



# New report of *Lolium multiflorum* and *Rumex crispus* as weed hosts of epiphytic populations of *Pseudomonas* sp., causal agent of yellow bud in onions in Georgia, USA

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Yellow bud, an emerging bacterial disease of onion (*Allium cepa*), has been spreading throughout the Vidalia onion-growing region in Georgia, USA since 2007 (Gitaitis, 2012). Symptoms of yellow bud include intense chlorosis in emerging leaves and severe blight in the older leaves leading to stand loss and reduced bulb size. The causal agent is a Gram-negative, ice-nucleating, rod-shaped, non-fluorescent, aerobic bacterium. The bacterium possesses all the phenotypic characteristics of *Pseudomonas syringae* van Hall LOPAT group Ia of Lelliott *et al.* (1966) as it produces levan, is negative for oxidase, potato rot, and arginine dihydrolase and produces a hypersensitive reaction in tobacco. Like some other species sharing these traits, yellow bud strains have the ability to produce the toxin coronatine. In the spring of 2012, weed samples (20 samples at each location) were collected from the edge of fields in three different counties (one fallow field where yellow bud was first observed in 2007 and two fields with onions currently expressing yellow bud symptoms). Samples were placed in 25 ml of phosphate-buffered saline and shaken overnight at 24°C. After incubation, 4.0 ml aliquots were removed and divided into two fractions (3.0 ml and 1.0 ml) that were placed in separate, sterile micro-centrifuge tubes. Total genomic DNA was isolated from the 3.0 ml fraction using commercial extraction kits and subjected to SYBR green-real-time PCR using primers that amplify the coronafacate ligase (cfl) gene (Gitaitis *et al.*, 2012). The remaining 1.0 ml fraction was spread-plated on nutrient agar (NA) supplemented with 0.5% yeast extract (NA+).

The PCR assay detected the bacterium on Italian ryegrass (*Lolium multiflorum*) from all three locations and on curly dock (*Rumex crispus*) from only the fallow field. Bacterial colonies isolated on NA+ had characteristics similar to previously reported yellow bud strains (Lelliott *et al.*, 1966). When 15-day old onion seedlings, cv. Century, (= 10/strain/experiment) were inoculated with a suspension of  $1 \times 10^8$  cfu/ml (using a hypodermic needle, with 1 ml of inoculum injected into the cavity of onion leaf), 100% of the seedlings developed yellow bud symptoms 14 days after planting under greenhouse conditions (~24°C and ~50% RH). Ten control plants inoculated with sterile water remained healthy.

Bacterial colonies were re-isolated from seedlings showing symptoms. The non-fluorescent colonies recovered had similar characteristics to those described above. Universal primers fD1 and rD1 (Weisberg *et al.*, 1991) and cfl1 (GGCGCTCCCTCGCACT) and cfl2 (GGTATTGGCGGGGTGC) were used to amplify the 16S rRNA and coronafacate ligase genes, respectively. Amplified products were sequenced and BLAST searched in GenBank. The nucleotide sequences of the weed strains (GenBank Accession Nos. KC786974 to KC786977) matched *Pseudomonas* sp.7Y-1 (JF939842.1; Gitaitis, 2012) and *P. syringae* pv. *atropurpurea* (AB001440.1) (97 to 98% similarity with 16S rRNA and 94 to 98% by coronafacate ligase gene). Inoculation of isolated strains on four-week old Italian ryegrass and curly dock seedlings under greenhouse conditions (both by injecting 1ml of inoculum at  $1 \times 10^8$  cfu/ml into the leaf blade or by spraying at the same inoculum concentration on the leaf surface until runoff) did not result in yellow bud symptoms, when incubated for 28 days. Our data suggest that these bacteria survive as epiphytes without inciting any symptoms on their weed hosts. However, they possess ability to cause disease on inoculated onion seedlings. Understanding the epidemiology of yellow bud should be helpful for developing disease management strategies.

## References

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