Characterization of *Xanthomonas campestris* pv. *vesicatoria* from *Capsicum annuum* L. in Italy

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ABSTRACT

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In the summer of 1991 and 1992, 38 strains of *Xanthomonas campestris* pv. *vesicatoria* were isolated from pepper leaves and fruits in a number of central (Latium, Marche, Tuscany, and Umbria) and southern (Apulia) Italian regions. Race and copper and streptomycin sensitivity of the bacterial strains were determined. Races 1, 2, and 3 were found to be present on pepper. Of the strains tested, 39, 16, and 45% belonged to races 1, 2, and 3, respectively. Of all strains tested, 45% were resistant to copper sulfate (200 μg/ml), while none were resistant to streptomycin sulfate (100 μg/ml). All of race 2 and 73% of race 1 strains were resistant to copper, while all race 3 strains were copper sensitive.

In Italy, pepper (*Capsicum annuum* L.) is a traditional crop cultivated on about 14,000 ha of open field and 2,200 ha in the greenhouse each year. Bacterial spot, caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye, is an important pepper disease in many production areas of the world (10). In Italy, the disease was first observed in 1964 and is currently present in both the southern and central regions (4,12,16).

Although several compounds for controlling this disease exist, only those containing copper are registered in Italy. However, copper tolerance has been reported among strains of *X. c. vesicatoria* in many production areas (1,13). Consequently, the efficacy of copper for controlling this disease may be questionable.

Pepper cultivars resistant to bacterial spot, not yet available in Italy, may be the best method to control the disease. It is known that single dominant genes *Bs1, Bs2,* and *Bs3* render pepper plants resistant (for hypersensitivity) to bacterial spot. Resistance to races 1 and 2 is conferred by both *Bs1* and *Bs3* genes, while the *Bs2* gene confers resistance to races 1, 2, and 3 (14). To determine which of these genes should be incorporated into commercial cultivars suitable for Italian growers, the races present in Italy and their relative occurrence need to be known.

On the basis of virulence on tomato or pepper plants, there are three groups of *X. c. vesicatoria.* These include the tomato group (XcvT), only virulent on tomato; the pepper group (XcvP), only virulent on pepper; and the pepper–tomato group (XcvPT), virulent on both pepper and tomato (17). Races of *X. c.*

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vesicatoria virulent on pepper (XcvP and XcvPT groups) can be differentiated using three near-isogenic derivative lines of the pepper cultivar Early Calwonder (ECW)—ECW-10R, ECW-20R, and ECW-30R—which carry the resistance genes *Bs1, Bs2,* and *Bs3,* respectively (14).

The purpose of this research was to determine which pepper races of *X. c. vesicatoria* were present in Italy, and whether these strains were copper and/or streptomycin resistant. To our knowledge, this is the first study conducted in a European country to determine the presence and distribution of *X. c. vesicatoria* races isolated from pepper plants.

MATERIALS AND METHODS

Field survey and sampling. Bacterial spot-affected pepper leaf and fruit samples were collected from 35 farms located in various regions of central (Latium, Marche, Tuscany, and Umbria) and southern Italy (Apulia), during June–September of 1991 and 1992. Samples of diseased leaves and fruits were collected in each field from four to five plants per row. Both row and plants to be sampled were selected at random. Diseased samples were processed within 24 hr, or leaves were pressed and dried and stored at 8 C until they were used.

Isolation and identification of the pathogen. To isolate the pathogen, small pieces of either leaf or fruit tissues of pepper plants, cut at the edge of a young lesion, were ground in a sterile mortar with a few drops of sterilized deionized water. A loop of the suspension was streaked on plates containing yeast extract nutrient agar (YNA) (7). The plates were incubated at 27 ± 1 C. Individual bacterial colonies characteristic of *X. c. vesicatoria* were selected and streaked onto YNA. The strains were suspended in sterile tap water and stored at 4 C.

Identification of 38 bacterial strains was performed according to the following biochemical and nutritional tests described by Dye (7) and Sands (19): Gram's stain; oxidative/fermentative metabolism; catalase, oxidase, and urease activities; nitrate reduction; hydrogen sulfide production from cysteine; aesculin, starch, gelatin, and casein hydrolysis; growth at 35 C; and acid production from arabinose, glucose, and mannose.

To determine the pathogenicity of the bacterial strains, pepper plants (cv. ECW) at sixth to seventh true-leaf stage were used. To prepare inoculum, bacteria were grown on nutrient agar (NA) for 48 hr at 27 ± 1 C, suspended in deionized water, and spectrophotometrically adjusted to 1×10^8 colony-forming units (cfu)/ml (optical density of 0.3 at 600 nm). The bacterial suspensions were then diluted to 1×10^6 cfu/ml.

The abaxial surfaces of the fourth and fifth true leaves of two pepper plants per strain were infiltrated with the bacterial suspension using a glass atomizer. After inoculation, plants were kept in a greenhouse at 20–28 C under natural light conditions. Pepper plants infiltrated with water served as controls. The strain Xcv 82-8 (pepper race 1) of *X. c. vesicatoria* was used as a reference strain for biochemical, nutritional, and pathogenicity tests.

Race determination. Races were determined using the procedures described by Minsavage et al (14). Bacteria were grown on NA for 48 hr, suspended in deionized water, and adjusted to about 1×10^8 cfu/ml. Leaves of the differential pepper cultivars (ECW, ECW-10R, ECW-20R, and ECW-30R) were infiltrated with bacterial suspensions by a syringe. Race determination was performed in two separate experiments. In the first experiment, inoculated plants were kept in a growth chamber at 25 C, 240 $\mu E \cdot m^{-2} \cdot s^{-1}$ illumination, 14-hr light period with 55% relative humidity (RH), and 70% RH during the dark period. In the second experiment, plants were incubated in a glasshouse at 22–28 C and 40–50% RH. The pepper race 1 (strain Xcv 82-8), race 2 (strain Xcv E3), and race 3 (strain Xcv 87-13) of *X. c. vesicatoria* were used as reference cultures. Symptom development was followed until the seventh day after inoculation. In incompatible interactions, characterized by hypersensitive reaction, cell collapse and necrosis appeared 12–36 hr after inoculation; while in compatible interactions, water-soaked and chlorotic lesions were evident 3–5 days after inoculation.

Copper and streptomycin sensitivity. Sensitivity of bacterial isolates to copper and streptomycin was assayed as described by Ritchie and Dittapongpitch (18). Five microliters of bacterial suspension for each strain was spotted on SPA medium contained in petri plates (20 g of sucrose, 5 g of peptone, 0.5 g of dibasic potassium phosphate, 0.25 g of magnesium sulfate, and 15 g of agar in 1 L of deionized water) amended with either $CuSO_4 \cdot 5H_2O$ (200 μg/ml) or streptomycin sulfate (100 μg/ml). Aqueous

Table 1. Races of *Xanthomonas campestris* pv. *vesicatoria* recovered from pepper plants in Italy in 1991–1992 and their sensitivity to copper (200 μg/ml) and streptomycin (100 μg/ml) sulfate

				Number of strains						
Year	Region	Location	Total strains	Pepper race 1	Pepper-tomato race 2	Pepper-tomato race 3	Copper resistant	Copper sensitive	Streptomycin resistant	Streptomycin sensitive
1991	Marche	Passatempo (AN)[a]	2	2	0	0	2	0	0	2
		S. Faustino (AN)	1	1	0	0	0	1	0	1
		S. Maria Nuova (AN)	1	1	0	0	1	0	0	1
	Tuscany	Montepulciano (SI)	1	1	0	0	0	1	0	1
		Ossaia (AR)	2	0	0	2	0	2	0	2
	Umbria	Gioiella (PG)	1	0	1	0	1	0	0	1
		Lisciano Niccone (PG)	5	1	4	0	4	1	0	5
		S. Nicolò di Celle (PG)	1	1	0	0	1	0	0	1
		Trestina (PG)	1	0	1	0	1	0	0	1
Total			15	7	6	2	10	5	0	15
1992	Apulia	Mesagne (BR)	3	3	0	0	3	0	0	3
	Latium	Tarquinia (VT)	2	0	0	2	0	2	0	2
		Torrimpietra (Roma)	1	1	0	0	0	1	0	1
	Tuscany	Camucia (AR)	2	0	0	2	0	2	0	2
		Mengaccini (AR)	2	0	0	2	0	2	0	2
		Mercatale (AR)	1	0	0	1	0	1	0	1
		Montalla (AR)	1	0	0	1	0	1	0	1
		Riccio (AR)	1	1	0	0	1	0	0	1
	Umbria	Lisciano Niccone (PG)	5	1	0	4	1	4	0	5
		Tuoro (PG)	5	2	0	3	2	3	0	5
Total			23	8	0	15	7	16	0	23
Total (2 yr)			38	15	6	17	17	21	0	38

[a]Province initials are in brackets. AN = Ancona, AR = Arezzo, BR = Brindisi, PG = Perugia, SI = Siena, and VT = Viterbo.

solutions of both filter-sterilized (0.45 μm pore size) compounds were added to SPA after autoclaving and cooled to 45 C.

Plates were incubated at 27 C for 48 hr, and the presence or absence of growth was recorded. Bacterial strains that grew on SPA medium, amended with either copper sulfate or streptomycin sulfate at the concentrations reported above, were considered resistant to copper or streptomycin, respectively.

RESULTS

Field survey and identification of the pathogen. Bacterial spot was present on pepper leaves on all of the farms surveyed, with a disease incidence ranging from 40 to 90%. Disease symptoms on pepper fruits were found only in fields located in Latium (Torrimpietra, Rome) and Marche (Passatempo, Ancona).

Biochemical and nutritional tests showed that all bacterial strains were gram-negative, aerobic, catalase-positive, and oxidase- and urease-negative. They hydrolyzed aesculin, gelatin, casein, and starch weakly; grew at 35 C; produced acid from arabinose, glucose, and mannose; and produced hydrogen sulfide from cysteine. They did not reduce nitrates. All bacterial strains were pathogenic on the pepper cultivar ECW. Based on biochemical, nutritional, and pathogenicity tests, it was confirmed that all bacterial strains belonged to *X. c. vesicatoria.*

Race, and copper and streptomycin sensitivity determination. Of the 38 strains of *X. c. vesicatoria* tested, 45% were race 3, 39% race 1, and 16% race 2 (Table 1). In 1991, 47% of the strains were race 1, 40% were race 2, and 13% were race 3. In 1992, 65% of the strains were race 3, and 35% were race 1, while race 2 was not detected. Of all strains tested, 45% grew on SPA amended with 200 μg/ml of copper sulfate and were therefore considered resistant to copper (Table 1). We did not find any streptomycin-resistant strains, since none of them grew on SPA containing 100 μg/ml of the antibiotic (Table 1). All race 2 strains and 73% of race 1 strains were resistant to copper, while no copper-resistant isolates of race 3 were found (Table 2).

Table 2. Race, number, and copper sensitivity of *Xanthomonas campestris* pv. *vesicatoria* strains recovered from pepper plants in Italy

Race	Total strains	Number of strains: Copper resistant	Number of strains: Copper sensitive
1[a]	15	11	4
2	6	6	0
3	17	0	17

[a] Race 1 is of the pepper group.

DISCUSSION

This study shows that pepper races 1, 2, and 3 of *X. c. vesicatoria* are present in Italy. Previously, the only indication of pepper races in Italy was provided by Cook and Stall (6), who identified only pepper race 1. Races 3 and 1 were prevalent, representing 84% of the strains obtained in both years. Race 2 was only detected in 1991. Although only 15 strains in 1991 and 23 in 1992 were used in this study, our data on race composition is similar to those recently observed in several other countries. However, in 1992, race 3 was predominant among strains recovered, accounting for 65%; while in the United States (15,18), Taiwan (9), and Barbados (21) it represented less than 20% of the total strains.

The spectrum of races observed has probably been influenced by the strains introduced into Italy by contaminated pepper seed and/or transplants, important sources of primary inoculum (3,8). In addition to these sources, primary inoculum of the bacterium may result from pepper and/or tomato residues present in the soil (2,11). In the Umbrian fields surveyed, where neither peppers nor tomatoes had been grown previously, primary inoculum was probably introduced by contaminated pepper seeds and/or transplants.

Pepper infection by race 3 of the pathogen occurred in several farms located in Latium, Tuscany, and Umbria (Table 1). Since tomato was previously cultivated in the fields located in Latium and Tuscany, it is possible that the infection by race 3, which is virulent on both tomato and pepper plants, could have come from the inoculum present on tomato debris in the soil.

Streptomycin-resistant strains were not detected in our study. Two plausible explanations for this are that the use of this antibiotic is not permitted in Italy, or that the pathogen was introduced on seed which had been produced in countries where the antibiotic is not used or permitted.

Our data, similar to results reported by Marco and Stall (13) and Ritchie and Dittapongpitch (18) on *X. c. vesicatoria* copper sensitivity, document that all race 2 and most race 1 strains were copper tolerant. It has been demonstrated that copper tolerance in race 2 is encoded by a gene cluster situated in a self-transmissible plasmid, which in turn is linked to the avirulence gene that determines race 2 (20). However, it is not known whether copper tolerance in the copper-tolerant race 1 strains is genetically similar to that of race 2 strains. The absence of copper tolerance in race 3 strains observed in the present study confirm the data of Ritchie and Dittapongpitch (18).

The impossibility of using streptomycin or copper mixed with maneb or mancozeb to control copper-resistant strains (5,13) on pepper in Italy, the relatively high percentage of copper-resistant strains, and the absence of resistant pepper cultivars make it very difficult to control pepper bacterial spot. The use and deployment of resistant cultivars to all three races of *X. c. vesicatoria* may provide the best disease-management strategy.

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