Description and Identification of the new Phytopathogenic Bacterium causing Bacterial Black Spots on Corn Salad (*Valerianella locusta*), as *Acidovorax valerianellae*

Grondeau C., Samson R., Gardan L., Comité Départemental de Développement Maraîcher / Institut National de la Recherche Agronomique, 42 rue Georges Morel, BP 57, 49071 Beaucozéd cedex, FRANCE. Email: grondeau@angers.inra.fr

Introduction

In France since 1985, corn salad (*Valerianella locusta*) production has been increasing by 820 t a year and now reaches nearly 15 000 t. This increase was followed by the outbreak of a new disease in the main production area. In 1991, a pathogenic bacterium was isolated from black and greasy leaf spots. Studies based on phenotypic and genetic characteristics have been carried out to describe this new bacterium first identified as a non-fluorescent *Pseudomonas* (1). As *Phoma valerianellae* is a fungus also responsible for black leaf spots on corn salad, a reliable method of diagnosis was needed to identify the disease in routine. Immunofluorescence performed on symptoms and double-diffusion on pure cultures were tested.

Description of *Acidovorax valerianellae*

Biochemical tests. Numerical analysis of phenotypic characteristics indicated that corn salad strains were clustered in one discrete phenon related to *Acidovorax* reference strain and distantly related to *Pseudomonas*, *Ralstonia* and *Burkholderia* reference strains.

DNA relatedness. DNA hybridizations showed that corn salad strains were 84 to 100% similar to the type strain CFBP4730 and constituted a discrete genomospecies.

16s rRNA gene sequence analysis. Corn salad strains, branched in the vicinity of *Acidovorax facilis* and *A. anthurii*, a new pathogenic bacterium of *Anthurium*, belonged to the genus *Acidovorax*.

Identification by Ouchterlony double-diffusion on pure cultures and path-test

Three hundred and ten strains isolated from corn salad which were non fluorescent, cytochrome c oxydase and HR on tobacco positive, levan sucrase and arginine dihydrolase negative, were inoculated by injection of a bacterial suspension in the oldest leaves of corn salad (Coquille de Louviers). Two hundred and fifty strains gave a brown and greasy necrosis of the infiltrated area within fifteen days, and were positive by double-diffusion with polyclonal antibodies. Sixty strains remained negative for the two tests.

Three strains isolated from water collected in pools near crops, that cross-reacted by double-diffusion, were negative to the pathogenicity test, and used

mannose, L+arabinose or glucose whereas the reference strain of *A.valerianellae* was negative for those three carbon sources.

Direct diagnosis in spots by immunofluorescence

From 1994 to 1998, immunofluorescence staining with polyclonal antibodies was performed directly on lesions cut in a drop of sterile water and taken on a large number of plants received at different times from different locations. The same drop was simultaneously streaked on King B medium and typical colonies of *Acidovorax* were identified by fluorescence, cytochrome c oxidase, levan sucrase, arginine dihydrolase, hypersensitivity on tobacco and double-diffusion. The two techniques gave the same result for more than 90% of the spots analysed.

Conclusion

Corn salad strains should be considered as a new phytopathogenic bacterium belonging to the β -subclass of Proteobacteria and the genus *Acidovorax*. The name *A. valerianellae* is proposed. In routine, *A. valerianellae* can be characterised by no fluorescent pigment, no levan sucrase and arginine dihydrolase, by the presence of a cytochrome c oxidase, by hypersensitivity on tobacco and double-diffusion positive. Double-diffusion can be replaced by pathogenicity test. Then, *A. valerianellae* was identified in Belgium (1998) and in Germany (2000).

Reference

1. Rat and Gardan, 1993. Fruits et Légumes 111, 42 - 43.