Supplemental Appendix for:

Identification of a virulence factor in the banana pathogen *Dickeya zeae* MS2

Luwen Feng, Amy L. Schaefer, Ming Hu, Ruiyi Chen,
E. Peter Greenberg and Jianuan Zhou

Figure S1. Virulence scoring for *Dickeya zeae* MS2 banana disease. Images are banana seedlings 7 days post-inoculation. The assays were based on those described elsewhere (1). The numbers (0-3) correlate to disease severity. (0) no visible infection symptoms; (1) discoloration only at the inoculation point on the stem; (2) discoloration and soft rot spread throughout the stem; (3) discoloration and soft rot spread from stem to leaves or growing point, and/or the plant can no longer support leaf weight.

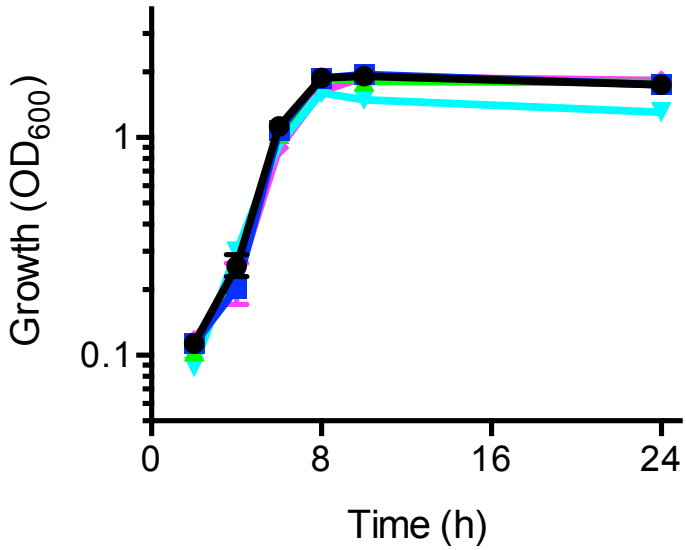

Figure S2. Growth of MS2 and MS2 mutants in LB at 30°C with shaking (25-ml volume). Wildtype MS2 (black), The ExpR mutant Δ*expR* (pink), The ExpI mutant Δ*expI* (teal), the PipR mutant Δ*pipR* (green), and the PipA mutant Δ*pipA* (blue). Data are means of three experiments and the error bars represent the range.

WT ΔexpR ΔexpI

Exoenzyme	WT	Δ*expR*	Δ*expI*
Cellulase	100	99.6 (0.001)	99.7 (0.003)
Pectate lyase	100	99.9 (0.002)	99.7 (0.002)
Polygalactouranase	100	100.0 (0.006)	99.8 (0.009)
Protease	100	99.9 (0.002)	99.8 (0.002)

Figure S3. (Top) Potato-slice infections with wildtype (WT) *D. zeae* MS2 and strains with mutations in *expR* (Δ*expR*) and *expI* (Δ*expI)* mutants. Potato tuber assays were as described previously (2), except that potato slices were 1 cm thick and incubated at 30°C. (Bottom) Exoenzyme activity measured as described previously (3). Data are mean values of 3 biological replicates (+/- SD) normalized to the MS2 wildtype (WT) strain.

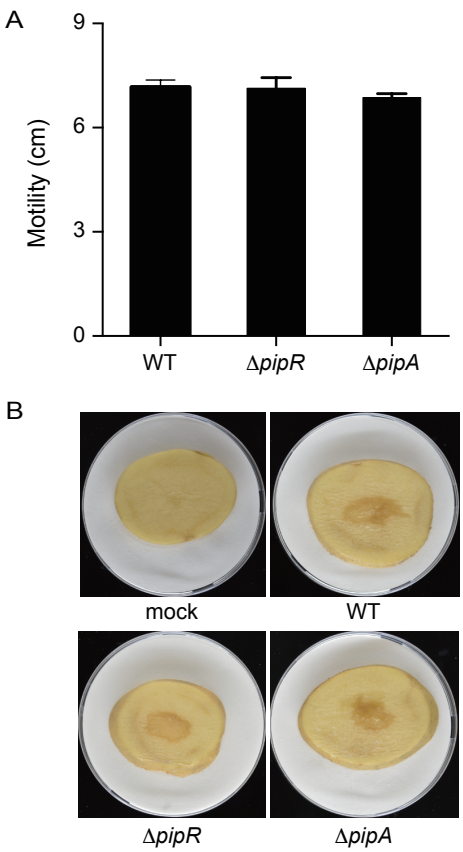

Figure S4. The PipR (Δ*pipR*) and PipA (Δ*pipA)* mutants are indistinguishable from the MS2 wildtype (WT) in swimming motility and potato tuber assays. (A) Swimming motility assays measuring the distance traveled by the indicated strain during 24 hours as described in MATERIALS AND METHODS. (B) Potato slice assays prepared as described in Figure S3 above.

SUPPLEMENTAL APPENDIX REFERENCES

1. Down, J. M., Clarke, B. R., Milligan, D. E., Tang, J. L. & Daniels, M. J. (1990) Extracellular proteases from *Xanthomonas campestris* pv. *campestris,* the black rot pathogen. *Appl Environ Microbiol* 56: 2994-2998.
2. Hussain, B. M., Zhang, H. B., Xu, J. L., Liu, Q., Jiang, Z. & Zhang, L. H. (2008) The acyl-homoserine lactone-type quorum-sensing system modulates cell motility and virulence of *Erwinia chrysanthemi* pv zeae. *J Bacteriol* 190: 1045-1053.
3. Hu, M., Li, J., Chen, R., Li, W., Feng, L., Shi, L., Xue, Y., Feng, X., Zhang, L. & Zhou, J. (2018) *Dickeya zeae* strains isolated from rice, banana and clivia rot plants show great virulence differentials. *BMC Microbiol* 18: 136.