Short Communication

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First Report of Bacterial Leaf Spot (Pseudomonas syringae pv. coriandricola) of Coriander in Spain

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Abstract

Bacterial leaf spot symptoms of coriander were first observed in January 2003 in three coriander fields in the valley region of the Axarquía (Málaga, Spain), showing a very high incidence. Pseudomonas syringae pv. coriandricola was consistently isolated from diseased plants, identified and its pathogenicity on coriander could be proved. The effective inoculum dose (ED50) of the isolated strains was estimated and it was very similar to those displayed by the P. syringae pv. coriandricola reference strains used as control. This is the first report of bacterial leaf spot on coriander in Spain.

Introduction

Bacterial leaf spot (also named bacterial umbel blight and seed decay) of coriander (Coriandrum sativum L.) is one of the most serious diseases of coriander, causing severe losses in open-field coriander production. It is caused by the bacterium Pseudomonas syringae pv. coriandricola (Psc), which has been described in different countries of Europe (Nemeth et al., 1969; Taylor and Dudley, 1980; Toben and Rudolph, 1996), North and Central America (Pérez-Valdez et al., 1990; Cooksey et al., 1991) and Australia (Gooden et al., 1995). Pseudomonas syringae pv. coriandricola causes necrosis, leaf spot, water-soaked lesions on foliage and reduced number of fruits in the umbels. The lesions develop a purplish margin with a tan, necrotic centre and they become manifested especially during flowering, when clusters of brownish plants appear in the field. These primary infections foci may spread very fast during cold and wet weather. Usually, the disease and yield losses increased from year to year (Toben and Rudolph, 1996).

The bacterium Psc is generally seed-borne, and water-splashed to the foliage of seedlings, mainly due to rain or sprinkler irrigation, spreads the bacteria. Only three plant species (Coriandrum sativum, Levisticum officinale and Ammi majus) has been described as susceptible to Psc (Toben and Rudolph, 1996). However, this bacterium can persist in the field on other plants, which harbour the pathogen as epiphyte for some time, but do not show symptoms (Al-Shinawi, 1996). Copper-based foliar treatments are not cost-effective for bacterial blight control, and treatments are ineffective when disease pressure is high (Dennis and Wilson, 1997). Clean seeds and furrow or drip irrigation to maintaining dry foliage are the most effective means of control, and in addition heat treatment of the ripe fruits before sowing (6 days at 65°C with dry heat) and other disinfection treatments have been developed (Toben and Rudolph, 1996).

The main objective of this work was to isolate and identify the causal agent of the bacterial leaf spot of coriander plants in Spanish fields by performing the Koch’s postulates. The quantitative relationship between the inoculum dose of the pathogen and the disease incidence was also studied.

Materials and Methods

Coriander plants showing leaf spot symptoms were observed and sampled in January 2003 from coriander growing fields at Axarquía (Málaga, Spain, Table 1). To isolate the causal agent, aerial plant parts showing symptoms were placed in sterile plastic bags, transported to the laboratory and processed at the day of sampling. The samples were processed by two methods: (i) a portion was surface-disinfected by immersion in a sterile aqueous solution of 0.1% (w/v) HgCl2, rinsed in sterile distilled water, and plated on King medium B (KB) and nutrient agar (NA; Difco, Detroit, MI, USA). (ii) The remainder portion was homogenized in a laboratory blender for 3 min with
10 ml of sterile phosphate buffer 0.1 m, pH 7.2 (PB) per g of fresh plant material, and 100 µl were spread onto KB and NA. Plates were incubated at 24°C for 2–3 days. A number of predominant colonies were selected and purified by repeated streaking on the same medium. The characteristics of the colonies were recorded after 4 days. The bacterial isolates were identified according to biochemical and physiological tests described previously (Toben and Rudolph, 1996), including Gram stain; LOPAT (levan production, oxidase reaction, potato soft rot, arginine dihydrolase [ADH], and tobacco hypersensitivity) tests; ice-nucleation activity (INA); metabolism of glucose and utilization of D-tartrate and D-arabinose. All inoculated media were incubated at 24°C for 3–5 days. Duplicate tubes or plates were run for each test, and tests were repeated at least twice. INA of the bacterial isolates was assayed following a tube test at 70% of relative humidity (RH). About 10 µl of bacterial suspensions with a cell density of 10^8 cfu/ml and injected into the intercellular spaces of young leaves with hypodermic needles. At least 15 inoculations were performed per every dose and assayed strain (three to four inoculated leaves in three to four independent plants). The experiment was carried out twice. Plants were maintained at growth chamber conditions (24°C, 16 : 8 ratio of light : dark and 70% of RH). Resulting symptoms were evaluated 18 days after inoculation. The percentage of necrotic spots on leaves from each dose assayed were calculated as the mean of more than 60 inoculated leaves for the Spanish Psc isolates, and 30 inoculated leaves for the reference Psc strains. A dose–response curve was produced by plotting the bacterial dose (log_{10} transformed) against a Weibull transformation of the proportion of disease spots (log_{10}([1 – P]/P)). Two straight lines (one for the Spanish Psc isolates and one for the reference Psc strains) were fitted to the data points that were not equal to a 0 or 100% response, and confidence limits (50%) corresponding to different inoculations per dose were calculated as previously described (Shortley and Wilkins, 1965; Cazorla et al., 1998) and showed as bars.

**Results and Discussion**

Symptoms resembling to those caused by bacterial leaf spot were first observed in January 2003 in 2-month-old coriander plants from coriander fields at Axarquía, a valley-area in Málaga, southern Spain. Coriander leaves showed small dark brown spots of 2–6 mm diameter that enlarged to angular lesions delimited by veins (Fig. 1a,b). Patches of severely infected plants

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**Table 1**

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NCPPB, National Collection of Plant Pathogenic Bacteria, United Kingdom.

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Fig. 1 Symptoms of bacterial leaf spot of coriander, indicated by black arrows. (a) Typical symptoms observed on 2-month-old plants in Spanish fields; (b) detail of the coriander leaf spot; (c) symptoms induced by artificial inoculation of wild isolates of *Pseudomonas syringae* pv. *coriandricola* from Spanish coriander plants. The white arrow shows the inoculation spot (IP) of a bacterial suspension of 10^8 cfu/ml.
had leaves with black veins and edges. Only few plant stems were affected in January, because the symptoms seemed to be in an initial stage. During flowering and ripening, the disease becomes manifested in the affecting stems and a clustering of plants showing a dark brown discoloring was observed. Latter symptoms appeared predominantly on the inflorescences. On infected umbels the white to red blossoms discoloured brown, and green, unripe fruits showed water-soaked lesions, which later turned dark brown and sometimes shrivelled, as previously described (Toben and Rudolph, 1996). Plants with advanced disease symptoms usually became stunted and sometimes wilted. In the surveyed Spanish fields, the disease incidence was very high, i.e. approximately 90% of the plants showed dark brown spots on leaves.

White and circular bacterial colonies were routinely isolated from symptomatic plants and inoculated onto KB. The bacteria were rod-shaped, Gram-negative and strictly aerobic, positive for INIA, produced levan and induced a hypersensitivity response (HR) on tobacco. The bacterial isolates were negative for oxidase and arginine dihydrolase tests, did not rot potato slices, and the detected –tartrate, but not –arabinose. Characteristically, the isolates did not produce fluorescent pigments on KB. Following isolation and identification, pathogenicity tests were carried out to determine the virulence of the isolates. All isolates caused disease symptoms on artificially inoculated coriander seedlings in growth-chamber assays (Fig. 1c). From the seedlings, the same bacterium could be reisolated, fulfilling the Koch’s postulates. Thus, it is concluded that the bacterium responsible for the bacterial leaf spot disease of coriander in Spain was identified as Psc (Toben and Rudolph, 1996).

The independent-action model has been used in the analysis of the probability of disease response as a function of bacterial dose concentration (Fig. 2). The median effective dose (ED$_{50}$) for four isolated strains was estimated from the straight line produced by adjusting the bacterial-inoculated dose (log$_{10}$-transformed) against the response data on a Weibull scale obtained from eight assays (r = 0.98, P < 0.001), and compared with that obtained for two Psc reference strains from four assays (r = 0.95, P < 0.001); the estimated ED$_{50}$ displayed by the Spanish isolates of Psc (2.5 × 10$^5$ cfu/inoculation point) were very similar to those observed for the reference strains (4 × 10$^5$, Fig. 2). These ED$_{50}$ values were similar to those observed for pathovars of P. syringae multiplying and inducing symptoms in different plants (Ercolani, 1973; Cazorla et al., 1998). As it has been shown previously, the symptoms produced at low inoculum dose (10$^3$–10$^4$ cfu) resulted in persistent water-soaked lesions resembling the symptoms observed under field conditions (Fig. 1), but lesions produced by higher inocula (approximately 10$^4$ cfu) resulted in necrosis (Toben and Rudolph, 1996). The obtained results showed a very high similarity between the strains isolated in Spain, and the reference strains, including the pathotype strain Psc NCPPB3681.

To our knowledge, this is the first report of the bacterial leaf spot of coriander in Spain. Because this is a very new crop in Spain, and the seeds are the main source of transferring the bacterium, it is most likely that it has been imported by contaminated seeds. The disease could become established in the above-mentioned valley region, however, it may still be possible to prevent the spread of the disease to other potential coriander growing regions along the southern coastal zone, by implementing suitable phytosanitary measures.

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References


