Bacterial Wilt of Sacha inchi (*Plukenetia volubilis*) Caused by *Ralstonia pseudosolanacearum* Phylotype I in Southern China

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Sacha inchi (*Plukenetia volubilis* L.) is a little known yet important perennial plant native to the Andes Mountains of Peru. It is a plant producing large, edible seeds very rich in omega-3 fatty acids and proteins usful for promoting human health. The economic importance of Sacha inchi has increased in recent years because its seeds produce significantly higher oil yields than those of other plants (Wong, 2017). Sacha inchi was first introduced to grow in Xishuangbanna, Yunnan Province, southwest China in 2006, and then to Lingshui County, Hainan Province, southern China in 2014. However, just two years later, in April 2016, unusual wilting was observed on many Sacha inchi plants in the fields of Lingshui County. The disease outbreak proved to be rapid and extensive, covering approximately 11 ha of Sacha inchi growth and causing an 80% production loss in that crop's area in just a few months. By 2017, the farmers had almost given up on continuing Sacha inchi production due to the severity and spread of this disease. In the fields, the disease caused the plant's stem and leaf to wilt. On seedlings, their leaves wilted and drooped while still photosynthetically active (i.e. green), followed by total plant collapse within a few days. Mature plants initially had symptoms of irregular, black necrotic lesions at their leaf base margins. But as the disease symptoms progressed, the necrotic leaves spread upward, and eventually the affected plants turned chlorotic and shed leaves and developed black stripes on their stems. Vascular necrosis and bacterial ooze were observed when longitudinal sections were obtained from the basal portion of infected stems (Fig 1A). For identification, a total of 10 plants with the typical wilting symptoms were collected from the Sacha inchi fields of Lingshui County. Six isolates were obtained from the roots and plantlets of six separate plants. All the isolates were gram negative, oblong to rod shaped, and 0.4 to 0.9 × 0.7 to 2.0 µm in size (n = 25) when viewed under electron microscopy (Fig. 1B). When cultured on Kelman's tetrazolium chloride medium, the colonies appeared round to oval, fluidal, and entirely white with a pale red center (Fig. 1C) after incubation at 30°C for 48 h. Three *Ralstonia solanacearum*-specific primer pairs, for the flagella subunit (Rsol_fliC-F/ Rsol_fliC-R) (Schönfeld et al. 2003), the polygalacturonase gene (pehA #3/ pehA #6) (Gillings et al. 1993) and 759/760 (ITO et al. 1998) yielded the expected amplicons. Two representative strains (ACCC60145 plu-3 and ACCC60146 plu-6) were identified as *R. pseudosolanacearum* phylotype I, sequevar 34, according to the phylotype-specific multiplex PCR assay (Fegan et al. 2005), and phylogenetic analysis of the partial *egl* gene sequences (GenBank Accession Nos. KY352419 and KY352421) (Safni et al. 2014). The two strains shared 99.9% and 100% sequence identity with *R. pseudosolanacearum* GMI1000 (AL646052), which had been identified using 16S ribosomal RNA (GenBank Accession Nos. KY346975 and KY346977) sequencing and sequence comparisons. For the pathogenicity test, stems of 30 one-month-old Sacha inchi (cv. Pinto Recodo) seedlings were injected with 40-µl suspensions (10^8 CFU/ml) of the representative isolates ACCC60145 plu-3 and ACCC60146 plu-6 (10 seedlings per isolate inoculation treatment). The injection point on the seedling stem was at 2–3 cm above the soil surface (Fig. 1D). Symptoms of wilt were observed 5 days after these inoculations of Sacha inchi. To serve as a negative control, 20 healthy Sacha inchi seedlings were likewise injected but with sterile water. No disease symptoms were observed on these control plants. Re-isolations were done twice, as outlined above, on the symptomatic seedlings and control plants at 3–4 cm above the original injection points. To complete Koch's postulates, the bacteria were then re-isolated from the inoculated Sacha inchi plants and re-identified by PCR. Following the same inoculation procedure, the bacterium also caused wilting in cherry tomato (*Lycopersivon esculentum* Mill.; 20 of 20 individuals), eggplant (*Solanum melongena* L. var. *esculentum* Nees.; 17 of 20), and pepper

(*Capsicum annuum* var. *conoide* Mill. Irish; 18 of 20). To the best of our knowledge, this is the first report of *R. pseudosolanacearum* phylotype I sequevar 34 causing bacterial wilt of Sacha inchi in China and around the world. These disease strain findings can be useful for developing effective strategies for control of the disease in the important oil and seed plant sacha inchi.

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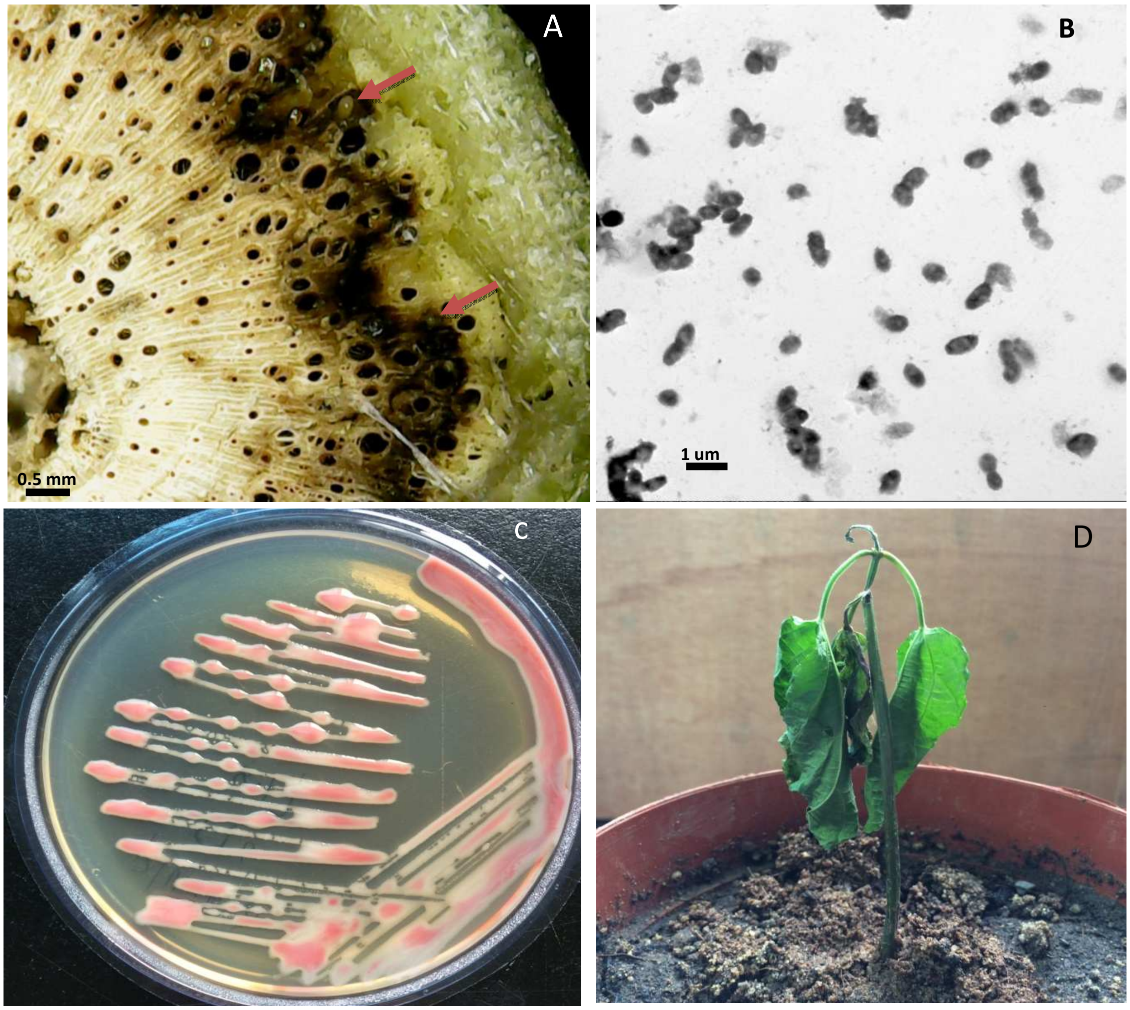

Fig. 1 (A) Bacterial ooze observed on the cross sections of Sacha inchi (*Plukenetia volubilis*) stems infected with *Ralstonia pseudosolanacearum*; (B) Under electron microscopy, observed ovoid-shaped rods 0.4 to 0.9 × 0.7 to 2.0 µm in size; (C) From symptomatic plant tissue, typical *R. pseudosolanacearum* colonies isolated on TZC medium were raised, fluidal, and white with pale red centers; (D) Symptoms of wilt observed on a Sacha inchi seedling 5 days after inoculation with *R. pseudosolanacearum*.