Occurrence in France of Pseudomonas syringae pv. papulans, the causal agent of blister spot on apple

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Abstract : Blister spot caused by *Pseudomonas syringae* pv. *papulans* (*PSP*) is an important disease that especially affects the apple cultivar Mutsu in eastern USA, in Canada (Ontario) and in Italy. In France, this disease has never been described. A study on epiphytic populations of *Pseudomonas syringae* isolated from apple and pear leaves in French orchards revealed a particular isolate called KA54. Its biochemical characterization showed high similarities with *PSP* strains isolated from blister spots in USA, Canada and Italy. Identical symptoms were obtained with KA54 and these *PSP* strains after inoculation of immature fruits of the cultivar Fuji, or inoculation of young leaves of the cultivars Fuji, Mutsu, Gala and Golden Delicious. In addition the Koch's postulate was verified. These results confirm the presence of *PSP* in France. In order to facilitate its diagnostic by PCR method, we developed primers by genomic comparison between *PSP* and *Pseudomonas syringae* pv. *syringae*. These primers allowed the clear-cut identification of KA54 and others *PSP* amongst a collection of *Pseudomonas syringae*.

Key words : Apple, blister spot, *Pseudomonas syringae* pv. *papulans*, PCR, primers.

Introduction

Pseudomonas syringae pv. *papulans (PSP)* is the causal agent of blister spot, an important disease of apple cv. Mutsu in Canada (Dhanvantari, 1969, 1977), the eastern USA (Burr & Hurwitz, 1979) and Italy (Bazzi & Calzolari, 1983); it has never been described in France. A survey of epiphytic populations of *Pseudomonas syringae* in French orchards of apple revealed the presence of an isolate, KA54, showing high similarities with *PSP* strains. In this study, we present the phenotypical, serological, pathological and molecular characterization of this isolate.

Materials and Methods

Strains

Isolate KA54 was compared with a collection of 10 strains of *PSP* originating from USA, Canada, Italy and diverse strains of *Pseudomonas syringae* pv. *syringae (PSS)* from the Collection Française de Bactéries Phytopathogènes (CFBP).

Phenotypical and serological characters

20 conventional biochimical tests were used according to Schaad (1988). In addition O-serogroups of the strains were assigned by Ouchterlony double diffusion (Saunier *et al.*, 1996).

Pathogenicity tests
Immature fruits cv. Mutsu and leaves of cv. Mutsu, Fuji, Golden Delicious and Gala were vacuum infiltrated by bacterial suspensions adjusted to 10^7 cfu /ml. This technique was selected because it allowed infection without mechanical injuries.

Molecular characterization
PCR method was performed using primers (named Pap) designed by genomic comparison between *PSP* and *PSS* (Kerkoud & Manceau, unpublished result).

Results

Phenotypical and serological characters
A high level of similarity was found between KA54 and others *PSP* strains except for lactate and tartrate utilisations. Two characters: production of levan from sucrose and polypectate gel pitting at pH5 (which were negative and positive respectively for *PSP)* discriminated *PSP* from *PSS*. All but one isolates (including KA54), except two rough strains (RIB), were serologically homogeneous. They belonged to a new O-serogoup (PST4) defined as giving a reaction with the antisera 196 and 287, but no reaction with 292.

Pathogenicity tests
KA54 produced typical blister spots on Fuji fruits, identical to spots caused by the *PSP* reference strain CFBP 3323. These blister spots were also clearly different from the hypersensitive necrosis induced by the *PSS* strain CFBP 3077 (2027.37). Typical *PSP* symptoms were also obtained after leaf inoculation of Mutsu, Fuji, Golden Delicious and Gala (Figure 1, 2 et 3). In addition, Koch's postulate was completed when KA54 was reisolated from the blister spot lesions of apples and leaves.

Molecular characterization
The Pap primers especially designed for this study amplified specifically a 240 pb DNA fragment of *PSP* strains (including KA54) amongst the collection of *P. syringae.* This result was confirmed with additional tests, using further isolates of *Pseudomonas* (data not shown). The same signal was obtained with strains reisolated from pathogenicity test.

Discussion

Phenotypical characters and pathogenicity tests undoubtedly showed that KA54 is identified as *PSP*. The specific Pap primers confirm theses results and could be used to facilitate the molecular diagnosis of this pathogen in further epidemiological studies. In addition, our results indicate that polypectate gel pitting at pH5 is a useful discriminating test in *PSP* identification. Finally, *PSP* appears to be present in France, although no natural symptoms have been reported in the country yet.

References

Bazzi, C. & Calzolari, A. 1983: Bacterial blister spot of «Mutsu» apples in Italy. Phytopath. Medit. 32: 19-21

Burr, T.J. & Hurwitz, B. 1979: The etiology of blister spot of «Mutsu» apple in New York State. Plant. Dis. Reptr. 63: 157-160.

Dhanvantari, B.N. 1969: Bacterial blister spot of apple in Ontario. Can. Plant. Dis. Surv. 49: 36-37.

Dhanvantari, B.N. 1977: A taxonomic study of *Pseudomonas papulans* Rose 1917. N.Z. Jl. Agric. Res. 20: 557-561.

Saunier, M. Malandrin, L. & Samson, R. 1996: Distribution of *Pseudomonas syringae* pathovars into twenty three O-serogroups. Appl. Environ. Microbiol. 62: 2360-2374.

Schaad, N.M. 1988: Laboratory guide for identification of plant bacteria. 2nd edition. American Phytopathological Society Press., St. Paul. MN., 164 pp.