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Interactions with hosts at cool temperature, not cold tolerance, explain the unique epidemiology of *Ralstonia solanacearum* Race 3 biovar 2

Fanhong Meng, Annett Milling and Caitilyn Allen, Department of Plant Pathology, University of Wisconsin-Madison

Introduction

Ralstonia solanacearum is a soil-borne plant pathogen that causes lethal bacterial wilt disease in more than 200 plant species around the world, including important crops such as potato, tomato, tobacco, banana and peanut (1). Due to its extreme aggressiveness, wide geographic distribution, and unusually broad host range, bacterial wilt is considered one of the most destructive plant diseases (2). The bacterium invades plant roots from the soil through wounds or natural openings, colonizes the root cortex and vascular parenchyma, and eventually enters the xylem vessel and spreads up into the stem and leaves (3). Affected plants suffer chlorosis, stunting, wilting, and usually die rapidly (Fig.2).

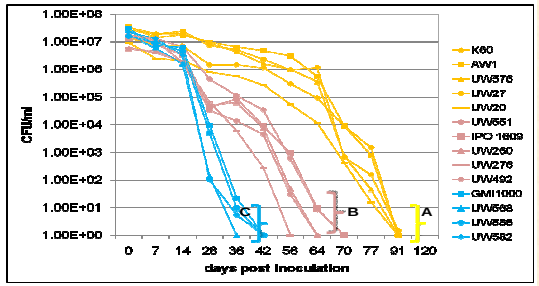
While most *R. solanacearum* strains are intolerant of cold and are limited to tropical and warm temperate zones, one subgroup known as race 3 biovar 2 (R3b2) causes severe losses in the cool highland tropics. R3b2 strains are listed as Select Agents in the US (4), making R3b2 subject to stringent security regulations. Although epidemiological data document the cold tolerance of R3b2 strains (5, 6, 7), there have been no studies of the mechanisms that underlie this distinctive ecological trait. Here we compared the survival, growth and virulence of R3b2 and non-R3b2 strains at different temperatures with and without host plants. Our data indicate that R3b2 strains are not distinctively cold tolerant in the absence of host plants, but they do survive better in host tissue in the cold, and are more virulent at cool temperatures than tropical or warm-temperate strains.

Table 1 Bacterial strains used in this study

	Strain	Race	Biovar	Phylotype[a]	Isolated from	Location
Group A	K60	1	1	II/7	Tomato	N.C., USA
	AW1	1	1	II/7	Tomato	Alabama, USA
	UW27	1	1	II/7	Tobacco	Florida, USA
	UW576	1	1	II/7	Tomato	Florida, USA
	UW20	2	1	II/6	Banana	Venezuela
Group B	UW551	3	2	II/1	Geranium	Kenya
	IPO1609	3	2	II/1	Potato	The Netherlands
	UW260	3	2	II/1	Potato	Peru
	UW276	3	2	II/1	Potato	Mexico
	UW492	3	2	II/1	Potato	Peru
Group C	UW582	1	1	II/5	Hydrangea	Florida, USA
	GMI1000	1	3	I/12	Tomato	French Guyana
	UW585	1	3	I/13	Pepper	Florida, USA
	UW568	1	3	I/14	Soil, tomato field	Guatemala

[a]Phylotype/ Sequevar. Phylotype was determined by multiplex PCR and sequevar was determined based on *egl* gene DNA sequence.

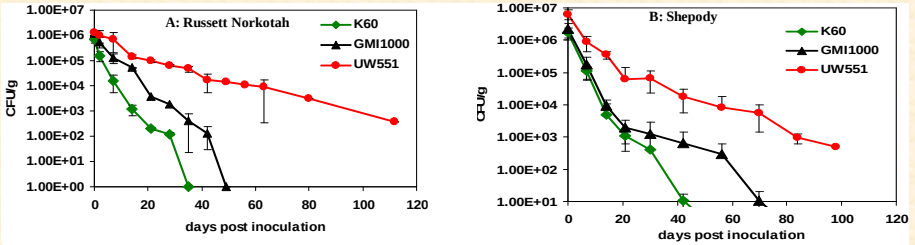
Fig. 1 Survival of *R. solanacearum* strains in water at 4°C



Bacterial cells were grown in rich broth at 28°C to OD600 = 0.1 and pelleted. The cell pellet was washed twice with 25°C sterile MilliQ water and used to inoculate 10-ml MilliQ water microcosms in 18 x 150 mm glass tubes to a density of 1- 2 x 10^7 cfu/ml. Cells were starved for 2 days at RT before incubation at 4°C . Culturable cells were enumerated at regular intervals by dilution plating on rich agar plates. Groups A, B and C were distinguished based on relative survival. This experiment has been repeated 3 times; typical results are shown.

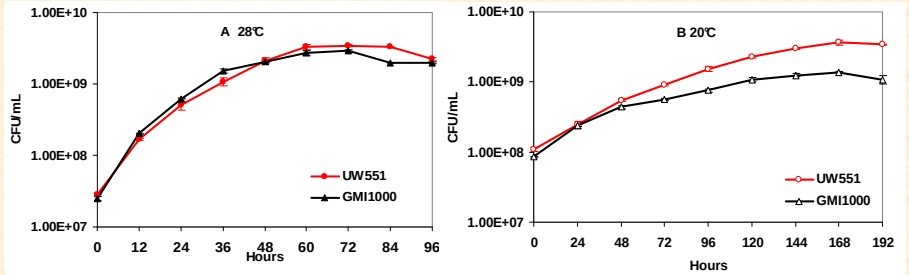
Fig. 2 Disease symptom of bacterial wilt on tomato

Fig. 3 A R3b2 strain (UW551) survived longer in potato minitubers at 4°C than strains native to the southeastern USA (K60) and the tropics (GMI1000)



Cells were grown in rich medium at 28°C to OD600 = 0.1 and pelleted. The cell pellet was washed twice with 25°C sterile MilliQ water and used to inoculate two varieties of potato minitubers to an initial cell density of 1 -2 x 10^6 CFU/ tuber. Tubers were immediately stored at 4°C, a typical storage temperature for seed potatoes. Culturable cells were enumerated at regular intervals by dilution plating on rich agar plates. 2-3 tubers were sampled each time. This experiment has been repeated twice; typical results are shown.

Fig. 4 R3b2 strain UW551 and tropical strain GMI1000 grow similarly in culture

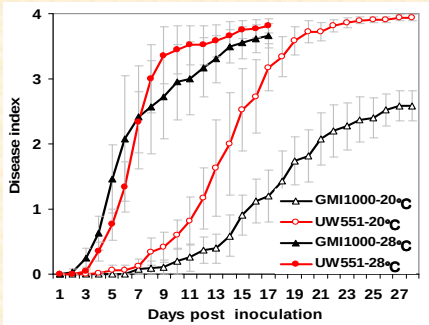


Cells were grown in minimal medium with 0.2% glucose. Cell counts were performed every 12 hours for cells grown at 28°C and every 24 hours for cells grown at 20°C . The growth curves show the average of three experiments, each containing three cell counts per strain per time point; bars indicate standard error.

Conclusions

- Diverse R3b2 strains didn't survive in water at 4°C as long as a group of strains that originated in the southeastern USA. However, R3bv2 strains survived slightly better than Group C strains, which mainly originated in the tropics.
- In contrast, R3b2 strain UW551 survived significantly longer in potato tubers at 4°C (the temperature of commercial seed potato storage) compared to tropical strain GMI1000 (Group C) and American strain K60 (Group A).
- There was no difference in growth rate of R3b2 strain UW551 and tropical strain GMI1000 at 28°C in minimal medium, though UW551 grew slightly faster than GMI1000 at 20°C .
- In a naturalistic soil soak inoculation of tomatoes, both GMI1000 and UW551 were highly virulent at 28°C . In contrast, at 20°C , UW551 still killed all the plants (though it took longer), but GMI1000 was much less virulent at the cooler temperature, killing less than half of the plants one month after inoculation.
- Thus, the "cold-tolerant" epidemiological trait of R3b2 is expressed only in interaction with host plants. These data suggest that R3b2 strains are not more likely than native US strains to survive in soil or water during cold temperate winters.

Fig. 5 Virulence of R3b2 strain UW551 and tropical strain GMI1000 on tomato plants at 20°C and 28°C



Sixteen-day-old unwounded tomato plants (cv. Bonny Best) were inoculated by pouring bacteria onto the soil to a final concentration of about 1x10^8 CFU/g soil. Plants were rated daily on a disease index scale from 0 to 4 where 0 indicated healthy and 4 indicated 100% wilted. Each point represents the mean disease index for three independent experiments each containing 20 plants, bars indicate standard error.

References

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