L-Histidine Induces Resistance in Plants to the Bacterial Pathogen Ralstonia solanacearum Partially Through the Activation of Ethylene Signaling

Journal or publication title: Plant and Cell Physiology
Volume: 57
Number: 9
Page range: 1932-1942
Year: 2016-09-01
URL: http://id.nii.ac.jp/1578/00000711/
doi: 10.1093/pcp/pcw114

Creative Commons license: http://creativecommons.org/licenses/by/3.0/deed.ja
**Supplementary Fig. S1.** Effects of a yeast cell extract on the inhibition of bacterial wilt disease in the tomato. The roots of hydroponically grown tomato plants with two to three true leaves were soaked in AgrevoEX, a liquid fertilizer originating from a yeast cell extract, diluted to 1:500 with H₂O in a test tube for 48 h. The treated plants were inoculated with *R. solanacearum* strain 8107S by submerging their roots into a bacterial suspension (5X10⁷ cfu ml⁻¹) for 24 h at 30°C. The inoculated plants were incubated at 30°C. Photographs were taken 7 dpi.
**Supplementary Fig. S2.** Flow diagram for the purification and isolation of a bacterial wilt disease-inhibiting compound from a yeast cell extract. (A) Extraction and purification procedures. Each fraction was subjected to a bioassay using a test tube to examine their ability to inhibit bacterial wilt disease in the tomato. Inhibitory activity was determined based on the degree of disease incidence that was evaluated using a disease index ranging from 0 to 4: 0, no wilted leaves; 1, up to 25% wilted; 2, up to 50% wilted; 3, up to 75% wilted; and 4, entirely wilted. Inhibitory activity for wilt disease in fractions obtained by ultrafiltration (B) and SPE (C). Data are the means ± SD of three independent assays, each performed with eight plants per treatment. Asterisks indicate significant differences from the H$_2$O treatment (**P < 0.01). Fractions that exhibited inhibitory activity are highlighted in red.
Supplementary Fig. S3. Separation of a bacterial wilt disease-inhibiting compound by HPLC. (A) HPLC chromatogram of the active fraction obtained by SPE. (B) Inhibitory activity in fractions obtained by the 1st HPLC. Chromatogram (C) of second round HPLC and inhibitory activity (D) in the fractions obtained. Data are the means ± SD of three independent assays, each performed with eight plants per treatment. Asterisks indicate significant differences from the H<sub>2</sub>O treatment (**P < 0.01). Fractions that exhibited inhibitory activity are highlighted in red. The peak with a retention time of 52.0 min is histidine.
Supplementary Fig. S4. Exogenously applied L-histidine inhibits wilt disease caused by *R. solanacearum* in the tomato. The roots of tomato plants grown in Jiffy-7 pots were soaked in 10 mM L-histidine or H$_2$O for 48 h, inoculated with strain 8107S, and photographed 7 dpi.
**Supplementary Fig. S5.** Effects of the duration of the L-histidine treatment on the inhibition of bacterial wilt disease. The roots of tomato plants grown in Jiffy-7 pots were soaked in 10 mM L-histidine or H₂O for 24, 48, or 72 h and inoculated with strain 8107S. Resistance was evaluated by determining the severity of wilt symptoms 14 dpi. Data are the means ± SD of three independent assays, each of which was performed with eight plants per treatment. Asterisks indicate significant differences from the H₂O treatment (**P < 0.01).
Supplementary Fig. S6. HPLC analysis of L-histidine in AgrevoEX. A 10 μl aliquot of a 500-fold diluted working solution of AgrevoEX was injected onto a reversed-phase HPLC column and monitored at 210 nm. A solution of 1 mM L-histidine was used as a standard.
Supplementary Fig. S7. Effects of exogenously applied L-histidine on phytohormone production in Arabidopsis plants. Hydroponically grown Arabidopsis (Col-0) plants were treated with 10 mM L-histidine or H₂O by submerging their roots in the solution for 48 h. L-histidine-treated roots and untreated leaves were detached and used for the measurement of SA, JA, and ABA. Data are the mean ± SD of three independent measurements.
Supplementary Fig. S8. Effects of exogenously applied L-histidine on the activation of AtMPK3 and AtMPK6 in Arabidopsis plants. Hydroponically grown Arabidopsis (Col-0) plants were treated with 10 mM L-histidine or H₂O by submerging their roots in the solution for the indicated time intervals. L-histidine-treated roots were detached and used for protein extraction followed by immune complex kinase assay with anti-AtMPK3 or anti-AtMPK6 antibodies. Arrows indicate phosphorylated MBP (P.MBP). Experiments were repeated three times with similar results.
Supplementary Fig. S9. Effects of exogenously applied L-histidine on the induction of TMV resistance in tobacco. Tobacco plants were treated with 10 mM L-histidine or H$_2$O by submerging their roots in the solution for 48 h. The leaves of the treated plants were inoculated with TMV, incubated at 22°C for 5 d, and subjected to diameter measurements of necrotic lesions (A) or an analysis of the amount of viral RNA (B). Data are the means ± SD of three independent assays, each of which was performed with six plants per treatment.