A *Streptomyces* sp. NEAU-HV9: Isolation, identification, and potential as a biocontrol agent against *Ralstonia Solanacearum* of tomato plants

Ling Ling¹, Xiaoyang Han¹, Xiao Li¹, Xue Zhang¹, Han Wang¹, Lida Zhang¹, Peng Cao¹, Yutong Wu¹, Xiangjing Wang¹, Junwei Zhao^{1,*} and Wensheng Xiang^{1,2,*}

- ¹ Key Laboratory of Agricultural Microbiology of Heilongjiang Province, Northeast Agricultural University, No. 59 Mucai Street, Xiangfang District, Harbin 150030, China; LLYNL2621161093@163.com (L.L.); hanxy139251@163.com (X.H.); Lx1244070003@126.com (X.L.); zhangxue_968425@163.com (X.Z.); wanghan507555536@gmail.com (H.W.); yone910310@163.com (L.Z.); cp511@126.com (P.C.); 18103699151@163.com (Y.W.); wangneau2013@163.com (X.W.)
- ² State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China
- * Correspondence: guyan2080@126.com (J.Z.), xiangwensheng@neau.edu.cn (W.X.)

| Medium | Growth | Aerial mycelium | Substrate mycelium | Diffusible pigment |
|--------|--------|----------------------|-------------------------|----------------------|
| ISP 1 | Good | White | Moderate Yellow | Vivid Yellow |
| ISP 2 | Good | White | Brilliant Orange Yellow | Light Orange Yellow |
| ISP 3 | Good | White | Brilliant Orange Yellow | Light Yellow |
| ISP 4 | Good | White | Brilliant Yellow | Dark Greenish Yellow |
| ISP 5 | Good | Moderate Yellow | Grayish Yellow | Moderate Yellow |
| ISP 6 | Good | None | Moderate Yellow | Moderate Yellow |
| ISP 7 | Good | Pale Greenish Yellow | Dark Olive | Moderate Olive |
| NA | Good | None | Moderate Yellow | Moderate Greenish |
| INA | Good | none | Moderate reliow | Yellow |
| BA | Good | None | Brilliant Orange Yellow | Light Orange Yellow |
| CA | Poor | White | Pinkish White | None |

Table S1. Growth and cultural characteristics of strain NEAU-HV9 after 2 weeks at 28 °C

| Characteristic | Result | Characteristic | Result |
|--------------------------------|--------|-----------------------------|--------|
| Growth at/with: | | Decomposition of | |
| Temperature range (°C) | 15-37 | Adenine | + |
| Growth pH range | 5-9 | Casein | — |
| Maximum NaCl | 7 | Hypoxanthine | + |
| Production of H ₂ S | + | Tyrosine | + |
| Reduction of nitrate | _ | Xanthine | + |
| Coagulation and peptonization | | Hydrolysis of | |
| of milk | — | Aesculin | + |
| Liquefaction of gelatin | — | Starch | + |
| Carbon source utilization | | Nitrogen source utilization | |
| L-Arabinose | _ | L-Alanine | + |
| Dulcitol | _ | D-Arginine | + |
| D-Fructose | _ | L-Asparagine | + |
| D-Galactose | + | L-Aspartic acid | + |
| D-Glucose | + | Creatine | — |
| Inositol | + | L-Glutamic acid | + |
| Lactose | — | L-Glutamine | + |
| D-Maltose | + | Glycine | + |
| D-Mannose | + | L-Proline | + |
| L-Raffinose | + | L-Serine | + |
| D-Ribose | — | L-Threonine | + |
| D-Sorbitol | _ | L-Tyrosine | + |
| D-Sucrose | + | | |
| D-Xylose | _ | | |

Table S2. Physiological and biochemical characteristics of strain NEAU-HV9, Abbreviation: +, positive; –, negative.

All data was obtained from this study.

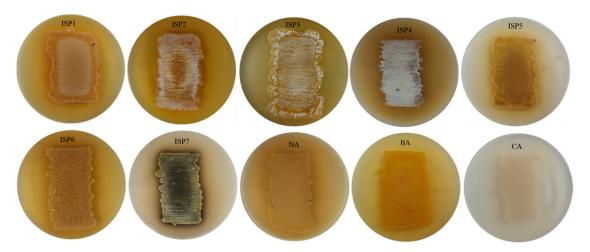


Figure S1. Cultural characteristics of strain NEAU-HV9 observed on ISP 1, ISP 2, ISP 3, ISP 4, ISP 5, ISP6, ISP7, Nutrient agar, Bennett's agar and Czapek's agar after being incubated at 28 °C for 2 weeks.

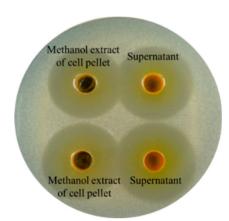


Figure S2. Bioactivities of the supernatant and cell pellet of NEAU-HV9 against R. solanacearum.

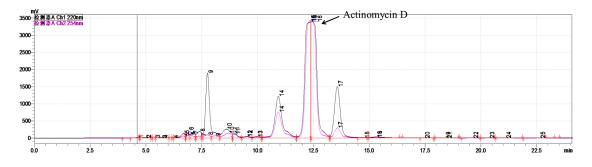


Figure S3. The HPLC profiles of crude extract produced by *Streptomyces* NEAU-HV9.

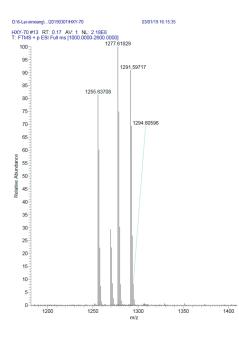


Figure S4. Mass Spectrometry of actinomycin D (C62H86N12O16Na : 1277.6).

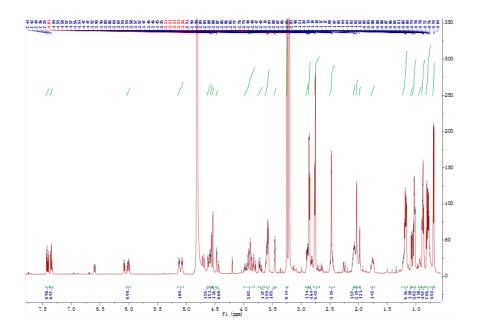


Figure S5. ¹H NMR of actinomycin D.

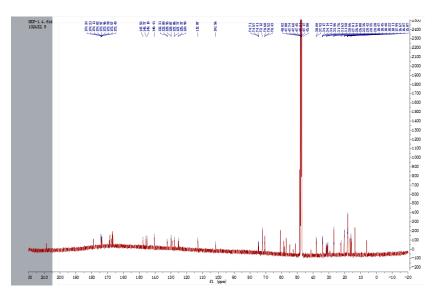


Figure S6. ¹³C NMR of actinomycin D.

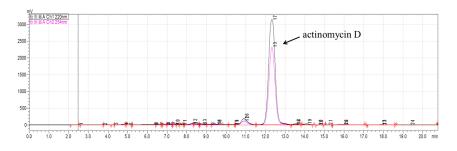


Figure S7. The HPLC profiles of commercial actinomycin D.



Figure S8. Bioactivities of commercial actinomycin D and the main product of NEAU-HV9 against *R. solanacearum.*

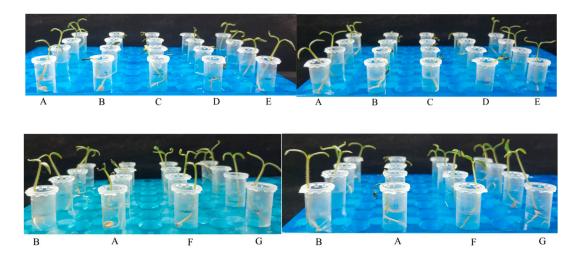


Figure S9. Control efficiency of strain NEAU-HV9 against *R. solanacearum*. A, tomato seedlings were inoculated with sterile water (CK 1); B, tomato seedlings only were inoculated with *R. solanacearum* (CK 2); C, tomato seedlings were preinoculated with suspension (10⁷ cfu mL⁻¹) of NEAU-HV9 and then inoculated with *R. solanacearum* (TR 1); D, tomato seedlings were preinoculated with suspension (10⁸ cfu mL⁻¹) of NEAU-HV9 and then inoculated with *R. solanacearum* (TR 1); E, tomato seedlings were preinoculated with suspension (10⁹ cfu mL⁻¹) of NEAU-HV9 and then inoculated with *R. solanacearum* (TR 1); E, tomato seedlings were preinoculated with suspension (10⁹ cfu mL⁻¹) of NEAU-HV9 and then inoculated with *R. solanacearum* (TR 1); F, actinomycin D at the concentration 1 × MIC (TR 2); G, actinomycin D at the concentration 2 × MIC (TR 2).

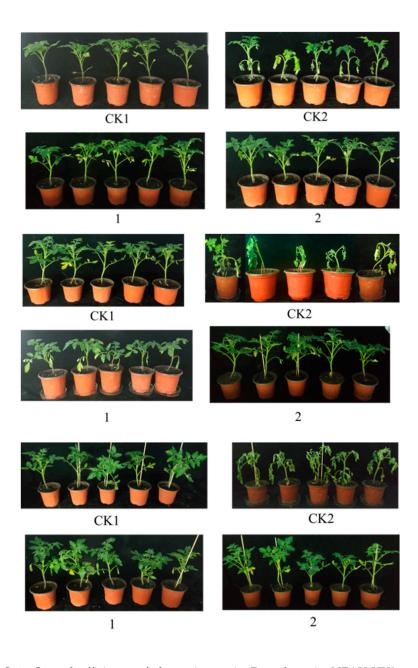


Figure S10. Control efficiency of the actinomycin D and strain NEAU-HV9 against *R*. *solanacearum*. CK 1, positive control; CK 2, negative control; 1, tomato seedlings were preinoculated with suspension (10^9 cfu g⁻¹) of NEAU-HV9; 2, actinomycin D at the concentration $1 \times MIC$.