T. guizhouense</i>	9185	23.5±7.2ab	4.9±4.1a	33.7±3.7ab	++

	9288	61.9±9.8d	25.2±7.7b	39.7±4.4ab	++
<i>T. paratroviride</i>	8942	38.6±9.1bc	31.1±5.9bc	33.2±7.6ab	++
	9766	26.3±8.7ab	0.6±0.6a	26.2±8.8a	+
<i>T. pyramidale</i>	8991	56.2±4.6d	26.9±1.8b	60.8±6.5c	++
<i>T. rufobrunneum</i>	8084	19.2±5.7a	49.1±4.4d	37.4±6ab	++
<i>T. simmonsii</i>	8702	48.3±17.5cd	35.9±6.9c	61.4±8.4c	+++
<i>T. thermophilum</i>	10127	37.4±10bc	1±0.7a	42±1.4b	+
<i>T. viridulum</i>	9026	29.4±4.9ab	7.7±3.8a	37.1±6.1ab	+

+, faint yellow color and small amount of ammonia production; ++, yellow color and medium amount of ammonia production; +++, deep yellow color and maximum amount of ammonia production. Different letters indicate significant differences among isolates according to Duncan test at $P < 0.05$.