DIVERSITY OF PLASMIDS AND PLASMID-ENCODED PHENOTYPIC TRAITS IN *Xanthomonas campestris* pv. *vesicatoria*

By

BLANCA ISABEL CANTEROS

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To the memory

of my father,

Jose Maria Canteros.

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BLANCA ISABEL CANTEROS

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Chairman: Robert E. Stall
Major Department: Plant Pathology

Plasmid profiles were determined in a world collection of 522 strains of Xanthomonas campestris pv. vesicatoria (Doidge) Dye (Xcv), causal agent of bacterial spot of tomato (Lycopersicon esculentum Mill.) and pepper (Capsicum annuum L.). Plasmids were grouped into 13 size classes. The sizes ranged from 2 to 300 kbp, but most plasmids were larger than 30 kbp. A few strains had no plasmid. Seventy-one different plasmid profiles were observed with a maximum of six plasmid size classes in each profile. Diversity was evident in strains from a culture collection as well as from freshly isolated strains. Some profiles were more dominant than others.

Thirty-eight percent of the strains examined were resistant to streptomycin. Seventy percent of the strains were resistant to copper. A DNA fragment encoding copper

resistance hybridized to large plasmid DNA of resistant strains only. Comparison of those plasmids by restriction analysis showed polymorphism and many of the plasmids were self-transmissible. Most strains from Argentina hydrolyzed starch and were pectolytic, whereas strains from USA and Taiwan were negative for those traits.

The plasmid-borne avirulence genes that determine races 1 and 2 of the pepper group of strains and the tomato group of strains were not randomly distributed. Race 2 strains were detected in strains from the USA but only rarely in strains from other parts of the world. Race 1 strains were found in all areas. The tomato group of strains were also found in all areas, but were isolated only from tomato fields.

Strains avirulent to tomato but virulent to pepper were distinguished with carborundum-ammended inoculum. The hypersensitive reaction in tomato caused by those strains was confirmed by electrolyte leakage analysis and multiplication of the bacterium in tomato leaves. The avirulence gene avrBsP was cloned from strain 87-7 and it converted strains virulent in tomato to avirulent. A 1.7 kbp subclone hybridized to plasmid bands in avirulent strains only. The avrBsP gene was linked in most strains to the avirulence gene, avrBs3, that determines race 1 on the pepper group of strains.

CHAPTER 1
INTRODUCTION

Bacterial spot of tomato (Lycopersicon esculentum Mill.) and pepper (Capsicum annuum L.) caused by Xanthomonas campestris pv. vesicatoria (Doidge) Dye (Xcv) is one of the most economically important diseases of those crops. Tomato and pepper are among the most economically important vegetable crops. Annually 261,000 tons of green pepper for the fresh market and for processing are produced in the USA (Greenleaf, 1986). In Florida alone 9,000 ha are planted, with an economic value of $93,000,000 (USDA, 1989). Tomato for fresh market is planted in 182,000 ha with a production of 7,000,000 ton in the USA (Tigchelaar, 1986). In Florida, in the 1987-88 season, 25,000 ha were planted, that produced 870,000 ton with an economic value of $535,000,000 (USDA, 1989). Bacterial spot affects production, occasionally resulting in heavy losses. In some fields 50-70% of foliage can be lost (Pohronezny et al., 1986). Experiments including inoculation of field plots have demonstrated the adverse impact of bacterial spot on tomato yield, fruit quality, and size (Pohronezny and Volin, 1983).

The climate in Florida is subtropical with predominantly mild temperatures and seasonal periods of high rainfall; the cropping patterns in southern Florida are reversed from those in temperate regions with the noncropping season in the hot, humid month of June, July and August (Pohronezny et al., 1986). These environmental conditions favor the continued presence of the bacterium in the field, with the disease being endemic in Florida and many other subtropical regions of the world. Survival of the bacterium from one season to another, in Florida, is mainly in tomato volunteer plants and crop residue (Jones et al., 1986); survival in other areas may be on alternate hosts (Srinivasan et al., 1962; Dye et al., 1964) or on stalk of dead tomato plants (Peterson, 1963; Goode and Sasser, 1980).

The endemic nature of the disease might favor diversity within the pathovar. The selection of organisms combining the many attributes necessary for epidemiological competence in the field is the product of long evolution (Crosse, 1966). It was postulated by Crosse (1966, 1968) that, since each separate growing area represents a unique ecological situation with different methods of cultivation, host varieties and climate conditions, a different variant or variants of the pathogen will be best fitted to colonize each

habitat and, therefore, survive in the field. The identification of the variants of the pathogen and knowledge of their relationship with the host and the environment provides for better understanding of the disease and, by consequence, for better control. Estimation of the variability of pathogenic populations as they occur in the natural habitat is a prerequisite to the epidemiological analysis of the disease as a system. Information on bacterial characterization is, thus, more meaningful if studies of pathogen variability are performed 1) in populations representing its geographical and temporal distribution and 2) in defined systems such as those provided by field strains in naturally infected plants.

Among the characteristics of bacteria that give useful information about variability is the presence and distribution of plasmids in strains. Bacterial plasmids encode functions required for the interaction with the environment, including the host. Eberhard (1989) suggested that genes coding local adaptations will reproduce more successfully when on plasmids than when on the chromosome due to plasmids having greater horizontal mobility. This advantage may be greatly increased if the plasmid already carries another gene or genes coding for adaptations to the same or similar local conditions (Eberhard, 1989). The presence of

plasmid (doexyribonucleic acid) DNA in Xcv strains is documented, as is the coding of important traits, such as copper resistance and avirulence in those plasmids (Stall et al, 1986). The reported studies were from a limited number of strains from relatively few locations, however. We ignore the distribution of these traits and the distribution of other plasmids that, although now defined as cryptic (with unknown function), may encode for other meaningful traits.

The purpose of this dissertation was to study variability in plasmid content and plasmid-encoded phenotypic traits of strains of Xcv that were obtained from different areas of the world as well as from commercial fields of pepper and tomato in several locations. The plasmid content of each strain and the frequency of determined plasmid-size classes and variability of profile patterns within and between populations was determined. The distribution of several phenotypic traits that were reported as being plasmid-borne or may be plasmid-determined was investigated. The distribution of the plasmid-mediated copper resistance phenotype was studied in detail. The copper-resistant plasmids were compared by restriction fragment analysis and DNA homology of a clone containing the copper resistance gene.

The interaction of strains incompatible with tomato was characterized by bacterial multiplication in inoculated leaves and by electrolyte leakage analysis. Genetic characterization of this relationship was performed by isolating the gene that codes for avirulence activity. Molecular characterization of the gene was performed by restriction mapping and hybridization experiments.

CHAPTER 2
REVIEW OF LITERATURE

Microorganisms need to adapt to extremely variable environments. A factor mentioned as very important in the development of better adapted types is the reassortment of chromosomal genes as a consequence of the exchange of genetic material between organisms (Beringer and Hirsch, 1984). According to Selander (1985), chromosomal recombination is restricted in natural bacterial populations, giving rise to populations that are mixtures of clones that are relatively independent. The enteropathogenic bacterium Escherichia coli was studied in this respect and stable phenotypes with wide geographical distribution were found, suggesting clonal population structure (Orskov and Orskov, 1983; Stenderup and Orskov, 1983). Five clonal groups were determined in Bacillus sphaericus populations by multilocus enzyme electrophoresis (Singer, 1988). The basic requirement for adaptation suggests that bacteria that are able to survive in different environments might be much more variable than those with narrower ecological ranges (Selander, 1985).

Several approaches have been proposed to examine diversity among strains. The collection of information at the cellular and molecular level and information on cellular products are used to describe differences between cultures (Singer, 1988). The knowledge of the basic population structure of a determined organism allows the incorporation of information on phenotypic characters or properties to give a better picture of the variability, especially of those characters that are important in pathogenic processes (Selander, 1985). Selander (1985) proposed the use of isozymes as markers in studies of population genetics and systematics. Isozyme analysis have been used to determine clonal lineages for many species. Among plant pathogenic bacteria, protein gel electrophoresis and DNA-DNA hybridization have been used to determine species limits and pathovar relationships (Vera Cruz et al., 1984; Hildebrand et al., 1987; Van der Mooter et al., 1987). Diversity within a pathovar was studied by RFLPs by Denny et al. (1988).

Many clonal lineages have been determined for most species studied. Several mechanisms for the exchange of genetic material between these clones may exist. Between them, the function of mobile genetic elements (plasmids, transposons carried by plasmids, and bacteriophages) is most important. Organisms can

increase their genetic diversity without chromosomal recombination by carrying genes on mobile genetic elements (Finlay and Falkow, 1989). The study of plasmids and the functions that they encode is meaningful in bacterial classification (Harwood, 1980) and in bacterial evolution, when they act as agents of genetic adaptation to the environment (Reanney, 1976; Campbell, 1981; Beringer and Hirsch, 1984; Freter, 1984; Summers, 1984; Eberhard, 1989). Stewart and Levin (1977) studied with theoretical models the probability of plasmid-carrying cells being established and maintained in a population under different physical conditions, concentration of resources, and dilution rates. They concluded that those cells will maintain high frequencies even if they have lower growth rate than cells carrying no plasmids and this will occur independently of the variation applied in the model.

Plasmids are also of great value as material for studying the structure and function of genetic material and as experimental tools for molecular biology (Meynell, 1973; Broda, 1979). According to Thomas (1987) and Couturier et al. (1988) bacterial plasmids are circular double-stranded, covalently closed deoxyribonucleic acid (DNA) molecules (although some plasmids from Streptomyces species are double-stranded, linear DNA molecules) that replicate autonomously in a

host cell. Plasmids contain genes essential for their maintenance (initiation and control of replication), and for their stable inheritance (equipartitioning during cell division or conjugal transfer). Some plasmids contain genes that confer on their host properties essential for survival in certain environments, although they are considered (by definition) a non-essential addition to the genetic information of the host bacterium. Plasmids are replicated and are inherited as genetic units physically independent of the bacterial chromosome, but biochemically their replication generally depends on many host-encoded proteins.

Among bacteria pathogenic to humans, molecular analysis of plasmids is being applied in the investigation of epidemiological problems (Farrar, 1983; Platt et al., 1984a, 1984b; Platt et al., 1986b). In these investigations, a large number of strains are examined to determine plasmid distribution; relationship to antimicrobial resistance; and restriction enzyme fingerprinting of medically important plasmids (Kraft et al., 1984; Lyon et al., 1984; Platt et al., 1984b; Beul et al., 1985; Townsend et al., 1985; Brown et al., 1986; Brunton et al., 1986; Platt and Brown, 1986; Platt et al., 1986a; Kraft et al., 1987; Abeck et al., 1988; Kraut and Meyer, 1988; Shears et al., 1988; Melo-Cristino et al., 1989).

Separation of plasmids in size classes and plasmid profile typing, together with other characteristics as serotyping and bacteriophage typing, provide valuable epidemiological data (Jamieson et al., 1979.; Helmuth et al., 1985; Noble and Rahman, 1986; Dodd et al., 1988; Gaston et al., 1988; Whiley et al., 1988; Threlfall et al., 1989; Haider et al., 1989). The study of plasmid distribution in representative collections of strains of a determined pathogen provides a fast method to compare strains isolated at different times and locations (Platt et al., 1984b; Platt et al., 1986a; Ling and Chau, 1987; Nastasi et al., 1987).

The investigation of plasmid distribution in natural populations of bacteria other than human pathogens is very recent. Implication of plasmids in resistance to antibiotics and heavy metals and other pollutants is evident in those bacteria (Baya et al., 1986; Fredrickson et al., 1988; Wickman and Atlas, 1988).

Plasmid profile analysis in field strains of plant-related bacteria have been performed mainly in the Rhizobiaceae. In these bacteria many important traits (host specificity, nodulation, and nitrogen fixation) are encoded by plasmids. Great diversity in both number and size of plasmids was observed in

native populations of strains (Bromfield et al., 1987; Mozo et al., 1988; Brockman and Bezdicek, 1989).

The study of plasmids in plant pathogenic bacteria has been restricted to detection, characterization and determination of their relatedness and involvement in specific phenotypes in a small number of strains with scarce information on plasmids in field strains (Coplin, 1982; Piwowarski and Shaw, 1982; Sato et al., 1982; Curiale and Mills, 1983; Civerolo, 1985; Morales and Sequeira, 1985; Panopoulos and Peet, 1985; Coplin, 1989). Recently, diversity was found in plasmid profiles in temporal and geographically representative strains of Pseudomonas syringae pv. tomato (Denny, 1988).

Resistance to heavy metals and antibiotics are some of the more important phenotypes encoded by genes carried in plasmids in many bacteria (Foster, 1983; Tetaz and Luke, 1983; Haefeliet al., 1984; Summers, 1985; Desomer et al., 1988; Silver and Misra, 1988). Among the plant pathogenic bacteria, streptomycin resistance was found to be encoded by plasmids in field strains of Pseudomonas syringae pv. papulans (Burr et al., 1988) as was copper resistance in several strains of Xanthomonas campestris pv. vesicatoria (Xcv) (Stall et al., 1986), Pseudomonas syringae pv. tomato (Pst) (Bender and Cooksey, 1986) and P. s. pv.

syringae (Pss) (Sundin et al., 1989). Plasmids encoding copper resistance in Pst were characterized and the responsible genes were found to be arranged in an operon with a copper-inducible promoter (Cooksey, 1987; Bender and Cooksey, 1987; Mellano and Cooksey, 1988a,; Mellano and Cooksey, 1988b). The extent of the copper resistance phenotype in Xcv in different areas of the world is not known, and no information exists about the degree of homology of the encoding sequences.

Other plasmid-borne traits related to ecological fitness are bacteriocin production in several species of Erwinia and Agrobacterium, catabolic pathways in Rhizobium, Rhodococcus and Pseudomonas solanacearum, pigmentation and thiamine prototrophy in Erwinia (Coplin, 1989). Toxin production in several P. syringae pathovars is also plasmid encoded (Coplin, 1989). The most extensively studied plasmids are those encoding plant hyperplasias as crown gall and hairy root in Agrobacterium and olive and oleander knot in P. s. pv. savastanoi (Coplin, 1982, 1989). Among the plasmids related to host specificity and pathogenicity are those encoding avirulence genes in Xcv, Pst, and P. s. pv. glycinea (Psg) (Keen and Staskawicz, 1988; Coplin, 1989) and the hrp (Lindgren et al., 1986, 1988) genes in P. solanacearum (Boucher et al., 1987).

Together with the study of plasmid distribution, the determination of other phenotypic traits helps in understanding the variation within a particular organisms. In Xcv several characters have been mentioned as being variable with respect to host and region of origin of the strains. Differences were found in pathogenic races, starch hydrolysis, serology and cultural characteristics (Burkholder and Li, 1941; Srinivasan et al., 1962; Dye et al., 1964; Lovrekovich and Klement, 1965; Cook and Stall, 1969; Mathew and Patel, 1977; Schaad, 1976; Cook and Stall, 1982; Rodrigues Neto et al., 1984; Bongiolo-Neto et al., 1986; Maringoni and Kimati, 1987a; Maringoni and Kimati, 1987b). Pathogenic variation has been studied in some detail in Xcv. Races of the bacterium have been described that possess avirulence (avr) genes that interact with resistance genes in pepper in a gene-for-gene (Flor, 1955) manner (Cook and Stall, 1969; Cook and Stall, 1982; Cook and Guevara, 1984; Stall et al., 1986; Hibberd et al., 1987a,; Hibberd et al., 1987b; Ronald and Staskawickz, 1988; Swanson et al., 1988; Whalen et al., 1988; Bonas et al., 1989). Information concerning the relationship of Xcv and tomato is more limited. No avr genes have been isolated that interact with genes in that host. Avirulence genes have been cloned in many bacteria (Keen and Staskawicz, 1988) but the

product of these genes is unknown, as is the product of plant resistance genes, and how the gene products interact. The Xcv-pepper interaction is already used as a model system to study this type of relationship. The elucidation of the interactions with the other host, tomato, will help in this understanding.

CHAPTER 3
DISTRIBUTION OF PLASMIDS

Introduction

Ecologically important traits have been found to be plasmid-encoded in *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye (Xcv) (Stall et al., 1986; see Chapter 5 and 6). In a previous analysis of seven strains of Xcv, 2 to 5 plasmids were detected (Dahlbeck et al., 1977; Dahlbeck, 1978). Plasmid DNA was determined in other Xcv strains when studying linkage of copper resistance determinants and the avirulence gene *avrBs1* (Stall et al., 1986).

The distribution of plasmid DNA in a large number of strains, the stability of plasmid patterns in strains in a collection and the variability of plasmid patterns in natural populations are information that would allow better understanding of the ecology of Xcv and its intrapathovar variation. The presence of plasmids within bacterial populations increase their genetic diversity.

Noble and Rahman (1986) determined plasmid profiles in Staphylococcus aureus strains isolated 25 years before. The epidemiological interpretation obtained with the plasmid data were consistent with those obtained at the time of isolation using data on phage type, antibiotic sensitivity and geographical location. In another epidemiological study large variety of R-plasmids were detected in salmonellae and it was found that they would spread only to certain strains. In that study large numbers of these bacteria that were isolated during a period of 10 years were analyzed with respect to their plasmid profiles (Ling and Chau, 1987). Epidemiological information on relationship between strains was also obtained after comparing the plasmid profiles of more than 80 strains of Salmonella dublin isolated during a period of 15 years (Nastasi et al., 1987). Studies on the distribution and frequency of plasmids in bacteria isolated from relatively deep subsurface strata and shallow aquifers were used to determine differences in the composition of the bacterial communities of those two systems (Fredrickson et al., 1988). In a similar study an increase was observed in the number of plasmids bands detectable in bacterial populations isolated from samples of chemically contaminated water in contrast to those bacteria from domestic sewage-impacted waters

or from uncontaminated open ocean sites (Baya et al., 1986). In contrast, in a study of plasmid frequency fluctuation in bacterial populations from chemically stressed soil communities, chemical stress did not necessarily cause an increase in plasmid-mediated multiple resistance, the change in plasmid frequency was dependent on both the amount and type of chemical stress (Wickmam and Atlas, 1988).

A great diversity in both number and size of plasmids was observed in native populations of strains of Rhizobium spp (Mozo et al., 1988). A total of 33 different plasmid profiles was observed in a natural population of about 200 strains of Rhizobium leguminosarum biovar viceae. The grouping by plasmid profiles was strongly associated with grouping by serogroup and by intrinsic antibiotic resistance (Brockman and Bezdicek, 1989).

Published information on plasmids in a large number of strains in natural populations of plant pathogenic bacteria are related only to surveys for the detection of specific plasmids involved in antimicrobial resistance (Burr et al., 1988, Sundin et al., 1989) with scarce information on characterization of total plasmid DNA.

A comprehensive study of plasmid profiles in a large number of strains of Xcv, representing its

temporal and geographical distribution, will help in understanding the variation and ecology of this pathovar.

Materials and Methods

Media. Bacteria were stored for long term (several month to years) in sterile tap-water in tubes kept at room temperature. For short term storage (less than six month) bacteria were kept at room temperature in Petri dishes on 1:1 Lima Bean Agar (DIFCO): water agar (DIFCO), pH 7.0 (Lima A).

Xcv strains stored in collections. Bacteria were received from other scientists in tap water, or lyophylized cultures. They were recovered by streaking on Lima A or nutrient agar. A single colony was selected and streaked again to further purify the cultures. Sometimes, when very different types of colonies were obtained from one culture, they were individually transferred and kept as different strains.

Strains of the UF collection were obtained from the laboratory of R. E. Stall, Department of Plant Pathology, University of Florida, Gainesville, and consisted of 218 strains obtained during the years 1960 to 1988. Most strains were isolated from pepper and tomato plants in Florida and other Southeastern states, but some strains were received from scientists in other parts of the country and the world. One

culture, Xv-3, was the type culture obtained from the American Type Culture Collection, Rockville, Maryland.

The TW collection was received from Dr. S. T. Hsu, National Chung Hsing University, Taichung, Taiwan and Dr. A. T. Tschanz, The Asian Vegetable Research and Development Center, Shinhua, Tainan, Taiwan, consisted of 28 strains isolated from pepper and tomato over several years.

The BA collection was received from Delia Erbaggi, Instituto de Patologia Vegetal, INTA, Castelar, Buenos Aires, Argentina, and they were from the collection of Adriana M. Alippi, Catedra de Fitopatologia, Facultad de Agronomia, Universidad Nacional de la Plata, Buenos Aires, Argentina. They were isolated from 1984 to 1987 in pepper and tomato fields in areas of Buenos Aires province, Argentina.

Xcv strains from natural populations. Bacteria were obtained from fields of naturally diseased pepper and tomato plants. Leaves and fruits with symptoms of bacterial spot were obtained in the field, and brought to the laboratory in plastic bags in ice chests. One lesion was selected from each sample and the bacteria were isolated by the puncture method (Goth, 1965). Isolations were made on nutrient agar (DIFCO) plates. A typical Xcv colony was selected after two days and single colonies were selected successively twice more.

Bacteria were checked for pathogenicity and other physiological characteristics as explained in Chapter 4.

The strains of the BV tomato field population were obtained in November, 1987, in a field of tomato cultivar Marmande in INTA Experimental Station, Bella Vista, Corrientes, Argentina. The collection originated from 20 samples from which 86 strains were obtained, by selecting different colony types. Only 50 strains were used for plasmid determination.

The strains of the Ohio tomato field population were obtained in August, 1987, from fields of tomato in Northwestern Ohio, USA. This population consisted of 15 strains isolated from fruits and leaves selected at random.

The strains of the FL tomato field population were obtained from commercial plots in Bradenton, Florida, USA in November, 1987. Two separate fields were sampled. Samples of the groups designated TA and TB were from one plot in one field from two samplings done transversal to the rows, and samples of the groups TC, TD, and TE were from three different plots located in another field.

The strains of the FL pepper field population were obtained in March, 1988 from commercial plots of different pepper cultivars in Delray Beach, Florida, USA. Samples of groups PA and PB were from two plots

in one field of Yolo Wonder. Samples of group PD were from one plot in one field of a Yolo Wonder hybrid and samples of groups PE and PC were from one plot in one field of Summer Sweet, a yellow-fruited hybrid.

Plasmid extraction and visualization. Bacterial strains were grown for 20-30 hours in 2 ml of nutrient broth at 30°C with vigorous shaking. The concentration of cells was standarized to an OD of 0.3A at 600nm and plasmid DNA was extracted using a modification of the method of Kado and Liu (1981) for large plasmids (Rodriguez and Tait, 1983). The modification consisted of the addition of 0.57M of sodium chloride in the lysing solution. Plasmid preparations were stored for several months at 4°C in disposable microfuge tubes after adding 25 mM of EDTA. Samples were loaded in gels of 0.5% agarose, DNA Grade (Sea Kem), in TE buffer (0.04M Tris-acetate, 0.001M EDTA). The separated DNA's in the gels were stained with ethidium bromide for 40 minutes and photographed with Type 55 Polaroid film, after visualizing with UV light.

Plasmid sizing. Plasmid DNA was subjected to electrophoresis in agarose gels until the bromthymol blue front moved five centimeters from the well. Occasionally, electrophoresis conditions were changed to resolve plasmids bands running too close to the linear DNA. Gels were of 0.5% agarose and the electrical

current was 5V/cm for the Mini-Sub cell (BIO RAD) gels and 3V/cm for the wide Mini-Sub cell (BIO RAD). In each gel a plasmid DNA preparation of Erwinia stewartii (Es) strain SW2 (Coplin et al., 1981) was included as a molecular weight marker. Molecular weight of the SW2 plasmids were calculated by Coplin et al. (1981) by measurement using electron microscopy. The same extraction method was used for Xcv and Es. Thirteen plasmids ranging from 2.7 to 210.0 megadaltons (Md) were visualized in Es strain SW2, as reported by Coplin et al. (1981). Relative mobility of the SW2 plasmids was measured for each gel. The $\log_{10}$ of the distance is inversely related to the $\log_{10}$ of the molecular weight of covalently closed circular (ccc) plasmids (Crossa and Falcow, 1981). Linear regression equations were calculated for each gel with the $\log_{10}$ of the distance as the independent variable and $\log_{10}$ of the molecular weight as the dependent variable. Molecular weight of the Xcv plasmids was calculated with this equation replacing the value for the distance migrated for each plasmid band in each strain. Two equations were calculated for each gel, one equation with the four larger SW2 plasmids and the other equation with the nine other plasmids. This was done in order to obtain a straighter line for the high molecular weight plasmids. Values obtained are

considered approximations, since it is known that sizing by measuring restricted plasmids gives 10-20% larger sizes than measuring the ccc form of the same molecules even using appropriate markers in each case. The R^2 values for all equations was always larger than 0.97. All values were calculated in megadaltons and later transformed to approximated size in kilobases (size in kb= size in Md/0.65). All sizing experiments were done at least two times for each strain.

Results

Plasmid size classes. Several methods of plasmid isolation were used, but the only method which allowed the visualization of the large size plasmids was that of Kado and Liu (1981). Many methods, including the miniprep method of Maniatis et al. (1982), selectively eliminated plasmids larger than 50 kbp in size. The existence of very large megaplasmids (larger than 500 kbp) can not be rule-out.

Sizes calculated from different gels were consistent, only small variations were noted. Three strains of Xcv (Xv75-3, 71-31, and E-3) were included in all sizing gels as reference markers. Only the plasmids of Es were used to calculate sizes. The variation observed in calculated sizes in the strains of Xcv in eight similar sizing gels are presented in Table 3-1.

The strains of the UF collection of Xcv were used for preliminary determination of plasmid-size classes in the bacterium, since it was thought to contain representatives of many populations. Plasmids of this collection were grouped into 13 different size classes (Table 3-2). Plasmids of the other groups strains were then placed in these groups. Representatives of some of the size classes are presented in Fig. 3-1. Plasmids were not detected in very few strains including Xv-3, the type strain of Xcv. Plasmidless strains were always from collections and were isolated several years ago. Strains freshly isolated from the field always carried plasmids.

As a criterion for determining classes, two plasmids were placed in different classes when they occurred together in any one of the strains. This criterion was fulfilled by all classes except class L. Class L plasmids were found in very few strains. In some cases, two plasmids classified as class L were in the same strain.

A diffuse band was consistently observed at the plasmid of 12-20 kb position in many strains which was thought to be linear, single stranded DNA. Disrupted chromosomal and plasmid DNA was observed in all sizing gels at the 40-45 kbp position. In some cases several different electrophoretic conditions had to be used in

order to obtain resolution of the plasmid class I from the linear chromosomal and plasmid DNA.

Plasmids profiles. Seventy-one different plasmid profile patterns were determined in strains of Xcv based on size of the plasmids. Each profile consisted of 0 (no plasmids) to five different plasmid size classes. Thirty of the 71 profiles had 3 size classes. Size classes included in each of the 71 profiles are presented in Table 3-3. The class M was not always clearly observed due to the preference given to the visualization of the larger plasmids. Thus, class M was not considered when determining profiles, except profile I-10 that includes few strains where only this class was visualized.

Restriction analysis. Plasmids in the same size class may not necessarily be identical and the fact that most of the strains carry several plasmids precluded performing restriction analysis to compare them individualy. Most of the large size self-transmissible plasmids were easily lost if further manipulation of the DNA was attempted in order to isolate individual plasmids (see Chapter 5 for comparison of three of the large plasmids).

Two strains carrying only one plasmid of the size class I were selected for restriction analysis. Purified plasmid DNA was obtained by the miniprep

alkaline extraction method (Maniatis et al., 1982). This method allowed the purification of relatively small plasmids. Endonuclease EcoRI and PstI were used. Restriction patterns were the same with EcoRI but polymorphism was evident with PstI (Fig. 3-2).

Plasmids in strains stored in collections. All 13 plasmid size classes were found in the UF collection. Size classes K, M, and I were the most abundant, and these were followed by B, A, and C. The size classes D, E, G, H, and L were rarely found (Fig. 3-3). The 13 plasmid-size classes were arranged in 45 patterns in strains of the UF collection (Fig. 3-4). Four plasmid profiles (II-1, II-2, III-1, and IV-1) comprised more than 40% of the strains, the other profiles were present in much less proportion.

Only nine of the described size classes, arranged in 15 different profiles occurred in the strains of the TW collection (Fig. 3-5, and 3-6). Strains were fairly evenly distributed among different profiles, thus high variation occurred in the relatively low number of strains of this collection. The plasmid bands in some of the strains are shown in Fig. 3-7 and 3-8.

Plasmids in the strains of the BA collection were much less diversee. Only five of the 13 size classes were found and these classes were arranged in

only five different profiles (Fig. 3-9, and Fig. 3-10).

Plasmids in strains from natural populations. Four natural populations of strains were studied, three from tomato fields and one from a pepper field.

Plasmids representing nine of the 13 size classes were detected in the FL pepper field population (Fig. 3-11). Ten different plasmid profiles were found, but one of them (IV-10) comprised 70% of the strains, whereas the rest of the strains were almost evenly distributed among the other nine profiles (Fig. 3-12). The plasmid content of most of the strains is presented in Fig. 3-13, 3-14, and 3-15, and a gel representing all the profiles found, together with the molecular weight markers (Es SW2) and reference markers (Xcv strains 75-4, 71-31, and E-3), is presented in Fig. 3-16.

Strains in the Florida tomato field were more variable in their plasmid content than those in the Florida pepper field and those in tomato fields from other areas. Plasmids could be distributed into 11 of the 13 classes (Fig. 3-17) and also 11 different plasmid profiles were obtained (Fig. 3-18). Profiles II-4 (40%) and III-20 (25%) were most predominant.

Much less diversity was observed in the strains from the Ohio tomato field. Plasmids belonging to six

different size classes were detected (Fig 3-19) and they were arranged in only four different profiles (Fig. 3-20).

Five size classes arranged in six profiles were detected in the BV tomato field population (Fig. 3-21 and 3-22). The different profiles observed in this group are shown in Fig. 3-23, with strains of the BA collection given for comparison.

Discussion

The survival of microorganisms in nature depends upon their ability to find suitable niches in which they can grow and survive. The roles of plasmids in evolution and ecology has been readily demonstrated (Reanney, 1976; Coplin, 1989). Ecologically important traits, such as copper resistance and host specificity are plasmid-borne in Xcv (Stall et al., 1986).

Plasmids of Xcv were grouped into 13 size classes and 71 profile patterns were identified in the more than 500 strains examined. Diversity was evident in strains stored in collections and in strains from natural populations. Variability is a characteristic of Xcv for several physiological traits (see Chapter 4) and plasmid composition appears to be a highly variable character. This may suggest that there are many more traits encoded by plasmids in this pathovar. This is probable because many of the plasmids are

relatively large (more than 30 kb) and because there are enough sequences for the coding of several genes. Some strains isolated in Florida 10 to 20 years ago (UF collection) carried as many plasmids as those isolated more recently, but the majority of the strains freshly isolated (2-3 years ago) carried 3 or 4 plasmid classes.

Differences were more related to geographical location. Most strains from Argentina were clearly different in plasmid composition compared to those from other areas. Further differences detected in these strains are discussed in Chapter 4.

Some plasmid size classes were highly conserved (I, K) among many strains from different geographical locations. It is not known if plasmids that were grouped in the same size class are actually identical. In one comparison of two strains, involving size class I plasmid, fragment length polymorphism was observed with one restriction enzyme but not with another. Those plasmids may be related, but differ in some of the sequences, which may be due to the presence of transposable elements. On the other hand, large plasmids, although conserved among strains had very few fragments in common (see Chapter 5 for large plasmids encoding copper resistance). Comparison of plasmids in Rhizobium by DNA hybridization experiments showed that

large symbiotic plasmids (187-349 Md) of the same size were distinct (Mozo et al., 1988). In these bacteria diversity in plasmid content was detected in many species (Mot et al., 1988). What is evident from the data presented here is that laboratory studies with a single or few strains of a pathovar are not representative of the diverse population present in the field.

An unanswered question is whether a gene encoding a trait necessary for survival in a determined environment and located in a self-transmissible plasmid will be transferred to other strains, or whether the original strain carrying the gene will become prevalent in that environment. In human pathogens there is evidence that antibiotic resistance genes located in plasmids and transposons are distributed to other species and even genera of bacteria (Farrar, 1983). Experiments that include the field inoculation of mixed strains are necessary to test this hypothesis in Xcv. In the case of the Florida pepper field population, few copper susceptible strains with very different plasmid profiles were present among the majority of copper resistance strains. This may indicate that they are being maintained as a reservoir probably because they encode some other useful phenotypes. Our knowledge of the functions that are plasmid borne is probably distorted by the lack of information on the

factors that are really important for survival in different habitats (Beringer and Hirsch, 1984). There is evidence that genes in organisms indigenous to natural ecosystems, especially those of soil microorganisms, are carriers of the ancestral genes that are now commonly found in drug resistance plasmids (Reanney, 1976; Freter, 1984; Clarke, 1984).

Plasmids are considered to be potential strain-markers and have been shown to be a useful tool in epidemiological studies of various microorganisms (Haider et al., 1989). The information presented here can serve as a starting point in the characterization of plasmids in Xcv and the inherent diversity observed may be useful in epidemiological studies. Studies with human-pathogenic bacteria (Kerry-Williams and Noble, 1986) used plasmids as epidemiological markers and suggested evolutionary events that resulted in a similar plasmid being present in a high proportion of the strains. A candidate for this type of study in Xcv would be those plasmids in size class I. The same class plasmid extracted from two strains isolated 10 years apart had differences in some restriction sites. This plasmid class is also ubiquotous in Xcv strains and presumably encodes the avrBsT gene (Minsavage et al., MPMI in press) and the avrBs3 gene (Bonas et al., 1989). Homologous sequences to avrBsP are also present

in this plasmid class (see Chapter 6). Bonas et al. (1989) demonstrated that the plasmid in size class I in the strain 82-8 is conjugative and if all plasmids in the class are related, this may explain its wide spread distribution among the Xcv strains. An observation that was not pursued further is that when atypical colonies were selected after recovery from long term storage in tap water, they were found to carry no detectable plasmids even though the more typical colonies of the same strain carried several plasmids. The atypical colonies were always less slimy, more darkened yellow and when inoculated were much less agressive. Wheather these changes in characteristics are due to the loss of plasmids needs to be investigated.

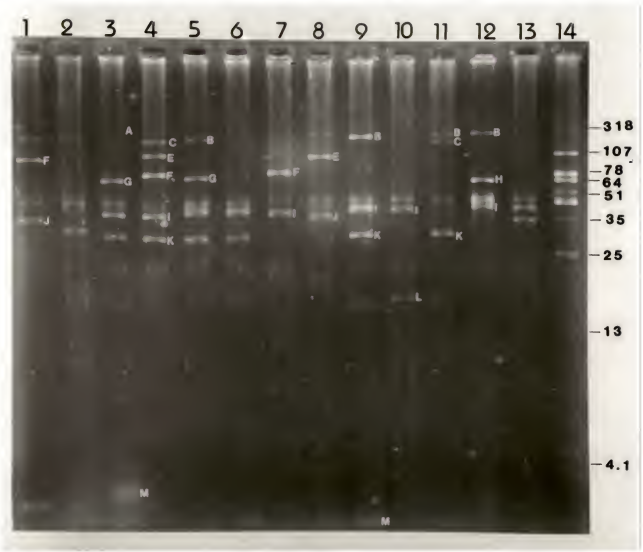
Table 3-1. Size in kilobase pair of plasmids in strains 75-3, 71-31, and E-3 of Xcv after agarose gel electrophoresis together with the strain SW2 of Erwinia stewartii used as size standard. Data are from eight sizing gels where the Xcv strains were included as reference markers.

Strain	Plasmid	Maximum	Minimum	Mean	SD
75-3	A	298.16	269.10	284.35	9.42
	I	42.91	39.26	40.75	1.52
	K	31.20	27.56	28.00	1.31
	M	3.02	2.63	2.85	0.14
71-31	B	243.18	214.22	232.57	10.51
	C	200.48	173.88	189.93	8.96
	K	42.91	39.26	40.75	1.31
	M	3.02	2.63	2.85	0.14
E-3	B	222.13	199.47	213.85	7.65
	I	42.91	39.26	40.75	1.52
	K	31.20	27.56	28.00	1.31
	M	3.02	2.63	2.85	0.14

Table 3-2. Size classes (in kilobase pair) in which plasmids of Xcv were grouped after agarose gel elctrophoresis.

Size class	Kilobase pair
A	>260.00
B	201.00-259.99
C	170.00-200.99
D	150.00-169.99
E	120.00-149.99
F	80.00-119.99
G	60.00-79.99
H	47.00-59.99
I	38.00-46.99
J	34.00-37.99
K	25.00-33.99
L	5.00-24.99
M	<5.00

Fig. 3-1. Agarose gel electrophoresis of plasmid DNA of some strains of Xcv from the UF collection. Letters indicate size class. Lane 14 is the strain SW2 of Erwinia stewartii and numbers indicate size in kilobase pair. Lanes 1 to 13 are: 87-18, 86-35, 86-36, 87-6, 87-13c, 87-13g, 84-1, 85-13, E-3, 80-5, 71-31, 79-2, and 65-4.


1
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3
4
5
6
7
8
9
10
11
12
13
14
318
107
78
64
51
35
25
13
4.1
A
B
C
D
E
F
G
H
I
J
K
L
M

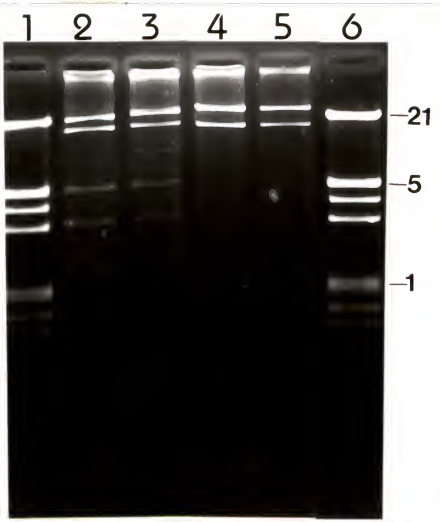
Table 3-3. Plasmid-size classes in each profile pattern determined in strains of Xcv after agarose gel electrophoresis. Roman numerals indicate numbers of clases in each profile. Arabic numerals identify different profiles.

Profile	Class	Profile	Class	Profile	Class
I-1=	K	III-1=	B,I,K	IV-1=	A,F,I,K
I-2=	I	III-2=	C,I,K	IV-2=	B,F,I,K
I-3=	B	III-3=	A,I,K	IV-3=	C,F,I,K
I-4=	D	III-4=	C,J,K	IV-4=	A,F,G,J
I-5=	C	III-5=	B,J,K	IV-5=	E,F,I,K
I-6=	L	III-6=	D,K,L	IV-6=	B,G,I,K
I-7=	J	III-7=	A,F,I	IV-7=	C,G,I,K
I-8=	H	III-8=	C,E,K	IV-8=	B,E,G,J
I-9=	G	III-9=	E,K,L	IV-9=	A,C,I,K
I-10=	M	III-10=	C,D,K	IV-10=	A,E,I,K
		III-11=	A,G,I		
II-1=	B,K	III-12=	F,I,K	V-1=	C,E,F,I,K
II-2=	A,K	III-13=	C,G,I	V-2=	A,C,G,I,J
II-3=	C,K	III-14=	C,F,K	V-3=	A,E,G,I,K
II-4=	B,J	III-15=	B,G,K		
II-5=	B,I	III-16=	B,G,H	O=	no plasmids

Table 3-3--Continued

Profile	Class	Profile	Class
II-6=	B,H	III-17=	B,H,K
II-7=	D,K	III-18=	B,G,I
II-8=	I,K	III-19=	B,E,K
II-9=	C,I	III-20=	B,E,J
II-10=	I,J	III-21=	B,F,K
II-11=	C,J	III-22=	B,F,G
II-12=	F,I	III-23=	B,H,J
II-13=	I,L	III-24=	C,G,K
II-14=	A,I	III-25=	B,C,K
II-15=	G,H	III-26=	B,F,J
II-16=	A,J	III-27=	B,I,J
II-17=	E,I	III-28=	C,F,I
		III-29=	B,F,I
		III-30=	B,J,L

Fig. 3-2. Agarose gel electrophoresis of plasmid size class I from strains 82-4n and 71-21 after restriction with endonucleases. Lanes 1 and 6= lambda DNA restricted with EcoRI and HindIII. Numbers indicate size in kilobase pair. Lanes 2 and 4= strain 82-4n, lanes 3 and 5= strain 71-21. Lanes 2 and 3 restricted with PstI and lanes 4 and 5 restricted with EcoRI.


1
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4
5
6
21
5
1

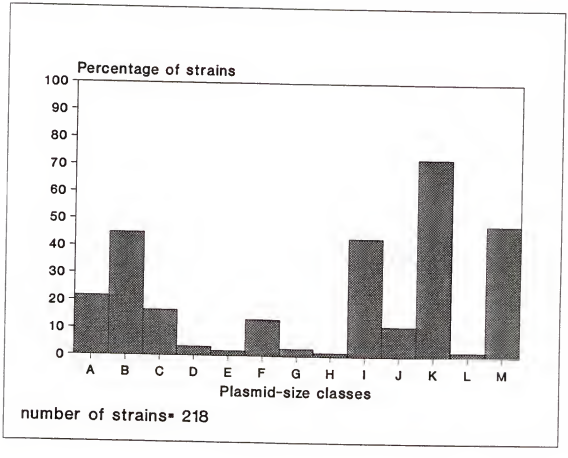


Fig. 3-3. Frequency of each plasmid size class in strains of Xcv from the UF collection.

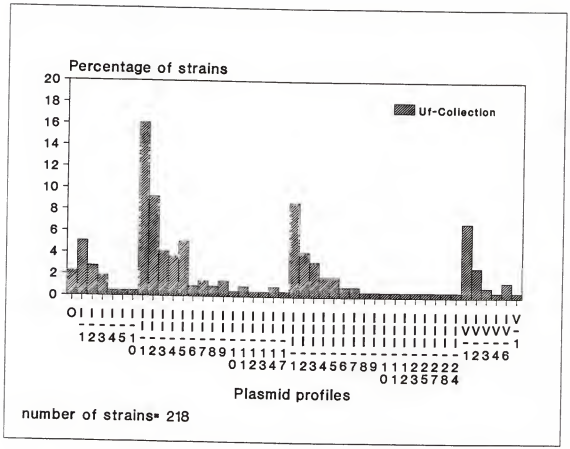


Fig. 3-4. Frequency of each plasmid profile pattern in strains of Xcv from the UF collection.

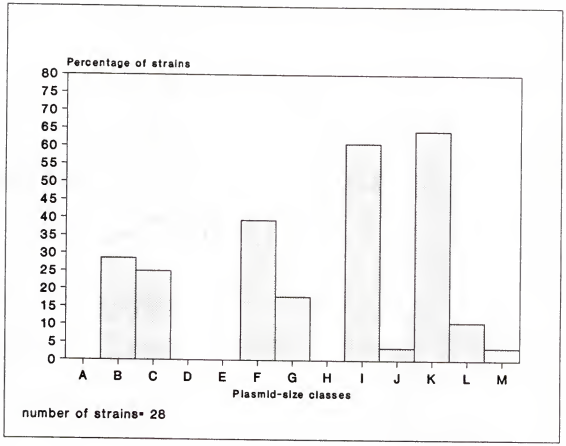


Fig. 3-5. Frequency of each plasmid size class in strains of Xcv from the TW collection.

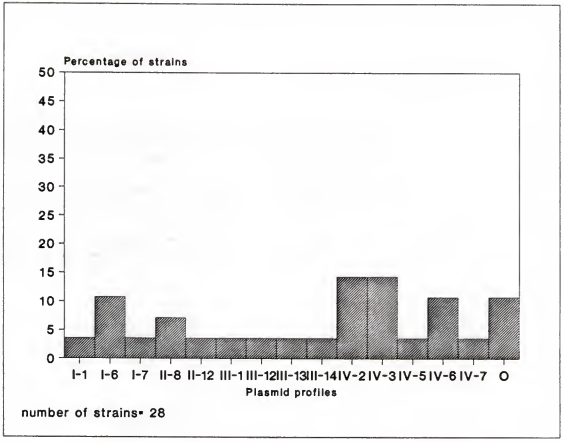


Fig. 3-6. Frequency of each plasmid profile pattern in strains of Xcv from the TW collection.

Fig. 3-7. Agarose gel electrophoresis of plasmid DNA of strains of Xcv from the TW collection and markers from the UF collection. Letters indicate size class. Lanes 1 to 26 are: 81-23, E-3, T87-50, T87-37, T87-35, T87-69, T87-66, T87-64, T87-64, T87-63, T87-61, T87-59, T87-56, T87-53, Es strains SW2, T87-48, T87-46, T87-42, T87-40, T87-39, T87-38, T87-36, 86-36, Xv70, 82-4n, 81-23, and E-3. Arrow indicates linear plasmid and chromosomal DNA.

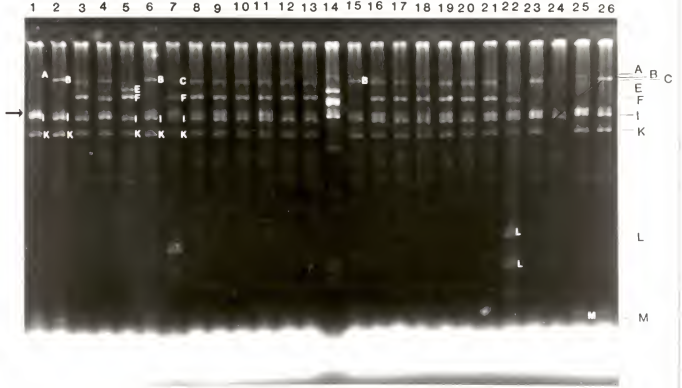
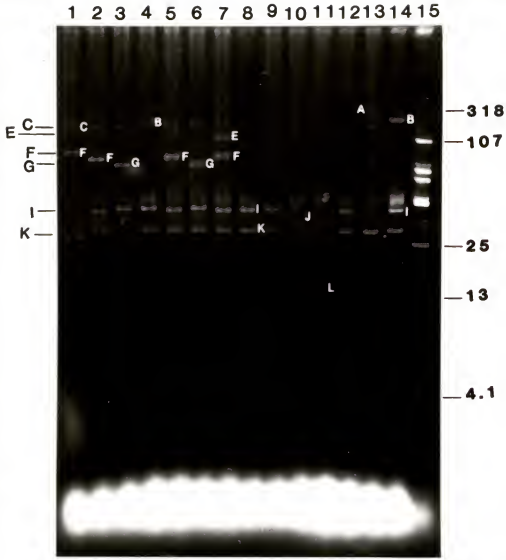

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
A
B
C
E
F
I
K
L
M

Fig. 3-8. Agarose gel electrophoresis of plasmid DNA of strains of Xcv from the TW collection with different profile patterns and strains used as markers. Letters indicate size class. Lane 15 is strain SW2 of Es and numbers are size in kilobase pair. Lanes 1 to 14 are: T87-62, T87-61, T87-58, T87-48, T87-38, T87-37, T87-35, T87-33, T87-30, T87-29, T87-27, 75-3, 71-31, and E-3.


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
E
C
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318
107
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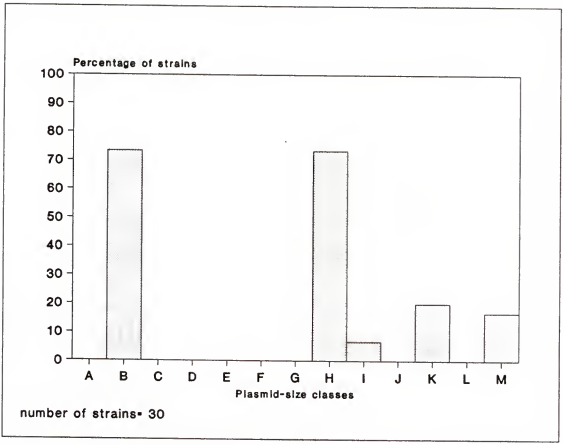


Fig. 3-9. Frequency of each plasmid size class in strains of Xcv from the BA collection.

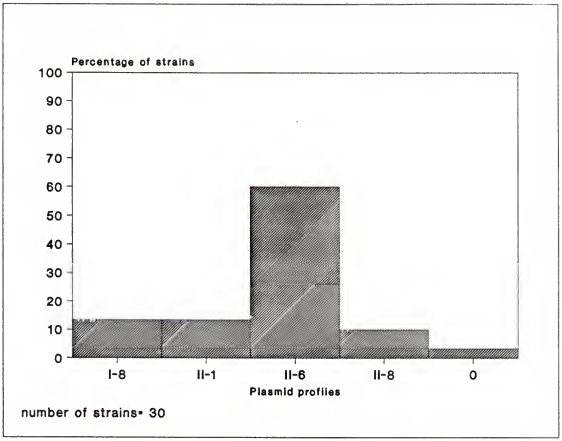


Fig. 3-10. Frequency of each plasmid profile pattern in strains of Xcv from the BA collection.

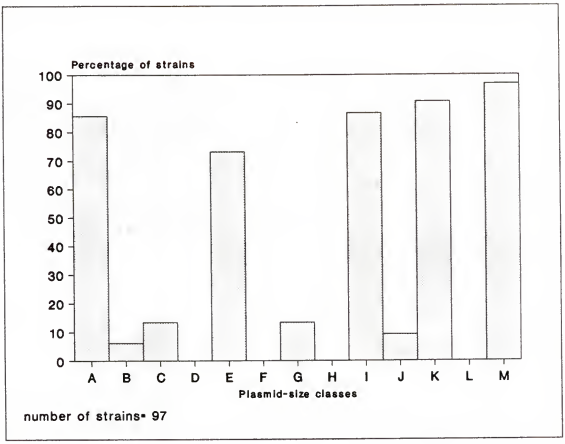


Fig. 3-11. Frequency of each plasmid size class in strains of Xcv from the FL pepper field population.

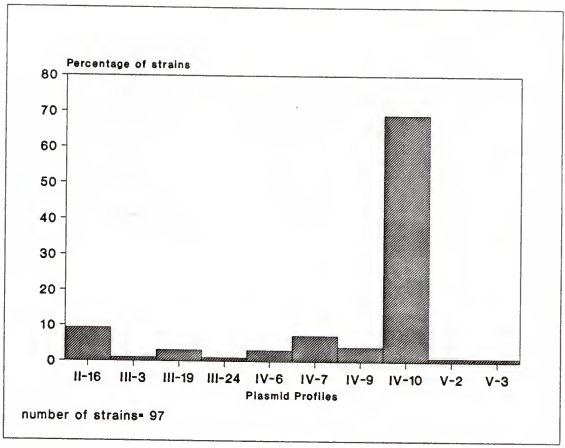


Fig. 3-12. Frequency of each plasmid profile pattern in strains of Xcv from the FL pepper field population.

Fig. 3-13. Agarose gel electrophoresis of plasmid DNA from strains of Xcv isolated from naturally infected plants of pepper in Florida fields. Letters indicate size classes. Lanes 1 to 30 are: strain PA-3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, PB-2, 3, 4, 5, 6, 7, 8, and 9. Arrow indicates linear plasmid and chromosomal DNA.

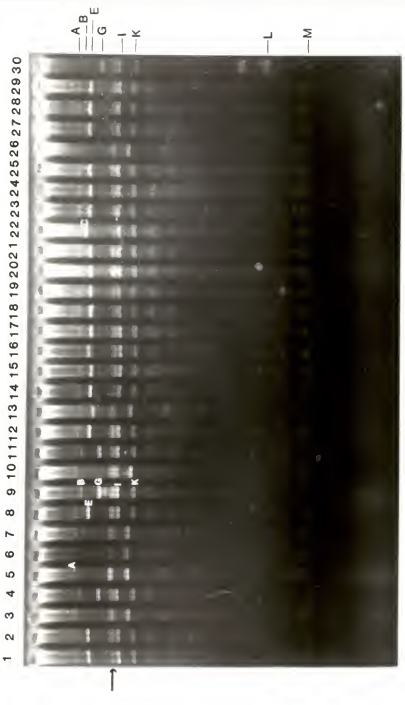

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
A
B
C
D
E
F
G
H
I
K
L
M

Fig. 3-14. Agarose gel electrophoresis of plasmid DNA from strains of Xcv isolated in naturally infected plants of pepper in Florida fields. Letters indicate size class. Lanes 1 to 30 are: strains PB-10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, PC-1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 17, and 18. Arrow indicates linear plasmid and chromosomal DNA.

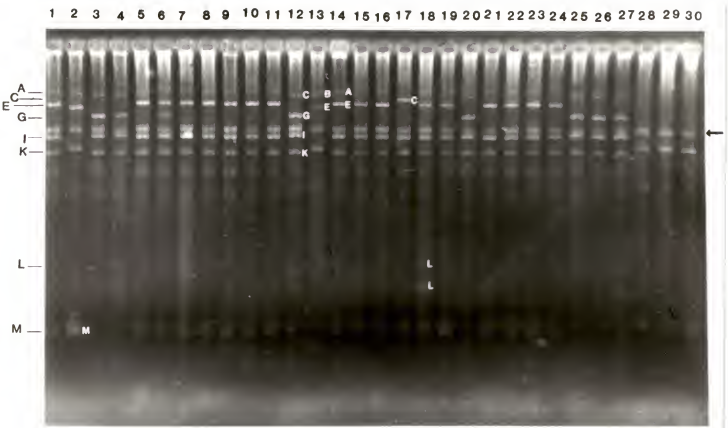

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
A
C
E
G
I
K
L
M
C
B
A
E
G
I
K
C
L
L
M

Fig. 3-15. Agarose gel electrophoresis of plasmid DNA from strains of Xcv isolated in naturally infected plants of pepper in Florida fields. Letters indicate size class. Lanes 1 to 30 are: strains PC-19, 20, 21, 22, 24, 25, PD-1, 2, 3, 4, 5, 6, 7, 8, 9, 10, PE-1, 17, 3, 4, 5, 19, 7, 8, undetermined, PE-20, 11, 13, and 14. Arrow indicates linear plasmid and chromosomal DNA.

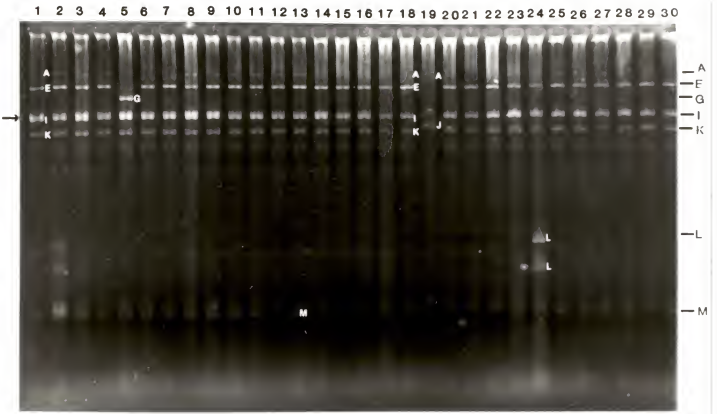
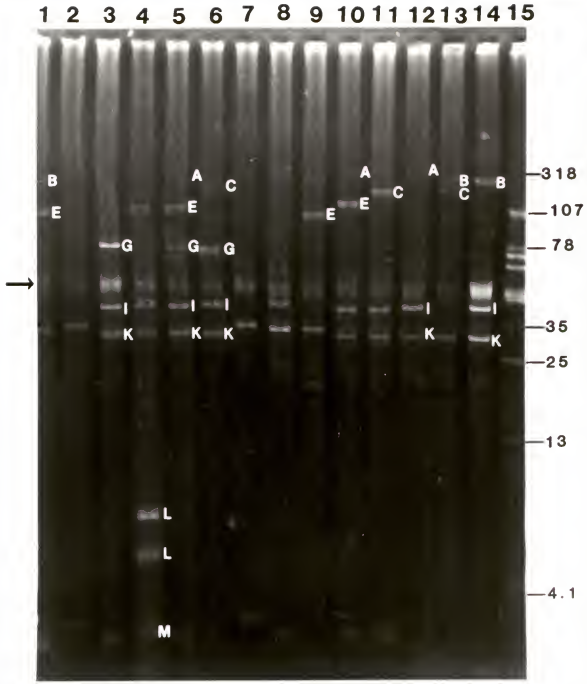

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
A
E
G
I
K
L
M
J

Fig. 3-16. Agarose gel electrophoresis of plasmid DNA of strains of Xcv from FL pepper fields with different profile patterns and strains used as markers. Letters indicate size class. Lane 15 is strain SW2 of Es and numbers are size in kilobase pair. Lanes 1 to 14 are: strain PC-17, PD-6, PE-20, PD-1, PC-10, PB-6, PB-5, PA-23, PA-20, PA-16, PA-11, 75-4, 71-31 and E-3. Arrow indicates linear plasmid and chromosomal DNA.


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
B
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G
I
K
L
L
M
A
E
G
I
K
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G
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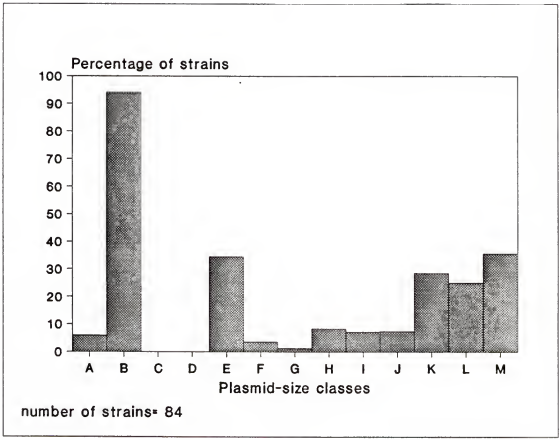


Fig. 3-17. Frequency of each plasmid size class in strains of Xcv from the FL tomato field population.

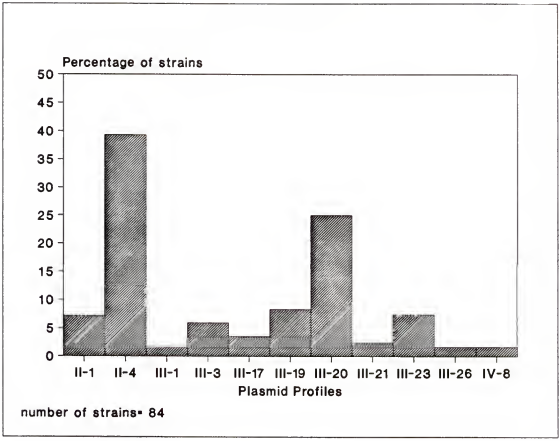


Fig. 3-18. Frequency of each plasmid profile pattern in strains of Xcv from the FL tomato field population.

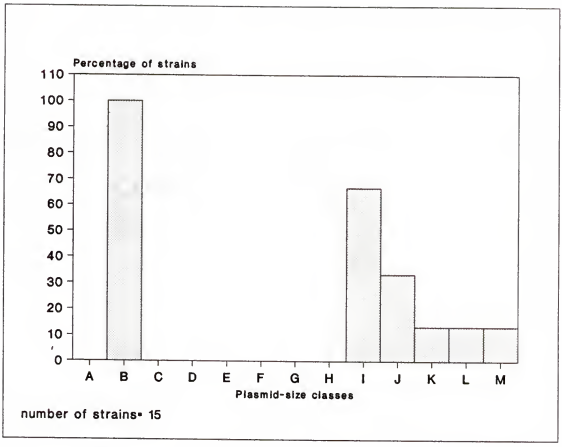


Fig. 3-19. Frequency of each plasmid size class in strains from the OH tomato field population.

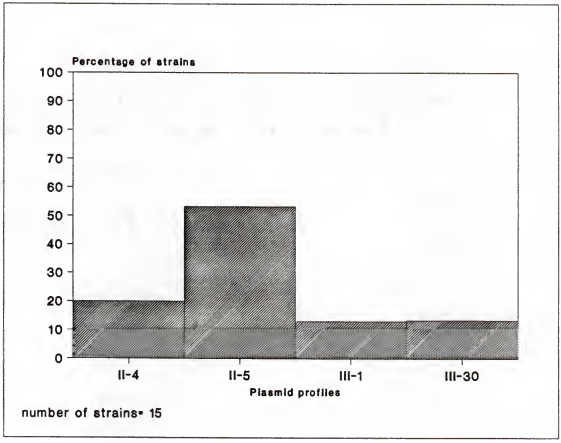


Fig. 3-20. Frequency of each plasmid profile pattern in strains of Xcv from the OH tomato field population.

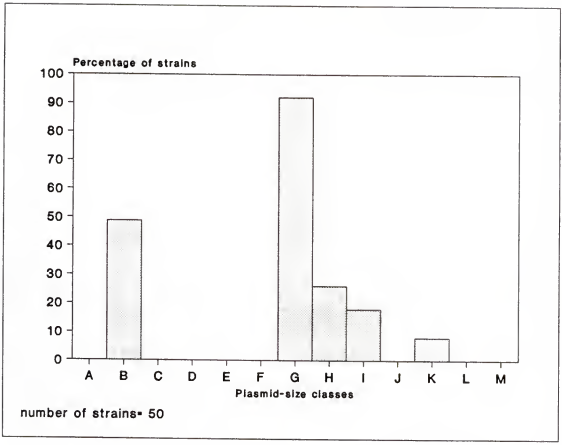


Fig. 3-21. Frequency of each plasmid size class in strains of Xcv from the BV tomato field population.

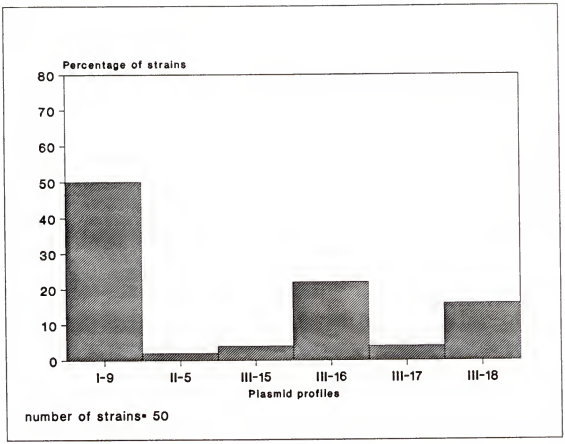


Fig. 3-22. Frequency of each plasmid profile pattern in strains of Xcv from the BV tomato field population.

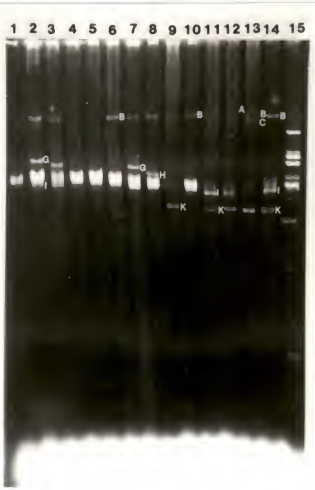


Fig. 3-23. Agarose gel electrophoresis of plasmid DNA of strains of Xcv from Argentina and strains used as markers. Letters indicate size class. Lane 1 to 15 are: strain 81-6 (from Indiana, USA, UF collection), strains BV 11-5, 20-3a, 6-3a, 5-3a, and 7-3b (BV tomato field), strains BA 23-1, 30-1, 24-1, and 26-1 (from the BA collection), strains 75-3, 71-31, and E-3 (UF collection), and strain SW2 of Erwinia stewartii.

CHAPTER 4
DIVERSITY OF PHENOTYPIC TRAITS

Introduction

Considerable variation had been reported within Xcv concerning different phenotypic traits even though a limited number of strains were examined. Often these variations were thought to be correlated with host of origin. Burkholder and Li (1941) found pathogenic variation between strains from the northeastern USA and those from the southeastern USA. They determined that strains isolated from tomato in the Northeast hydrolysed starch, but they did not test any strains isolated from tomato in Florida. Srinivasan et al. (1962) described two types of strains of Xanthomonas isolated in India from Physalis minima L. In cross-inoculation experiments they found that some of those strains would readily infect pepper, whereas the others would not. They named the latter X. physalidis, and among its characteristics was a strong hydrolysis of starch. The true strains of Xcv would infect P. minima and hydrolyse starch to a lesser extent. A thorough study of Xcv initiated by Dye et al. (1964) was related to the possibility of Xcv strains infecting another solanaceous plant, Nicandra physalodes Pers. in New

Zealand. They compared strains of Xcv from all over the world and found that the bacterium infecting N. physalodes was not different from Xcv and that starch hydrolysis was variable (from strongly hydrolytic to negative) with the Xcv strains. The strains from pepper in general were weaker starch hydrolyzers than the tomato strains. They suggested epidemiological relationships among some of the strains to explain similarity, especially in relation to phage susceptibility. Lovrekovich and Klement (1965) studied the variation within Xcv with respect to phage sensitivity, serology, and starch hydrolysis and came to the conclusion that bacteria in the Xcv group are not uniform. The pepper strains were distinguished from the tomato strains by the use of phages, and the tomato strains were divided into two groups corresponding to their ability to hydrolyze starch. Charudattan et al. (1973) in an investigation of serological differences in 72 Xcv strains found no correlation between serology and pathology. Two serotypes were found which included tomato and pepper strains. The ability of the strains to hydrolize starch, their resistance to streptomycin, or age in culture had no correlation with the serotypes. Schaad (1976) performed immunological comparisons of ribosomes of a world collection of Xcv from pepper and tomato and found three

serological groups that were correlated with starch hydrolysis but not with pathogenicity. Mathew and Patel (1977) studied Xanthomonas isolated from various solanaceous plants in India and found pathogenic specificity. Pepper strains could infect pepper and tomato, whereas tomato strains could not infect pepper. Rodriguez Neto et al. (1984) and Maringoni and Kimati (1987a, 1987b) investigated Xcv strains isolated from pepper and tomato in Brazil in relation to starch hydrolysis, serology and pathogenicity. Strains from pepper were serologically different from those from tomato. Only the strains isolated from tomato hydrolysed starch, and Xcv strains caused more disease in their host of origin. These same results with respect to disease intensity in the host of origin were found by Lucero (1967) in strains from pepper and tomato in Mendoza, Argentina. Pathogenic difference based on avirulence to pepper was found to be determined by a hypersensitive reaction (Cook, 1973).

Pathogenic variation was also studied in Xcv in an effort to develop resistant varieties of pepper. Races of the bacterium were described that infect cultivars in a differential manner (Cook and Stall, 1969; Cook and Stall, 1982; Rodriguez Neto et al., 1984; Reifschneider et al., 1985; Bongiolo Neto et al., 1986; Hibberd et al., 1987b). As more races are

described and other variable characteristics are reported, it is of interest to determine if strains currently classified as Xcv, based on their pathogenicity on pepper and/or tomato, are as variable as those previously studied and if differences can be found in other traits.

A limited number of strains were tested in the studies mentioned above. Insufficient information exists in the extent of diversity within the pathovar, and this precludes the selection of representative strains for genetic studies for taxonomic purposes. This work was initiated to study the phenotypic diversity in Xcv in a large number of strains representing wide temporal and geographical diversity. Phenotypic traits that were reported as being variable in Xcv were selected for this study.

Materials and Methods

Bacterial strains. Strains of Xcv used in this study were the same as those presented in Chapter 3.

Pathogenic variation. Plants of tomato cultivars Walter and Bonny Best, and pepper cultivar Early Calwander (ECW) and its near isogenic lines ECW 10R, ECW 20R, and ECW 30R, were inoculated. The latter three lines contained the resistance genes Bs1, Bs2, and Bs3, respectively. Seedlings of each were transferred to plastic pots of 10-cm in diameter. The potting soil

was a peat-vermiculite mixture (Metromix 300). The seedlings were fertilized every two weeks with a complete soluble fertilizer (Peters 20:20:20) (1.4g/pot).

Bacteria were grown in nutrient agar or Lima Bean agar for 24 hours at 30°C and resuspended in 1-2 ml of sterile tap-water at a concentration of 10^8-10^9 colony forming units (cfu)/ml. A small area of pepper leaves about 2 cm in diameter was infiltrated with a bacterial suspension and plants were then kept at 30°C in growth chamber (Hibberd et al., 1987b). Plant reactions were recorded at 18, 24, 36 and 48 hours after inoculation. Very young leaves of tomato, just after unfolding, were inoculated by rubbing a cotton swab previously dipped in the bacterial suspension ($\sim 10^8$ cfu/ml.) supplemented with carborundum. Plants were kept in the greenhouse at 22-35°C for 3 weeks before recording disease development.

Starch hydrolysis. The medium BS of Mulrean and Schroth (1981) was used for determination of starch hydrolysis. Plates containing the BS medium were spot-inoculated and incubated for 2 days at 30°C. A cloudy halo around the spot was recorded as positive for hydrolysis of starch. The plates were then flooded with iodine solution (1% KI) to confirm the utilization of starch. Clear zones were observed around the positive strains, whereas a dark brown

color developed around negative strains and in the rest of the medium.

Pectolytic activity. Pectolytic activity was detected by spot-inoculation on plates of sodium polypectate medium (Schaad, 1988) without the addition of crystal violet. Plates were incubated for 48 hours at 30°C and formation of a pit was recorded as a positive reaction.

Resistance to streptomycin. Bacterial strains were streaked onto nutrient agar plates amended with 25 ug/ml of streptomycin sulfate and incubated for 48 hours at 30°C. Copious growth was recorded as resistant, and no growth as sensitive.

Results and Discussion

Pathogenic variation. In preliminary tests with control strains and inoculation techniques, the inoculation of tomato by swabbing with carborundum was the only method to determine the strains not pathogenic to tomato. This method also allowed the visualization of two different types of symptoms: those produced by some of the strains from Argentina (larger spots and yellow halo) and those produced by the rest of the strains (smaller spots and almost no halo). Those strains not pathogenic on pepper were easily determined after infiltration by injection of leaves with inoculum. The reaction was described by Cook

(1973). Frequencies of each race in each group of strains are shown in Table 4-1. Races in Xcv were determined according to their reaction in the tomato cv. Bonny Best and the pepper cv. ECW and its near isogenic lines carrying resistance genes ECW 10R (Bs1), ECW 20R (Bs2), and ECW 30R (Bs3). Hypersensitive reaction in these plants is determined by avirulence genes in the bacterium, giving rise to different races: tomato race (avrBsT), pepper race 1 (avrBs3), race 2 (avrBs1), race 3 (avrBs2) (Minsavage et al., MPMI in press). Bacteria that give hypersensitive reaction in tomato carry the gene avrBsP (see Chapter 6). Of the strains of Xcv examined 38.43% were found to correspond to the tomato race, 36.14% were pepper race 2, 19.31% were pepper race 3, 6.12% were pepper race 1 and 11.15% were avirulent to tomato. Only 278 strains were screened for hypersensitivity to tomato, and 523 strains were screened for the other races.

The UF collection was the only group where all known races were represented. Forty five percent of the strains were of pepper group race 2, over 30% were of the pepper group race 3 and 10% of the pepper group race 1. The tomato group comprised only a little more than 10% of the strains.

Of the strains from the Taiwan collection 46% were of pepper group race 3, and decreasing

proportions were of pepper group race 1 and the tomato group. The pepper group race 2 was not present in this group. Fifty percent of the strains of this collection were hypersensitive to tomato.

Eighty eight percent of the strains of the BA collection were of pepper group race 3 and the rest of pepper group race 1. Strains of pepper group race 1, in this collection, were also hypersensitive to tomato. No pepper group race 2 or tomato group were found in this collection.

Strains from natural populations were less variable. All the BV tomato strains were of the tomato group. In the Ohio population 56% were of pepper group race 2 and the rest of the tomato group. Of the strains collected in Florida fields, 98% were of the tomato group in the tomato field and only 2% were of pepper group race 2; whereas 89% of the strains from the pepper field were of pepper group race 2, and the remaining were of the pepper group race 3. All the strains isolated from tomato were pathogenic to tomato. Strains isolated from pepper were not screened for reaction on tomato.

The data on distribution of races confirmed the report of Cook and Stall (1982) that pepper group race 2 is very common in Florida and occurs infrequently outside the USA.

Strains of the tomato group from Taiwan, Argentina, and USA gave different reactions when inoculated into pepper. The hypersensitive reaction (HR) obtained with the strains from Argentina was faster and resulted in a more intense necrosis, than the other strains. The genotype of these strains, with respect to the avirulent reaction may be different.

The majority of the strains in the Florida pepper fields were of the pepper group race 2 and only very few were of pepper group race 3. The selective advantage of two races being present in the same plot of plants of the same variety is not known. The absence of the plasmid that carries the avrBs1 or mutation for a race change (Dahlbeck and Stall, 1979) does not seems to be the only difference between the strains of these two races in those fields. These strains had different plasmid profiles (Chapter 3) with diversity in size and number.

For the strains in the UF collection the reaction in pepper was scored according with the phenotypes of the different avirulence genes involved (Hibberd et al., 1987b; Minsavage et al., MPMI in press). This allowed the determination of the distribution of the avrBs1 gene in the different size classes of large plasmids, i. e., by combining data from Chapter 3 with the reactions for race 2 obtained in this study

(Table 4-2). Sixty-eight percent of the large plasmids carry the avrBs1 gene, and the larger plasmids are more prone to carry the gene (86% of class A against 60% for B and C).

Starch hydrolysis and pectolytic activity. These two traits were linked in all strains examined. Only the strains from Argentina were positive and there was also a total correlation with the type of symptom in tomato. Some few strains from the BV group lost the pectolytic activity capability after storage, but the starch hydrolysis capability of these strains was intact.

There was no correlation between host of origin and starch hydrolysis or pectolytic activity. For example, in the BA collection there are strains from pepper and tomato. This is not in agreement with previous reports that starch hydrolysis is related to host of origin.

To determine if the strains from Argentina were pathogenic in other solanaceous plants as reported for strains of Xcv from New Zealand and India (see Introduction) seeds of Solanum sisymbriifolium Lamarck and Physalis viscosa L. were collected in Argentina from the same area where the Xcv strains were isolated. Despite repeated inoculations with those strains no pathogenic reaction was observed in these plants or in

those of Physalis sp. and Solanum nigrum L. obtained from Florida.

Further comparison by DNA-DNA homology experiments should give a better understanding of the relationships among strains from Argentina and the other strains and may finally determine if all of these strains should be considered as Xcv.

Streptomycin resistance. The frequency of streptomycin resistant strains for the different groups is given in Table 4-3.

Resistance to streptomycin was widespread. Of the strains from Argentina, only strains from the BA collection were sensitive, whereas approximately half of the BV strains were resistant. One third of the strains in the UF collection were resistant. The small group of strains from Ohio were all resistant to streptomycin. Less than ten percent of the strains from the TW collection were resistant. The distribution of streptomycin resistance in the strains from fields in Florida was markedly different in relation to the host. In the tomato field, 92% of the strains were resistant, whereas only 4% of the strains in the pepper field were resistant. Information on the history of streptomycin application to the sampled plants was not obtained which could have aided in the interpretation of this difference.

Phenotypic diversity was observed within Xcv for all strains examined. This diversity was related more to geographical origin of the strains than to host of origin.

Genetic analysis of phenotypic diversity in the group of strains called Xcv may support the hypothesis that different taxonomic units are being considered as Xcv. However, one can not reject the hypothesis that Xcv may be a very diverse pathovar with different clonal lines (Orskov and Orskov, 1983) or ecotypes (Crosse, 1966) as members. Even further diversity may be encountered if strains from other regions of the world are surveyed. Greater genotypic diversity was found in Pseudomonas syringae pv. syringae (Pss) than in P. s. pv. tomato (Pst) (Denny et al., 1988), whereas phenotypic diversity was high only for some traits within Pst (Denny, 1988). Genetic variation, measured by isozyme analysis, was also found in natural populations of Rhizobium leguminosarum biovar trifolii and the degree of diversity varied with the location of isolation (Harrison et al., 1989).

Table 4-1. Frequencies of strains of Xcv for each pathogenic race in different populations.

Strain population	Tomato Race	Pepper Race 1	2	3	P
UF collection	20/180[a]	19/180	92/180	49/180	14/158
TW collection	6/28	9/28	0/28	13/28	14/28
BA collection	0/32	4/32	0/32	28/32	3/13
BV tomato field	86/86	0/86	0/86	0/86	0/79
OH tomato field	7/16	0/16	9/16	0/16	ND
FL tomato field	82/84	0/84	2/84	0/84	ND
FL tomato field	0/97	0/97	86/97	11/97	ND
Total	201/523	32/523	189/523	101/523	31/278
Percentage	38.43	6.12	36.14	19.31	11.15

(a) Number of strains of race x / Total number of strains screened.

Table 4-2. Frequencies of strains of Xcv from the UF collection with the avrBs1 gene among size classes of large plasmids.

Size class	Frequency with avrBs1	Frequency in population
A	43/50[a]	50/217[b]
B	61/98	98/217
C	21/35	35/217
Total	125/183[c]	183/217[d]

(a) Number with the gene and plasmid A/Number with plasmid A.

(b) Number with plasmid A/Total number of strains screened.

(c) Total number with the gene/Total with large plasmids.

(d) Total with large plasmids/Total number of strains screened.

Table 4-3. Frequency of strains of Xcv with resistance to streptomycin in different populations.

Population	Frequency
UF collection	72/217[a]
BA collection	0/32
TW collection	2/28
BV tomato field	40/85
OH tomato field	16/16
FL tomato field	77/84
FL pepper field	4/97

(a) Number of resistant strains/Total number of strains screened.

CHAPTER 5
DISTRIBUTION OF PLASMID-ENCODED COPPER RESISTANCE

Introduction

Bacterial spot of pepper and tomato is an endemic disease in several regions of the world where these plants are cultivated. Copper-containing bactericides are commonly used for control. Differences in sensitivity to copper were detected among strains of Xcv isolated in Florida, and copper resistant strains were thought to be endemic to Florida (Marco and Stall, 1983). Copper resistance in the Florida strains was found to be encoded by a 200 kbp plasmid (pXvCu) present in all copper resistant strains, and in some copper sensitive strains, this plasmid was found to be self-transmissible (Stall et al., 1986).

Plasmid-mediated heavy metal resistance has been determined in many other bacteria (Foster, 1983; Tetaz and Luke, 1983; Haefeli et al., 1984; Robinson and Tuovinen, 1984; Summers, 1984; Summers, 1985; Desomer et al., 1988; Silver and Misra, 1988).

Copper resistance was found to be plasmid mediated in the plant pathogens Pseudomonas syringae pv. tomato (Bender and Cooksey, 1986) and in P. s. pv.

syringae (Sundin et al., 1989). It seems as more bacteria are studied, copper resistance is found to be a common occurrence and this resistance is generally found to be plasmid-borne.

This work was initiated to determine the distribution of copper resistance in a world-wide collection of Xcv strains stored for varying numbers of years and in strains recently isolated from the field.

Materials and Methods

Bacterial strains, media and conjugation experiments. Strains of Xcv included in this study were the same as those screened in plasmid profile determinations (see Materials and methods, Chapter 3). Bacteria were maintained in sterile tap-water for long term storage or on Lima Bean Agar (Difco), pH 7.0, for short term storage. Copper resistance was determined by growth on nutrient agar supplemented with 0.8 mM of $CuSO_4.5H_2O$. A nalidixic acid resistant mutant of Xanthomonas sp. strain T-55 (Stall, 1987), used as recipient in conjugation experiments, was obtained by selection on nutrient agar plates supplemented with 25 ppm of nalidixic acid (nal).

For conjugation experiments bacteria were grown with vigorous shaking at 30°C for 16 to 24 hrs in nutrient broth tubes, mixed and incubated with very gentle shaking for 8 hrs, then plated on appropriate

selective media and incubated at 30°C for 2-3 days until transconjugants developed.

Plasmid DNA isolation and sizing. Plasmid extraction was by a modification of the alkaline lysis method for large plasmids according to the procedure of Kado and Liu (1981). Preparations were maintained for several months at 4°C after adding 25 mM of EDTA. Large scale plasmid preparations were by the total DNA extraction method of Marmur (1961) and followed by cesium chloride centrifugation (Maniatis et al., 1982). Plasmid sizing was done as explained in Chapter 3.

Hybridization experiments. Southern blots were prepared according to Maniatis et al. (1982) and hybridization was done with the GENIUS nonradioactive DNA labeling and detection kit (Boehringer Mannheim Biochemicals) following the manufacturer's instructions. Restriction endonuclease analysis was performed in accordance with the manufacturer's recommendations.

Results and Discussion

Distribution of copper resistance and large plasmids. Large plasmids (170-300 kbp) were detected in eighty-four percent of the strains. They were separated into three size classes (Table 5-1). Seventy percent of all strains grew on a copper-supplemented medium (Table 5-2). Only strains carrying large

plasmids were copper-resistant but large plasmids were also detected in copper-sensitive strains (14 percent of the strains) (Table 5-2). Those strains with the largest plasmids (class A) were almost always copper resistant (99.3%). In contrast, the percentage of copper resistant strains was less in those in class B (82.3%), and only half (51.8%) of those in class C were copper resistant.

Conjugative properties of plasmids. Selected strains were used in conjugation experiments. They were mated to the plasmidless strain T-55 nal^R and transconjugants were selected on a copper and nal supplemented medium. Only large plasmids were detected in transconjugants (Fig.5-1), although occasionally the very small plasmid in size class M (less than 5 kbp, see Chapter 3) was apparently mobilized by the large plasmids. Its small size would not allow for coding of conjugative properties. Some plasmids were transmissible at high frequencies, whereas others were either transmitted at low frequencies or not transmissible to strain T-55 nal^R.

Restriction pattern comparison. The pXvCu plasmids in 15 strains were conjugated into T-55 nal^R. Total DNA was extracted from the transconjugants and the plasmid DNA was recovered after CsCl centrifugation. Only DNA of plasmid size class B from three

strains could be recovered intact. The plasmid DNA was subjected to restriction with the endonuclease XbaI and SmaI and concentrated by ethanol precipitation as the plasmid DNA concentration recovered was very low. Restriction patterns for the three plasmids in an agarose gel were not the same with either enzyme (Fig. 5-2).

DNA homology of the resistance determinants. A DNA fragment coding for copper resistance in Xcv strain 81-23, provided by Dr. B. J. Staskawicz, Univ. of California, Berkeley, was used as a probe in hybridization experiments. Several copper resistant strains and one copper-sensitive strain containing a large plasmid were probed. The probe hybridized to the large plasmid band in all copper resistant strains and also to a 55-75 kbp plasmid (class G and H) in the BV group of strains from Argentina. No hybridization occurred with the copper-sensitive strain (Fig. 5-3).

To further determine the degree of homology of the sequences encoding for copper resistance, the SmaI digest of the CsCl extracted plasmids was probed with the fragment encoding copper resistance. The probe hybridized to fragments of different sizes within the digested plasmid DNA from Florida and Argentina (Fig. 5-4).

Apparently, polymorphism exists among these large plasmids in Xcv in spite of their conserved size and their conjugative properties. In hybridization experiments polymorphism also occurred in the DNA fragments encoding copper resistance in two strains from Florida (TB-1 and 71-31, isolated 15 years apart) and one strain from Argentina (BV5-4a). Some of the polymorphism may result from an insertion sequence (IS element) present in at least some of the pXvCu plasmids (Kearney et al., 1988). A smaller plasmid present only in strains from Argentina also hybridized to the fragment encoding copper resistance, but it is not known if it contains an active gene for copper resistance.

Plasmid-determined resistance to copper was found to be widespread in Xcv strains isolated in several areas of the world. Moreover, hybridization occurred with all the copper resistance strains when a DNA fragment encoding copper resistance from a strain isolated in Florida was used as a probe. This suggests that genes coding for copper resistance in different strains of Xcv share at least some sequence homology. Whether the copper resistance sequences were distributed from a single source or arose independently in every location could not be determined from this study. As in the case of antibiotic resistance, heavy

metal resistance in natural environments is generally plasmid-encoded, and the mechanisms of resistance are different from chromosomal mutation resistance that is laboratory-produced (Silver and Misra, 1988). There is evidence that many soil bacteria carry antimicrobial resistance genes located generally in plasmids and/or transposons, and they may be a source of resistance genes probably conserved in several bacteria (Foster, 1983). The occurrence of this phenomenon with copper resistant determinants would explain its widespread distribution.

Table 5-1. Frequencies of strains of Xcv having large plasmids in various size classes

Population	Size class[a] A	B	C
UF collection	47/218[b]	98/218	36/218
TW collection	0/28	8/28	7/28
BA collection	0/30	22/30	0/30
BV tomato field	0/52	26/52	0/52
FL tomato field	5/84	79/84	0/84
FL pepper field	83/97	6/97	13/97
OH tomato field	0/15	15/15	0/15
Total	135/524	254/524	56/524
Percentage	25.8	48.5	10.7

(a) A= >260 kbp; B= 201-259.9; C= 170-200.9

(b) No. positive strains/total number in collection

Table 5-2. Frequencies of strains of Xcv with copper resistance among size classes of large plasmids.

Population	Size class A	B	C	Total Cu^R
UF collection	47/47[a]	76/98	21/36	144/218[b]
TW collection	0/0	5/8	4/7	9/28
BA collection	0/0	5/22	0/0	5/30
BV tomato field	0/0	26/26	0/0	26/52
FL tomato field	5/5	79/79	0/0	84/84
FL pepper field	82/83	4/6	4/13	86/97
OH tomato field	0/0	14/15	0/0	14/15
Total	134/135	209/254	29/56	368/524
Percentage	99.3	82.3	51.8	70.2

(a) Number of copper resistant (Cu^R) strains/ number of strains in class

(b) Number of Cu^R strains/number of strains in population.

Fig. 5-1. Agarose gel electrophoresis of plasmid DNA of Xcv strains and copper resistant transconjugants resulting from the mating with the plasmidless Xanthomonas sp. strain T-55.

SW2
75-3
71-31
E3
TB-1xT55
PB-11xT55
75-3xT55
71-31xT55
TB-1xT55
PB-11xT55
75-3xT55
68-1xT55
71-31xT55
BV5-4axT55
SW2
323kb

Fig. 5-2. Agarose gel electrophoreis of ccc plasmid DNA of Xcv strains and restriction fragment pattern of copper resistance plasmids from those strains after digestion with SmaI and XbaI. Bacterial source of the plasmids: pXvCu15 (BV5-4a); pXvCu10 (TB-1); pXvCu2 (71-31). Molecular weight markers were: SW2 for ccc plasmids, and lambda restricted with EcoRI and HindIII for fragments.

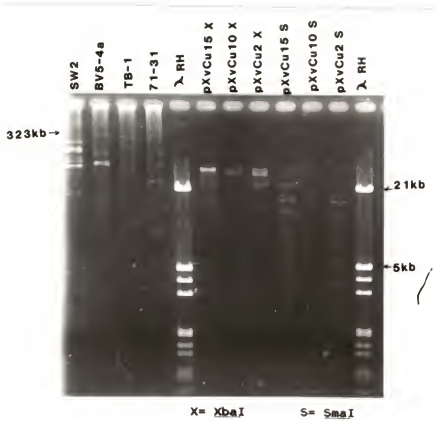
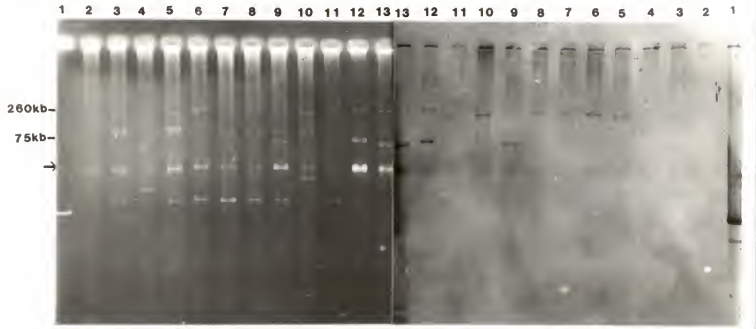

SW2
BV5-4a
TB-1
71-31
λ RH
pXvCu15 X
pXvCu10 X
pXvCu2 X
pXvCu15 S
pXvCu10 S
pXvCu2 S
λ RH
323kb→
21kb
5kb
X= XbaI
S= SmaI

Fig. 5-3. Hybridization of the copper resistance clone pXvCu1-13 to the plasmid DNA from strains of Xcv. A= gel stained with ethidium bromide. B= Southern blot of the gel in panel A probed with pXvCu1-13. Concentration of DNA in lane 4 (PB-11) was very low. Arrow indicates linear plasmid and chromosomal DNA.

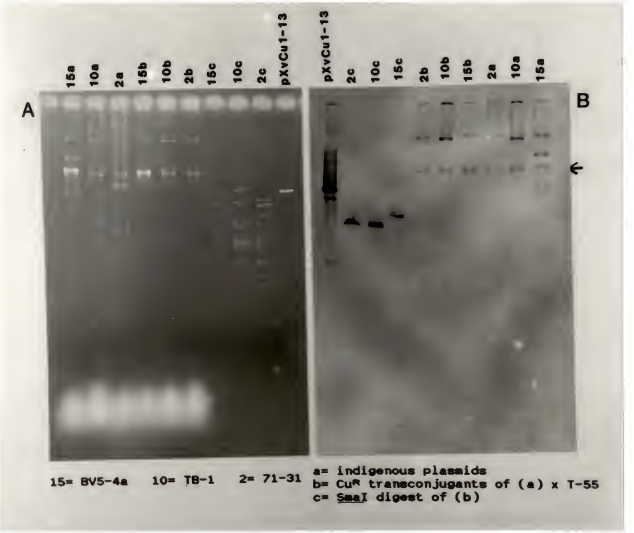


A

1= pXvCu1-13 Cu^R
2= 88-5 Cu^S
3= 87-56 TW Cu^R
4= PB-11 FL pf Cu^R
5= 87-7 FL Cu^R
6= E-3 FL Cu^R
7= 71-31 FL Cu^R
8= BA30-1 ARG Cu^R
10= TB-1 FL tf Cu^R
11= 68-1 FL Cu^R
12= 79-2 ARG Cu^R
13= BV5-4a ARG Cu^R

B

Fig. 5-4. Hybridization of the copper resistance clone pXvCu1-13 to the plasmid DNA from three strains of Xcv. A= gel stained with ethidium bromide. B= Southern blot of the gel in panel A probed with pXvCu1-13 clone. Lanes in both panels are; 1, 2, 3= total plasmid DNA; 4, 5, 6= transconjugants from the mating to the strain T-55; 6, 7, 8= SmaI digested plasmid DNA of the transconjugants. Lanes 1, 4, 7= BV5-4a; 2, 5, 8= TB-1; 3, 6, 9= 71-31. Lane 10= pXvCu1-13. Arrow indicates chromosomal and linear plasmid DNA.



15= BV5-4a 10= TB-1 2= 71-31 a= indigenous plasmids
b= Cu^R transconjugants of (a) x T-55
c= SmaI digest of (b)

CHAPTER 6
IDENTIFICATION, CHARACTERIZATION AND CLONING OF THE AVIRULENCE GENE avrBsP

Introduction

Bacterial spot is one of the most destructive diseases of pepper and tomato in warm, humid areas; control by the application of chemicals is inadequate especially in the rainy season (Stall and Cook, 1966; Marco and Stall, 1983; Pohronezny et al., 1986). Control of the disease by genetic resistance is the most cost-efficient method, and understanding the host-parasite relationship is essential for the application of this method. Information on the Xcv-pepper pathosystem is extensive. Quantitative resistance (Dahlbeck et al., 1979; Stall, 1981; Hibberd et al., 1988) and single gene resistance (Cook and Stall, 1963; Stall and Cook, 1966; Cook and Stall, 1968, 1969, 1982; Dahlbeck et al., 1979; Dahlbeck and Stall, 1979; Cook and Guevara, 1984; Kim and Hartmann, 1985; Hibberd et al., 1987a, 1987b; Ronald and Staskawicz, 1988; Swanson et al., 1988; Kearney et al., 1988; Bonas et al., 1989; Minsavage et al., MPMI in press) have been described and characterized. Published information on the Xcv-tomato pathosystem is much more limited.

Quantitative resistance exist in many cultivars; hypersensitive resistance was deccribed in one genotype (Jones and Scott, 1986). Single gene resistance to the bacterial speck pathogen (Pseudomonas tomato) has been described in L. pimpinellifolium, a relative of the tomato (Pilowsky and Zutra, 1982).

Pathogenic variants of Xcv have been described that cause disease in pepper but not in tomato (see Chapter 4). Consistent isolation of these strains was reported from Brazil and referred to as the "Rio strain"; hypersensitive resistance to the strain was found in the only two cultivars tested, Santa Cruz and Grothen's Globe (Rodriguez Neto et al., 1984; Reifschneider et al., 1985; Bongiolo Neto et al., 1986).

Single gene resistance to Xcv in pepper follows a gene-for-gene system (Flor, 1955), and complementary genes in the pathogen and in the host have been identified (Swanson et al., 1988; Bonas et al., 1989; Minsavage et al., MPMI in press). This work was initiated to determine if avirulence (avr) genes were involved in the interaction of Xcv with tomato and to characterize genetically this relationship.

Materials and Methods.

Bacterial strains and media. Strains of Xcv and their reactions in pepper and tomato are listed in Table 6-1. Strains of Xcv were kept in sterile

tap-water and were subcultured on nutrient agar (NA) medium. Rifampicin resistant strains were obtained by plating 10^9 colony-forming-units (cfu)/ml on NA containing 50 μg/ml of rifampicin. Escherichia coli (Ec) strains were maintained on Luria-Bertani medium (Maniatis et al., 1982). Conjugations were performed on nutrient yeast glycerol agar (NYGA, Daniel et al., 1984). The media were amended, when necessary, with antibiotics at the following concentrations: rifampicin, 50 μg/ml; tetracycline, 10 μg/ml; and kanamycin, 40 μg/ml.

Growth of plants and inoculum preparation. Plants were grown in steamed peat-vermiculite mix in 10-cm (diameter) pots in a greenhouse at 20-35°C. Plants were fertilized biweekly with a complete soluble fertilizer (Peters 20:20:20). Only the tomato cv. Bonny Best and pepper cv. Early Calwonder were used in the cloning, population, and electrolyte leakage experiments.

Bacterial strains were grown in nutrient broth, with shaking, for 24 hours at 30°C, centrifuged and resuspended in sterile tap-water to 0.3A at 600nm OD measured with a Spectronic 20. This corresponds to approximately $5x10^8$ cfu/ml. Subsequent dilutions in sterile tap-water were made as necessary. In other experiments bacteria were grown on solid media,

resuspended in sterile tap-water, washed by centrifugation as before or used directly.

Electrolyte leakage experiments. Electrolyte leakage was measured as conductivity of baths containing inoculated plant tissue (Cook and Stall, 1968; Hibberd et al., 1987b). Six leaf disks of 0.5 cm^2 area each were placed in 3 ml of distilled water in each assay. Experiments were repeated at least two times and the data were combined. Three replicates per treatment were used, each replicate in different leaves of the same plant. Plants were maintained in a growth chamber at 30°C or 24°C.

Bacterial growth curves. Bacterial multiplication in leaves was determined after infiltration of a bacterial suspension containing $1x10^4$ cfu/ml into the mesophyll with a syringe. Sampling was at regular intervals, number of colonies that developed in solid media after serial dilution were counted (Stall and Cook, 1966; Hibberd et al., 1987b). Three different leaves were inoculated in each assay and three areas of one cm^2 each were sampled. Plants were maintained in a greenhouse at 20-35°C.

Recombinant DNA techniques. Techniques used for cosmid cloning, enzyme digestion, ligation, map construction, subcloning, Southern transfer, plasmid alkaline lysis, and agarose gel electrophoresis were

essentially as described by Maniatis et al. (1982). The genomic library of Xcv strain 87-7 was constructed in the vector pLAFR-3 (Ditta et al., 1980; Friedman et al., 1982; Staskawicz et al., 1984, 1987) and individual clones were maintained in Ec DH5α (BRL). Packaging into lambda phages was with the DNA packaging kit of Boehringer Mannheim. The helper plasmid pRK2013 in Ec HB101 was used in conjugations by triparental mating.

Transposon Tn5 mutagenesis. Mutagenesis of the avirulence gene subclone in Ec was performed essentially as described by Ruvkun and Ausubel (1981).

Hybridization experiments. All hybridization experiments were performed on nitrocellulose membranes using the GENIUS nonradioactive DNA labeling and detection kit (Boheringer Mannheim) according with the manufacturer instructions. Genomic DNA extraction for hybridization experiments was by the method of Boucher et al. (1987).

Results

Selection of strains avirulent to tomato. Identification of the strains that were avirulent to tomato was possible after swabbing very young tomato leaves with suspensions of high concentrations of bacteria with carborundum. Typical bacterial spot symptoms developed after inoculation with the virulent strains, but no symptoms developed with the avirulent

strains, except small (1-3 mm diameter) hypersensitive flecks. These latter strains gave susceptible reactions when inoculated in Early Calwander pepper. Strains giving strong and consistent susceptible and hypersensitive reactions were selected to determine optimal conditions for development of the HR. In further experiments 6-week old tomato plants were used that had the main stem excised 1-2 weeks before being used. The stems were cut between the 8^{th} and 9^{th} true leaves. The youngest three leaves were then inoculated. In the beginning plants were kept in a growth chamber at 30°C and HR necrosis was visible at 24-36 hours after inoculation, whereas necrosis in susceptible reactions appeared after 48 hours. In later experiments the growth chamber temperature was changed to 24°C to better distinguish the symptoms. At the latter temperature, HR developed in 36-48 hours with strong necrosis, whereas the susceptible reaction expressed as water soaking that lasted several days.

To further characterize the avirulent and virulent reaction in pepper and tomato, strain 87-7 was selected. The time course of electrolyte leakage from tomato and pepper leaves inoculated with 10^8 cfu/ml was compared (Fig. 6-1). Conductivity of water containing disks of inoculated tomato tissue was higher at an earlier time than those containing inoculated

pepper tissue, indicating earlier onset of necrosis. In leaves of pepper inoculated with $1x10^4$ cfu/ml, populations of strain 87-7 increased to 10^9-10^{10} cfu per cm^2 by 6 days and these numbers continued at least until day 14 (Fig. 6-2); whereas in leaves of tomato bacterial populations reached 10^6 cfu/cm^2 by 4 days, and these numbers decreased after 6 days. Thus, the tomato plant reaction was typical of resistance and the pepper plant reaction was typical of susceptibility.

Identification and cloning of avrBsP. To determine if an avirulence gene in strain 87-7 prevented it from causing disease on tomato, a library of DNA from 87-7 was mobilized from Ec strain DH5α into a normally virulent strain Xv56. Of the 1195 library colonies obtained, 1102 were successfully conjugated independently into Xv56, three clones (p629, p965, and p1119) with inserts of 6.4, 29.08, and 25.21 kbp, respectively, converted the normally virulent Xv56 to avirulence on tomato. The p965 clone was chosen for detailed analysis, and the avirulence locus carried by it was designated avrBsP. The insert DNA of p965 was digested with HindIII and religated to pLAFR-3, and the resulting clones were mobilized into Xv56. The recombinant plasmid p965-2, carrying an insert of 11.37 kb still had the avrBsP activity. Further

subcloning was done by partial digestion of this insert with Sau3A and religating in the BamHI site of pLAFR-3. This gave rise to p965-2-4 with an insert of 1.7 kb which retained the avirulence gene activity. This clone was characterized by restriction mapping (Fig. 6-3).

To obtain further evidence that the cloned fragment contained a gene required for HR induction in tomato, the inactivation of the gene by transposon insertion mutagenesis was attempted. Of 120 insertions obtained in the plasmid, 12 eliminated the gene activity. The positions of 9 independent insertions by the transposon Tn5 are illustrated in Fig. 6-3.

Analysis of electrolyte leakage. The time course of electrolyte leakage from tomato infiltrated with Xv56(p965-2-4), Xv56(p965-2-4::Tn5) and the wild type strains Xv56 and 87-7 were compared at 24°C. The timing and rate of electrolyte leakage induced by Xv56 carrying the fragment encoding avrBsP and the wild type 87-7 were very similar. These data for Xv56 carrying the transposon-inactivated form of avrBsP and the wild type Xv56 were similar among themselves and clearly different from those bacteria carrying the active gene (Fig. 6-4).

DNA homology of avrBsP to other Xcv strains. Total genomic DNA digested with EcoRI and plasmid DNA

of selected strains were separated in agarose gel electrophoresis and hybridized in a Southern blot experiment to the clone carrying avrBsP (p965-2-4) (Fig. 6-5). No hybridization occurred to the strain E-3 that is virulent in tomato but strong hybridization was evident with a fragment of the same size in strains 87-7, 87-56, and 86-69. All the latter strains are avirulent to tomato. Hybridization occurred with two bands in the genomic DNA and with the plasmid size class I (38 to 46.9 kb) and F (80 to 119.9 kb) in the plasmid DNA of the three avirulent strains.

Strains 71-21 and 82-4n only carry plasmids of the size class I (see Chapter 3) and induce an HR in tomato. Strain 82-4j is a spontaneous mutant of 82-4n that carries no detectable plasmid. A hybridization experiment was performed to determine if there was homology with the avrBsP clone (Fig. 6-6). Total genomic DNA digested with EcoRI of strains 87-7, 82-4n, 82-4j, and Xv56 was separated in an agarose gel and hybridized to a 1.0 kb HindIII-BalI fragment of the avrBsP clone. As expected, no hybridization occurred to the DNA of Xv56, but varying degrees of hybridization occurred with DNA of the other strains. The strongest hybridization was to a large fragment in strains 87-7 and 82-4n. A lighter band appeared in a

smaller fragment in those same strains and a third fragment of about 2.0 kb hybridized lightly to the probe in 87-7, 82-4n, and 82-4j.

Interaction of avrBsP with other tomato cultivars. Strains 87-7 and Xv56(p965-2-4) were infiltrated into leaves of tomato cultivars Homestead 500, Sunny, Euromech, Saturn, Walter, Floradade, Manalucie, Malentha, Marglobe, CRA-66, MH-1, and 19 other breeding lines. An HR similar to that observed in Bonny Best was obtained in all plants. The reaction in MH-1 was delayed with respect to the others.

Discussion

A method was developed that allowed the identification of strains that induce an HR (Klement et al., 1964) in leaves of several cultivars of tomato. Characterization of the reactions of these strains was possible after comparing the rates of electrolyte leakage and bacterial multiplication in tomato and pepper leaves. The rates were characteristic of an incompatible (HR) reaction in tomato and compatible (susceptible reaction) in pepper (ECW) as described previously for other strains of Xcv with pepper (Stall and Cook, 1966; Cook and Stall, 1968; Hibberd et al., 1987b).

The avirulence gene, avrBsP isolated from 87-7 and located on a 1.7 kbp DNA fragment in clone

p965-2-4 was demonstrated to be responsible for the induction of the HR in tomato. Electrolyte leakage patterns of a previously virulent strain carrying the cloned gene gave reactions similar to those of the avirulent strain that was the source of avrBsP. Mutagenesis by several independent insertions of Tn5 into different sites of the clone resulted in complete inactivation of the gene as demonstrated by the electrolyte leakage curves obtained in tomato after incoculation of the strain carrying the intact gene and the one carrying the mutated gene (Fig 6-4).

Other strains of Xcv that induce HR in tomato had sequences homologous to avrBsP. Support for the gene-for-gene model of resistance in the Xcv-pepper interaction has been developed by genetic (Hibberd et al., 1987a, 1987b) and molecular (Minsavage et al. MPMI in press) analysis. The study of the inheritance of the resistance (Crute, 1986) to Xcv strains carrying avrBsP was not possible since no tomato line was found to be susceptible to those strains. The host range of Xcv includes pepper and tomato and strains carrying avrBsP are avirulent to tomato, but are capable of causing disease in specific pepper lines (Table 6-1). Moreover, strains normally virulent to tomato were converted to avirulence after complementation with a plasmid carrying avrBsP. This is evidence

for the existence of a dominant avirulence gene in Xcv that may interacts with a single, complementary, resistance gene in tomato, as is the case for all avirulence genes presently described (Keen and Staskawicz, 1988).

Precise localization of the avrBsP sequences in the genome of Xcv was not possible due to the hybridization of the specific probe to several DNA fragments. Homologous sequences were located in two plasmids in three strains and less strong hybridization occurred with sequences located in the chromosome (Fig. 6-6). It may be possible that inactive copies of the gene also exist and/or that homology exists with other avr genes. Indeed, in hybridization experiments, homology to the clone carrying the avrBs3 gene was detected (data not shown). The avrBs3 gene is located in the plasmid size class I in strains 82-8 and 71-21 (Bonas et al., 1989). Sequences homologous to the avrBsp clone were detected in plasmid size class I in strains 87-7, 86-67, and 87-56. There is a possibility that this gene, avrBsP, may be located in a transposable element. Gene replacement experiments by marker exchange may help to locate the gene in the genome of Xcv, although the existence of homologous sequences in several locations of the genome might make the task difficult. Better results could be

obtained with the isolation of the plasmids in strain 87-7 that carry sequences homologous to the avrBsP clone and construction of plasmid libraries to determine whether a plasmid carries the active copy of the gene or the gene is located in the chromosome.

Table 6-1. Phenotypes of tomato Bonny Best and lines of pepper Early Calwonder (ECW) inoculated with different strains and transconjugants of Xcv.

Xcv strains	Tomato	Lines of ECW pepper			
	Bonny Best	ECW	10R	20R	30R
E-3 pepper race 2	S[a]	S	HR[b]	HR	S
71-21 pepper race 1	HR	S	S	HR	HR
82-4n pepper race 1	HR	S	S	HR	HR
82-4j pepper race 3	HR[c]	S	S	HR	S
87-56 pepper race 1	HR	S	S	HR	HR
86-69 pepper race 1	HR	S	S	HR	HR
87-7 pepper race 1	HR	S	S	HR	HR
Xv56 tomato race 2	S	HR	HR	HR	HR
Xv56(p965-2-4)	HR	HR	HR	HR	HR
Xv56(p965-2-4::Tn5)	S	HR	HR	HR	HR

(a) Susceptible reaction

(b) Hypersensitive reaction

(c) Week hypersensitive-like reaction

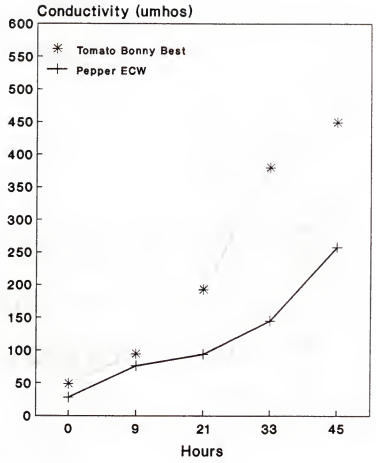


Fig. 6-1. Electrolyte leakage from leaves of pepper ECW and tomato Bonny Best plants inoculated with strain 87-7 of Xcv at a concentration of $5x10^8$ cells per milliliter. Plants were kept in growth chamber at 30^oC.

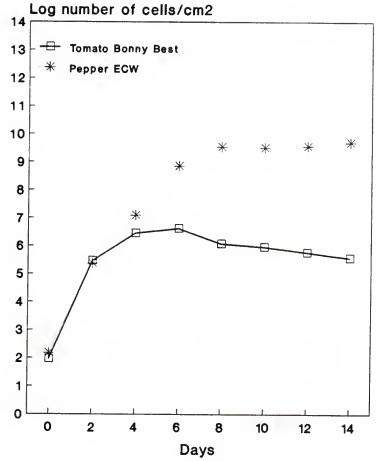


Fig. 6-2. Populations of bacteria per cm^2 of leaf tissue at increasing times after inoculation. Tomato Bonny Best and pepper ECW were inoculated with $1x10^4$ cells per milliliter of Xcv strain 87-7.

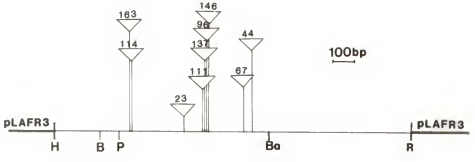


Fig. 6-3. Restriction map of the DNA region containing avrBsP activity. The 1.7 kbp Sau3A partial restriction fragment was subcloned two times from the original Sau3A partial restriction clone (p965) and HindIII subclone (p965-2) into the BamHI site of pLAFR 3 (p965-2-4). The position of independent Tn5 insertions into avrBsP are indicated by triangles. All the insertions shown eliminated the avirulence activity. H= HindIII; R= EcoRI; P= PstI; B= BamHI; Ba= BalI.

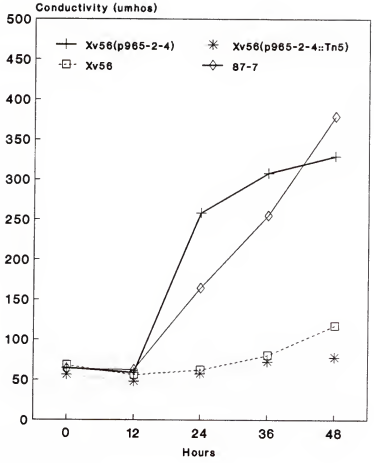


Fig. 6-4. Electrolyte leakage from leaves of tomato Bonny Best inoculated with strains and trans-conjugants of Xcv at a concentration of $5x10^8$ cells per milliliter. Plants were kept in growth chamber at 24^oC.

Fig. 6-5. Hybridization of avrBsP specific probe (p965-2-4) to total genomic DNA and plasmid DNA of Xcv strains. Lane 1= p965-2-4 linearized with HindIII; 3, 7= Xcv E-3; 4, 8= Xcv 87-56; 5, 9= Xcv 86-69; 6, 10= Xcv 87-7. Lanes 3, 4, 5, 6= genomic DNA restricted with EcoRI. Lanes 7, 8, 9, 10= plasmid DNA. A= Ethidium bromide stained gel. B= Southern blot of gel in panel A. Numbers indicate size in kbp of plasmid DNA only. Arrow indicates chromosomal DNA of plasmid samples.


10 9 8 7 6 5 4 3 2 1
200
80
40
30
A
1 2 3 4 5 6 7 8 9 10
B

Fig. 6-6. Hybridization of the HindIII-BalI fragment in avrBsP to total genomic DNA of Xcv strains digested with EcoRI. Lanes 1= 87-7; 2= 82-4n; 3= 82-4j; 4= Xv56. Lane 6= DNA from p965-2 and p18-4-20 (insert from p965-2-4 in pUC18) digested with EcoRI and HindIII. Numbers 1 and 2 indicate the respective inserts. A= Ethidium bromide stained gel. B= Southern blot of gel in panel A.


1 2 3 4 5 6
1 2 3 4 5 6
A
B
1
2

CHAPTER 7
DISCUSSION

Diversity was evident in the large number of strains of Xcv studied with respect to plasmid profiles, pathogenic races, resistance to streptomycin and copper, starch hydrolysis, and pectolytic activity.

Strains of Xcv that were stored for varying number of years after their isolation from several parts of the world served as a representative collection for the characterization of this important plant pathogen. Strains freshly isolated from naturally infected fields of pepper and tomato provided useful information on the bacterial population as its exist in nature. Crosse (1968) and Schroth (1968) have mentioned the importance of knowing the degree of homogeneity of the pathogen population occurring in a given host in a given locality and how similar they are to populations occurring in the same host in other localities, or in other hosts in the same locality. They proposed the possible inaccurate representation of the organisms, as they occur in nature, if studies are confined to very few strains.

Although many geographical regions were not represented in the group of strains examined, the information obtained can be considered a starting point to build an accurate characterization of Xcv. The addition of more strains from Australia, New Zealand, India, and Brazil, where variation was reported previously (Dye et al., 1964; Srinivasan, 1962; Reifschneider et al., 1985) may confirm and even increase the genetic base of this pathovar.

The information obtained on plasmids demonstrated the diversity that exists within Xcv. Plasmids ranging in size from a few kbp to 300 kbp were observed. Most plasmids were larger than 30 kbp. The plasmids were arbitrarily grouped into 13 size classes to facilitate comparison. The fragility of the plasmid DNA preparations and the fact that most strains carried several plasmids precluded further comparison by restriction fragment analysis and hybridization analysis. Combinations of the 13 size classes gave a total of 71 different plasmid profiles observed. This high diversity in profiles may be useful in epidemiological studies in which profiles can be used as strain marker to monitor the pathogen population.

The most predominant profiles in each host-locality combination studied were not the same, suggesting that different groups of strains (as defined

by their plasmid profiles) are the most dominant in each place. The same type of data was obtained when plasmids in strains of Rhizobium leguminosarum biovar trifolii (Harrison et al., 1989) were studied; a few distinct variants were found to be predominant in each of several particular sites sampled.

Although some plasmids of Xcv were found to be self-transmissible (Stall et al., 1986; Bonas et al., 1989; Chapter 6) some of the plasmids may be preferably maintained by some strains. Young and Wexler (1988) found that in Rhizobium leguminosarum biovar viceae some plasmids were present only in specific chromosomal types, whereas others were widespread.

The apparent differential variation in plasmid profiles for a host-locality combination may contribute to the existence of ecotypes (Crosse, 1966, 1968) in Xcv. Further evidence in this line was provided by the data obtained from starch hydrolysis and pectolytic activity characterization. Only strains from Argentina were positive for both traits, but in this case strains from pepper and from tomato were similar. Maringoni and Kimati (1987b) found that only some strains from Brazil could hydrolyse starch, although correlation was only with host of origin and not place of origin in the few strains tested. Lange and Knosel (1970) detected strong pectin methylesterase, pectic

transeliminase, and some polygalacturonase activity in one strain (NCPPB 1616) of Xcv.

Streptomycin and copper-containing compounds have been used for many years for control of bacterial spot in probably all pepper and tomato growing areas of the world. Streptomycin resistance occurs in many bacterial species and among them the plant pathogens (Foster, 1983; Schroth et al, 1979; Stall and Thayer, 1962). Thirty-eight percent of the Xcv strains tested were resistant to streptomycin; 27% from collections and 49% of the strains from the field. In almost every group examined (except Ohio) there were some strains that were still sensitive to streptomycin. Burr et al. (1988) also found that streptomycin sensitive strains were present together with the resistant strains in apple orchards even on trees that were sprayed with streptomycin several times. Resistance to streptomycin in Xcv strains was previously reported only from Florida (Stall and Thayer, 1962), the detection of the widespread occurrence of this resistance in other parts of the world has certainly an important implication in the use of this antibiotic for control of bacterial spot.

Resistance to copper in Xcv was first reported by Marco and Stall (1983) in strains from Florida. Resistance in those strains were found to be

plasmid-encoded (Stall et al., 1986). The widespread occurrence of copper resistance in Xcv was not expected, although copper compounds are used probably everywhere for control of bacterial spot. Seventy percent of all strains tested were copper resistant and they were from every group of strains studied. As expected, most Florida field strains were copper resistant although some copper sensitive strains were found in the pepper field. In other areas surveyed (except Ohio) copper resistant was less frequent. Most surprising, a probe containing sequences responsible for copper resistance obtained from a Florida strain hybridized to plasmid DNA from copper resistant strains from every other area, suggesting homology of the copper resistance gene or genes. In one strain from Argentina two plasmid bands hybridized to the probe, raising the possibility that copper resistance may be coded for by sequences in two plasmids. In yeast copper resistance is mediated by tandem gene amplification (Fogel and Welch, 1982). In strains of Escherichia coli trimethoprim resistance was encoded for by 12 different plasmids (Kraft et al., 1983). In a limited comparison by restriction fragments analysis of some of the copper resistance plasmids polymorphism was evident. Although many of the copper resistance plasmids were found to be self-transmissible, the

polymorphism observed in restriction fragments is evidence against the possibility that copper resistance was recently aquired by conjugation mediated by the same plasmid. The possibility of copper resistance being a common feature of many soil or plant surface inhabiting bacteria can not be eliminated (Silver and Misra, 1988). Those bacteria may serve as sources of sequences coding for copper resistance (Foster, 1983).

Attempts to correlate the phenotypic traits examined with the presence of specific plasmids were fruitless. The high diversity detected in plasmids complicated the task. Even for copper resistance many strains carried plasmids similar in size to those in the resistant strains and were copper sensitive. The allocation of a determined phenotype to a plasmid in Xcv can not be performed only by examining the presence or absence of the plasmid in strains positive and negative, respecyively, for that phenotype. It may be necessary to clone the sequences first and then locate them, by hybridization analysis, to the plasmid. If plasmids are self-transmissible conjugation experiments can be performed to determine the association of the plasmid and the phenotype. The strain of Xanthomonas sp. T-55 (Stall, 1987) was an excellent recipient for conjugation experiments with the plasmids encoding copper resistance. This strain may be used to

test the possibility that other traits may be plasmid-encoded in Xcv.

The distribution of pathogenic races in the Xcv strains studied was different for each collection and field population. Different races were predominant in different areas. The data confirmed a previous report (Cook and Stall, 1982) on the distribution of pepper race 2 that is restricted to the USA. Race 1 was not found in the fields surveyed in Florida, but was very common in strains from Taiwan. Race 3 was seldom found in USA but was common in other areas of the world. Most strains isolated from tomato in any area were of the tomato race, probably indicating some kind of selective advantage for this character. It is difficult to explain why this happens since it is not known what selective advantage avirulence genes have for the bacterium.

Pathogenic races in Xcv are determined by the presence or absence of determined avirulence genes (see Chapter 2). Most of these genes have been characterized genetically and molecularly. Strains of Xcv that infect pepper but are unable to infect tomato were previously described (Reifschneideret al., 1985) in Brazil but their avirulence was not characterized. Half of the strains from Taiwan that were included in this study were of this type, as were some other

strains from the UF collection isolated previously in Florida and in other areas. These strains may be common in the field but were not easily identified previously due to the difficulty of recognizing the phenotype through artificial inoculation. The method described here, the inoculation of plants with high bacterial concentrations containing carborundum, gave excellent results in distinguishing these strains. Recognition of the HR and separation of this reaction from the susceptible one by injection-infiltration of the inoculum is very difficult and should be used only after the strains were inoculated by the carborundum method.

The reaction of the strains that do not cause disease on tomato was characterized by electrolyte leakage analysis and bacterial multiplication in host tissue. The avirulence gene responsible for this reaction was identified and isolated by cloning and it was named avrBsP. The introduction of the cloned gene in strains of several other races converted them from virulent to avirulent to tomato. The avrBsP was found to be linked to avrBs3 (Bonas et al., 1989) in many strains. Hybridization occurred with the plasmid carrying the active avrBs3 and with another plasmid carrying sequences homologous to it (Bonas et al., 1989), but the relationship between these two genes at

present is not clear. No homology was previously reported between avirulence genes of Xcv. Sequence analysis of the avrBsP may give more information on the degree of homology since the sequences of the avrBs3 are known (Bonas et al., 1989). The size of both genes is very different (avrBs3 3.7 kbp; avrBsP <1.8kbp) which suggest that, although they share some sequences they are different from each other. Why these two genes are linked in many strains is not known and further speculations are difficult to make since the products of avirulence genes are unknown and they are defined only by their reaction on the host plant. Moreover, the product and function of the resistance genes in the plant are also unknown. Each gene in either member of a host-parasite system (avirulence in the parasite, resistance in the host) may be identified only by its counterpart in the other member of the system (Flor, 1971). The identification and isolation of this other gene in Xcv, the first identified to interact with tomato, should help in understanding the relationship of this important plant pathogen with it hosts, tomato and pepper, and will help in developing better methods for control of the disease by host resistance. Control of bacterial spot by the use of resistant varieties may be the only method available due to the widespread occurrence of

resistance to copper compounds and streptomycin currently used for control of the disease.

APPENDIX

Table 1. Phenotypic traits determined in strains of Xcv of the UF collection. Abbreviations and symbols are as follows: Gr Cu= growth in copper-ammended medium; Gr st= growth in streptomycin-ammended medium; Pc ac= pectolytic activity; Sr hy= starch hydrolysis; If= reaction of tomato after inoculation by infiltration; Cb= reaction of tomato after inoculation by carborundum; ECW, 10R, 20R, 30R= reaction of different pepper lines after inoculation by infiltration; (+)= positive reaction; (i) intermediate reaction; (-)= negative reaction; (ND)= no data; (s) susceptible reaction; (P)= pathogenic reaction; (n)= non-pathogenic reaction; (x)= atypical pathogenic reaction; (HR)= hypersensitive reaction; (1) phenotype given by the avrBs1 gene; (2)= phenotype given by the avrBs2 gene; (3)= phenotype given by the avrBs3 gene; (t)= phenotype given by the tomato races.

Strain	Plasmid profile	Gr Cu	Gr st	Pc ac	Sr hy	Tomato If	Tomato Cb	Pepper ECW	Pepper 10R	Pepper 20R	Pepper 30R	Host
Xv0623	III-5	+	-	-	-	ND	p	s	s	2	s	ND
Xv0683	III-5	+	-	-	-	ND	p	s	s	2	s	ND
Xv0245	III-5	i	-	-	-	ND	n	s	s	ND	3	ND
Xv84-1	II-12	-	-	-	-	HR	n	s	s	2	3	ND
XvE-3	III-1	+	+	-	-	s	p	s	1	2	s	Tomato
Xv61-1-3	II-1	i	+	-	-	ND	p	s	1	2	s	Tomato
Xv61-38	II-1	+	+	-	-	ND	p	s	1	2	s	Tomato
Xv61-51-2	III-5	+	-	-	-	ND	p	s	1	2	s	Tomato

Table 1--continued.

Strain	Plasmid profile	Gr Cu	Gr st	Pc ac	Sr hy	Tomato If	Tomato Cb	Tomato ECW	Pepper 10R	Pepper 20R	Pepper 30R	Host
Xv61-56	II-1	-	-	-	-	ND	p	s	s	2	ND	Tomato
Xv62-2-1-5	III-4	+	-	-	-	ND	ND	s	1	2	ND	Tomato
Xv62-3	II-11	ND	ND	ND	ND	ND	ND	s	1	2	s	ND
Xv62-4	III-2	-	-	-	-	ND	ND	s	1	2	s	ND
Xv62-27	II-1	+	-	-	-	ND	ND	s	s	2	s	ND
Xv65-1	III-4	-	-	-	-	ND	ND	s	1	2	s	Tomato
Xv65-1-3	III-23	-	-	-	-	ND	ND	s	1	2	s	ND
Xv65-2	II-11	-	-	-	-	ND	p	s	1	2	s	Tomato
Xv65-3	II-4	-	-	-	-	ND	p	s	1	2	ND	Tomato
Xv65-4	II-10	-	-	-	-	ND	ND	s	ND	ND	ND	Tomato
Xv65-7	III-1	-	-	-	-	ND	p	s	1	2	ND	ND
Xv65-11	II-1	+	+	-	-	ND	p	s	s	2	s	Tomato
Xv66-10	III-4	+	-	-	-	ND	ND	t	t	t	t	Tomato
Xv67-10	III-1	+	+	-	-	ND	p	t	t	2	t	Tomato
Xv67-11	II-1	i	+	-	-	ND	p	s	s	ND	s	Tomato
Xv67-13	III-1	+	+	-	-	ND	p	t	t	2	t	Tomato
Xv67-15	I-1	-	+	-	-	ND	p	s	s	2	s	Tomato
Xv67-21	II-1	+	-	-	-	ND	p	s	1	2	ND	Tomato

Table 1--continued.

Strain	Plasmid profile	Gr Cu	Gr st	Pc ac	Sr hy	Tomato If	Tomato Cb	Tomato ECW	Pepper 10R	Pepper 20R	Pepper 30R	Host
Xv68-1	II-3	+	-	-	-	ND	p	s	s	ND	ND	Pepper
Xv68-3	II-1	+	-	-	-	ND	p	s	s	ND	ND	Tomato
Xv68-5	IV-1	+	-	-	-	ND	ND	s	1	2	s	Tomato
Xv68-7	II-1	+	-	-	-	ND	ND	s	1	2	s	Tomato
Xv68-8	III-1	-	-	-	-	ND	ND	t	t	2	t	Tomato
Xv69-1	II-7	-	-	-	-	ND	p	s	s	2	s	Pepper
Xv69-3	III-4	-	-	-	-	ND	ND	s	1	ND	ND	Pepper
Xv69-4	II-7	-	-	-	-	ND	ND	ND	ND	2	ND	Pepper
Xv69-6	III-6	-	-	-	-	ND	n	s	s	2	s	Pepper
Xv69-7	III-9	-	-	-	-	ND	n	s	s	2	s	Pepper
Xv69-8	III-6	-	-	-	-	ND	ND	s	ND	2	s	Pepper
Xv69-10	II-1	i	-	-	-	ND	p	t	1	2	t	Tomato
Xv69-11	II-3	+	-	-	-	ND	p	s	ND	2	ND	Tomato
Xv69-12	II-9	ND	ND	ND	ND	ND	ND	s	1	2	ND	ND
Xv69-13	II-2	+	+	-	-	ND	p	s	1	2	s	Tomato
Xv69-15	II-3	-	-	-	-	ND	p	s	s	2	s	Tomato
Xv69-16	II-1	-	-	-	-	ND	p	s	s	2	s	Tomato
Xv69-20	II-3	-	-	-	-	ND	p	s	ND	2	s	Pepper
Xv69-21	I-1	-	-	-	-	ND	ND	s	s	2	s	Pepper
Xv69-23	II-1	+	-	-	-	ND	p	s	1	2	s	Pepper
Xv69-24	II-3	+	-	-	-	ND	p	s	s	2	s	Pepper

Table 1--continued.

Strain	Plasmid profile	Gr Cu	Gr st	Pc ac	Sr hy	Tomato If	Tomato Cb	Tomato ECW	Pepper 10R	Pepper 20R	Pepper 30R	Host
Xv69-27	III-8	+	-	-	-	ND	p	s	s	2	s	Pepper
Xv69-28	III-1	-	-	-	-	ND	p	s	1	2	ND	Pepper
Xv69-29	III-2	+	i	-	-	ND	p	s	1	2	s	Pepper
Xv70	III-2	+	+	-	-	ND	ND	s	1	2	s	Pepper
Xv70-1	II-3	i	-	-	-	ND	p	s	1	2	s	Tomato
Xv70-2	II-2	-	-	-	-	ND	p	s	1	2	ND	Tomato
Xv70-5	II-4	-	-	-	-	ND	n	s	1	2	s	ND
Xv70-7	II-1	+	-	-	-	ND	p	s	1	2	ND	Pepper
Xv70-8	II-6	i	+	-	-	ND	ND	s	1	2	ND	Pepper
Xv70-9	III-1	-	+	-	-	ND	n	s	s	2	ND	Tomato
Xv70-10	II-2	+	+	-	-	ND	x	s	s	2	s	Tomato
Xv70-11	II-2	+	+	-	-	ND	p	s	1	2	s	Tomato
Xv71-1	II-7	-	-	-	-	HR	n	s	s	2	s	Pepper
XV71-4	II-9	+	-	i	+	ND	p	t	t	2	t	Tomato
Xv71-11	I-2	-	-	-	-	ND	ND	s	s	2	s	Tomato
Xv71-12	O	-	-	-	-	ND	ND	t	t	2	t	Tomato
Xv71-13	II-4	-	-	-	-	ND	ND	t	t	2	t	Tomato
Xv71-14	II-4	-	-	-	-	ND	p	s	s	2	s	Tomato
Xv71-17	O	-	-	ND	-	ND	p	s	s	2	s	Pepper

Table 1--continued.

Strain	Plasmid profile	Gr Cu	Gr st	Pc ac	Sr hy	Tomato If	Tomato Cb	Tomato ECW	Pepper 10R	Pepper 20R	Pepper 30R	Host
Xv71-19	II-5	i	-	ND	-	ND	p	s	s	2	s	Pepper
Xv71-21	I-2	-	-	-	-	HR	n	s	s	2	3	Pepper
Xv71-26	I-1	-	-	-	-	ND	p	s	s	2	s	Tomato
Xv71-30	II-1	+	-	-	-	ND	p	s	s	2	s	Tomato
Xv71-31	III-25	+	-	-	-	ND	p	s	ND	2	s	Tomato
Xv71-32	II-3	+	-	-	-	ND	p	s	1	2	ND	Tomato
Xv71-34	III-2	i	+	-	-	ND	p	s	1	2	s	Pepper
Xv71-35	III-2	-	-	-	-	ND	ND	s	1	2	s	Tomato
Xv71-37	II-1	+	-	-	-	ND	ND	s	1	2	ND	Tomato
Xv71-38	II-2	+	-	-	-	ND	ND	s	1	2	ND	Tomato
Xv72-1	II-9	-	-	-	-	ND	ND	t	1	2	t	Tomato
Xv72-5	II-14	+	-	-	-	ND	ND	s	1	2	ND	Tomato
Xv72-6	II-1	+	-	-	-	ND	p	s	1	2	ND	Pepper
Xv72-9	III-2	+	+	-	-	ND	ND	s	s	2	3	Pepper
Xv73-1	II-4	+	+	-	-	ND	ND	s	1	2	s	Tomato
Xv73-2	I-3	+	-	-	-	ND	p	s	1	2	s	Tomato
Xv73-3	I-5	+	-	-	-	ND	ND	s	1	2	s	Tomato
Xv73-5	II-6	+	-	-	-	ND	p	t	1	t	t	Tomato
Xv73-6	II-1	-	-	-	-	ND	ND	s	1	2	s	Tomato
Xv73-7	III-1	+	-	-	-	s	p	s	s	2	s	Pepper

Table 1--continued.

Strain	Plasmid profile	Gr st Cu	Gr ac	Pc hy	Sr	Tomato If	Tomato Cb	Tomato ECW	Pepper 10R	Pepper 20R	Pepper 30R	Host
Xv75-3	III-3	+	-	-	-	s	p	t	1	t	t	Tomato
Xv75-4	III-3	+	-	-	-	s	p	t	1	t	t	Tomato
Xv76-1	II-2	i	+	-	-	ND	p	s	1	2	s	Pepper
Xv76-1A	II-1	+	i	-	-	ND	ND	s	1	2	s	ND
Xv76-2n	I-1	-	+	-	-	ND	p	s	ND	2	s	Pepper
Xv76-3	II-3	-	+	-	-	ND	p	s	1	2	s	Tomato
Xv76-4R	III-12	-	+	-	-	ND	p	s	s	2	s	Pepper
Xv76-10	II-1	+	-	-	-	ND	p	s	1	2	ND	ND
Xv76-11	III-1	+	-	-	-	ND	p	s	1	2	ND	ND
Xv77-2	II-1	+	+	-	-	ND	p	s	1	2	ND	ND
Xv77-3	I-3	+	+	-	-	ND	n	s	1	2	s	Pepper
Xv77-8	II-2	+	+	-	-	ND	p	s	1	2	s	Tomato
Xv77-12	III-1	+	-	-	-	ND	p	s	1	2	s	Tomato
Xv78-1	I-1	-	-	-	-	ND	n	s	s	ND	s	ND
Xv78-2	III-1	+	-	-	-	ND	p	s	1	2	s	ND
Xv78-3	II-2	i	+	-	-	ND	ND	s	1	2	s	ND
Xv79-2	III-11	+	+	+	+	s	x	t	t	t	t	Tomato
Xv79-4	III-1	+	-	-	-	ND	p	t	1	2	t	ND
Xv79-5	II-1	+	-	-	-	ND	p	s	1	2	s	ND
Xv80-1	III-1	+	-	-	-	ND	p	s	1	ND	ND	Tomato
Xv80-5	II-13	-	-	-	-	ND	ND	s	s	2	ND	Pepper
Xv80-6	I-2	-	-	-	-	ND	p	s	s	2	s	Pepper
Xv80-7	III-1	+	-	-	-	ND	p	s	1	2	s	Tomato

Table 1--continued.

Strain	Plasmid profile	Gr Cu	Gr st	Pc ac	Sr hy	Tomato If	Cb	ECW	Pepper 10R	20R	30R	Host
Xv80-11	II-2	ND	ND	ND	ND	ND	p	s	1	2	s	Tomato
Xv80-12	III-2	i	-	-	-	ND	p	s	1	2	s	ND
Xv81-1	II-1	+	+	-	-	ND	p	s	s	2	s	Pepper
Xv81-3	II-1	+	+	-	-	ND	p	s	s	2	s	Pepper
Xv81-4	II-8	-	-	-	-	ND	p	s	s	2	3	Pepper
Xv81-5	II-8	-	-	-	-	ND	p	s	s	2	3	Pepper
Xv81-6	O	-	-	+	+	s	x	s	s	2	s	ND
Xv81-8	II-5	-	-	-	-	ND	p	t	1	t	t	Tomato
Xv81-9	II-2	+	+	-	-	ND	ND	s	s	2	s	Tomato
Xv81-12	III-1	+	-	-	-	ND	p	s	s	2	s	Pepper
Xv81-17	II-17	-	-	+	+	ND	ND	s	s	2	s	Tomato
Xv81-18	III-1	+	-	-	-	ND	p	t	1	t	t	Pepper
Xv81-19	III-3	+	-	-	-	ND	p	s	s	2	s	ND
Xv81-21	III-2	+	-	-	-	ND	p	s	s	2	s	Tomato
Xv81-22	I-2	-	-	-	-	ND	p	s	s	2	s	Tomato
Xv81-23	III-3	+	-	-	-	s	p	s	1	2	s	Tomato
Xv82-1	IV-2	+	-	-	-	ND	ND	s	s	2	s	Pepper
Xv82-3	II-1	+	-	-	-	ND	ND	s	1	2	s	Pepper
Xv82-4n	I-2	-	-	-	-	HR	n	s	s	2	3	Pepper

Table 1--continued.

Strain	Plasmid profile	Gr Cu	Gr st	Pc ac	Sr hy	Tomato If	Tomato Cb	Pepper ECW	Pepper 10R	Pepper 20R	Pepper 30R	Host
Xv82-4	0	-	-	-	-	ND	ND	s	s	2	s	Pepper
Xv82-5	III-27	+	-	-	-	ND	ND	s	s	2	s	Pepper
Xv82-7	II-14	-	-	-	-	ND	ND	s	1	2	s	Pepper
Xv82-8	III-28	-	-	-	-	HR	n	s	s	2	3	Pepper
Xv82-8L	II-12	-	-	-	-	ND	ND	s	s	2	s	Pepper
Xv82-9	I-4	-	-	-	-	ND	p	s	s	2	s	Pepper
Xv83-3	0	-	-	-	-	ND	p	s	s	2	s	Tomato
Xv83-5	II-2	+	-	-	-	ND	p	s	1	2	s	ND
Xv83-12	II-2	+	+	-	-	ND	p	s	1	2	s	Tomato
Xv83-13	II-2	+	-	-	-	ND	p	s	1	2	s	Tomato
Xv83-14	II-2	+	-	-	-	ND	p	s	1	2	ND	Tomato
Xv84-10	II-2	+	+	-	-	ND	p	s	1	2	ND	ND
Xv84-11	II-2	+	+	-	-	ND	p	s	1	2	s	ND
Xv85-1	II-1	+	+	-	-	ND	p	s	1	2	s	Pepper
Xv85-2	II-3	-	+	-	-	ND	p	s	1	2	ND	Tomato
Xv85-4	II-2	+	+	-	-	ND	p	s	1	2	ND	Tomato
Xv85-5	0	-	-	-	-	ND	p	s	s	2	s	Tomato
Xv85-6	III-1	+	+	-	-	ND	p	ND	1	2	ND	Pepper
Xv85-7	II-1	+	-	-	-	ND	p	s	s	2	s	Pepper
Xv85-8	II-2	+	+	-	-	ND	p	ND	1	2	ND	Pepper

Table 1--continued.

Strain	Plasmid profile	Gr Cu	Gr st	Pc ac	Sr hy	Tomato If	Tomato Cb	Tomato ECW	Pepper 10R	Pepper 20R	Pepper 30R	Host
Xv85-9	II-4	-	+	-	-	ND	p	s	1	2	s	Pepper
Xv85-10	II-4	-	+	-	-	s	ND	s	1	2	s	Pepper
Xv85-11	I-2	-	-	-	-	ND	n	s	s	2	s	Pepper
Xv85-12	IV-1	+	-	-	-	ND	p	s	1	2	s	Pepper
Xv85-13	II-4	-	+	-	-	ND	p	t	1	2	t	Tomato
Xv85-14	IV-1	+	+	-	-	ND	p	t	1	2	t	Tomato
Xv85-15	II-5	-	+	-	-	ND	p	s	1	2	s	Tomato
Xv86-1	II-1	i	+	-	-	ND	p	s	1	2	s	Tomato
Xv86-2	II-2	+	+	-	-	ND	p	s	s	2	s	Tomato
Xv86-12	IV-1	+	-	-	-	ND	ND	s	1	2	s	Tomato
Xv86-13	II-4	i	+	-	-	ND	ND	s	1	2	s	Tomato
Xv86-16	II-4	+	+	-	-	ND	ND	t	1	t	t	ND
Xv86-22	I-3	-	+	-	-	ND	ND	s	1	2	s	Pepper
Xv86-23	II-1	+	+	-	-	ND	ND	s	1	2	s	Pepper
Xv86-24	II-1	-	+	-	-	ND	ND	s	1	2	s	Pepper
Xv86-25	III-24	-	+	-	-	ND	ND	s	1	2	s	Pepper
Xv86-26	III-3	+	+	-	-	ND	p	s	1	2	ND	Tomato
Xv86-27	II-1	-	+	-	-	ND	p	s	1	2	s	Tomato

Table 1--continued.

Strain	Plasmid profile	Gr Cu	Gr st	Pc ac	Sr hy	Tomato If	Tomato Cb	ECW	Pepper 10R	Pepper 20R	Pepper 30R	Host
Xv86-29	II-2	+	-	-	-	ND	p	s	1	2	s	Tomato
Xv86-30	III-1	i	-	-	-	ND	ND	s	1	2	s	Tomato
Xv86-31	III-2	i	-	-	-	ND	p	s	1	2	s	Tomato
Xv86-32	III-10	i	-	-	-	ND	p	s	1	2	s	Tomato
Xv86-33	II-5	+	+	-	-	ND	p	s	1	2	s	Pepper
Xv86-34	II-5	+	+	-	-	ND	p	s	1	2	s	Pepper
Xv86-35	II-2	+	+	-	-	ND	p	s	1	2	s	Pepper
Xv86-36	IV-4	+	+	-	-	ND	p	s	1	2	s	Pepper
Xv86-37	IV-1	+	-	-	-	ND	ND	s	1	2	s	Pepper
Xv86-38	IV-1	+	-	-	-	ND	n	s	1	2	s	Pepper
Xv86-39	IV-1	+	-	-	ND	ND	ND	s	1	2	s	Pepper
Xv86-40	IV-1	+	-	-	ND	ND	ND	s	1	2	s	Pepper
Xv86-41	IV-1	+	-	-	ND	ND	p	s	1	2	s	Pepper
Xv86-42	IV-1	+	-	-	ND	ND	p	s	1	2	s	Pepper
Xv86-43	III-7	+	-	-	ND	ND	ND	s	1	2	s	Pepper
Xv86-44	III-7	+	-	-	-	ND	ND	s	1	2	s	Pepper
Xv86-46	II-1	+	+	-	-	ND	n	s	1	2	s	Pepper
Xv86-51	II-5	+	-	-	-	ND	ND	s	s	2	s	Pepper

Table 1--continued.

Strain	Plasmid profile	Gr Cu	Gr st	Pc ac	Sr hy	Tomato If	Tomato Cb	Pepper ECW	Pepper 10R	Pepper 20R	Pepper 30R	Host
Xv86-59	II-1	+	+	-	-	ND	ND	s	s	2	s	Pepper
Xv86-61	IV-3	+	-	-	-	HR	n	s	s	2	3	Pepper
Xv86-69	IV-1	i	-	-	-	HR	n	s	s	2	3	Pepper
Xv86-72	III-3	+	+	-	-	ND	p	s	1	2	s	Pepper
Xv86-79	II-2	+	+	-	-	ND	p	s	1	2	s	Pepper
Xv86-87	II-2	+	+	-	-	ND	p	s	1	2	s	Pepper
Xv86-97	III-1	+	-	-	-	ND	p	s	1	2	s	Pepper
Xv87-2	IV-6	+	-	-	-	s	p	s	1	2	s	Pepper
Xv87-3	IV-2	+	-	-	-	HR	n	s	s	2	3	Pepper
Xv87-4	IV-2	+	-	-	-	HR	n	s	s	2	3	Pepper
Xv87-5	IV-2	+	-	-	-	HR	n	s	s	2	3	Pepper
Xv87-6	IV-1	+	-	-	-	HR	n	s	s	2	3	Pepper
Xv87-7	IV-2	+	-	-	-	HR	n	s	s	2	3	Pepper
Xv87-8	IV-2	+	-	-	-	HR	n	s	s	2	3	Pepper
Xv87-9	IV-3	+	-	-	-	HR	n	s	s	2	3	Pepper
Xv87-10	IV-1	+	-	-	-	ND	ND	s	1	2	s	Pepper
Xv87-11	IV-1	+	-	-	-	ND	p	s	1	2	s	Pepper
Xv87-12	IV-1	+	-	-	-	ND	p	s	1	2	s	Pepper

Table 1--continued.

Strain	Plasmid profile	Gr Cu	Gr st	Pc ac	Sr hy	Tomato If	Tomato Cb	Tomato ECW	Pepper 10R	Pepper 20R	Pepper 30R	Host
Xv87-13	IV-1	-	-	-	-	ND	p	s	1	2	s	Pepper
Xv87-13c	IV-6	-	-	-	-	ND	ND	s	s	2	s	Pepper
Xv87-13g	IV-6	+	-	-	-	ND	p	s	1	2	s	Pepper
Xv87-14	II-5	i	+	-	-	ND	p	s	1	2	s	Tomato
Xv87-15	II-5	i	+	-	-	ND	p	s	1	2	s	Tomato
Xv87-16	II-5	-	+	-	-	ND	p	s	1	2	s	Tomato
Xv87-17	II-5	+	+	-	-	ND	p	s	1	2	s	Tomato
Xv87-18	II-5	i	+	-	-	ND	p	s	1	2	s	Tomato
Xv87-19	I-3	+	+	-	-	ND	ND	s	1	2	s	Tomato
Xv87-20	III-3	+	+	-	-	ND	ND	s	1	2	s	Tomato
Xv87-21	I-1	-	-	-	-	ND	n	s	s	2	s	ND
Xv87-22	I-1	-	-	-	-	ND	ND	s	s	2	s	ND
Xv87-23	I-1	-	-	-	-	ND	ND	s	s	2	s	ND
Xv87-24	II-1	+	+	-	-	ND	p	s	s	2	3	ND
Xv87-25	II-1	+	+	-	-	ND	p	s	s	2	3	ND
Xv87-26	II-1	+	+	-	-	ND	p	s	s	2	3	ND
Xv-3	O	-	-	-	-	ND	n	s	s	2	s	ND
Xv-56	I-3	+	+	+	+	s	p	t	t	t	t	Tomato
Xv88-5	I-3	-	ND	-	-	s	p	s	s	2	s	Pepper

Table 2. Phenotypic traits determined in strains of Xcv of the Taiwan collection. All abbreviations and symbols are as in Table 1.

Strain	Plasmid profile	Gr Cu	Gr st	Pt ac	Sr hy	Tomato If	Cb	Pepper ECW	10R	20R	30R	Host
T87-27	I-6	-	-	-	-	-	p	+	+	+	+	Tomato
T87-28	O	-	-	-	-	-	ND	-	-	+	-	Pepper
T87-29	I-7	-	-	-	-	-	p	+	+	+	+	Tomato
T87-30	III-12	-	-	-	-	+	n	-	-	+	-	Pepper
T87-31	I-6	-	-	-	-	-	p	+	+	+	+	Tomato
T87-32	I-6	-	-	-	-	-	p	+	+	+	+	Tomato
T87-33	II-8	-	-	-	-	-	p	+	+	+	+	Tomato
T87-34	II-8	-	-	-	-	-	p	+	+	+	+	Tomato
T87-35	IV-5	-	-	-	-	+	n	-	-	+	-	Pepper
T87-37	IV-6	-	-	-	-	-	ND	-	-	+	-	Pepper
T87-38	IV-2	+	-	-	-	+	n	-	-	+	+	Pepper
T87-40	IV-2	+	-	-	-	+	n	-	-	+	+	Pepper
T87-41	II-12	-	-	-	-	+	n	-	-	+	+	Pepper
T87-42	IV-2	+	-	-	-	+	n	-	-	+	+	Pepper
T87-44	IV-6	-	-	-	-	+	n	-	-	+	-	Pepper
T87-45	IV-6	-	-	-	-	+	n	-	-	+	-	Pepper
T87-46	IV-2	+	-	-	-	+	n	-	-	+	+	Pepper

Table 2--continued.

Strain	Plasmid profile	Gr Cu	Gr st	Pt ac	Sr hy	Tomato If	Cb	Pepper ECW	10R	20R	30R	Host
T87-47	O	-	-	-	-	-	p	-	-	+	-	Pepper
T87-48	III-1	+	+	-	-	-	p	-	-	+	-	Pepper
T87-49	O	-	-	-	-	-	ND	-	-	+	-	Tomato
T87-50	IV-3	+	-	-	-	+	n	-	-	+	+	Pepper
T87-51	I-1	-	-	-	-	-	ND	-	-	+	-	Pepper
T87-56	IV-3	+	-	-	-	+	n	-	-	+	+	Pepper
T87-58	III-13	-	-	-	-	-	ND	-	-	+	-	Pepper
T87-61	IV-3	+	-	-	-	+	n	-	-	+	+	Pepper
T87-62	III-14	-	+	-	-	-	p	-	-	+	-	Pepper
T87-66	IV-3	+	-	-	-	+	ND	-	-	+	+	Pepper
T87-67	IV-7	-	-	-	-	+	n	-	-	+	-	Pepper

Table 3. Phenotypic traits determined in strains of Xcv of the BA collection. All abbreviations and symbols are as in Table 1.

Strain	Plasmid profile	Gr Cu	Gr St	Pt ac	Sr hy	Tomato If	Tomato Cb	Pepper ECW	Pepper 10R	HR 20R	HR 30R	Host
BA21-1	II-6	-	-	+	+	s	x	-	-	+	-	Pepper
BA21-2	II-6	-	-	+	+	ND	ND	-	-	+	-	Pepper
BA21-3	II-6	-	-	+	+	ND	ND	-	-	+	-	Pepper
BA21-4	II-6	-	-	+	+	ND	ND	-	-	+	-	Pepper
BA23-1	II-6	-	-	+	+	s	x	-	-	+	-	Tomato
BA23-2	II-6	-	-	+	+	ND	ND	-	-	+	-	Tomato
BA23-3	II-6	-	-	+	+	ND	ND	-	-	+	-	Tomato
BA23-4	II-6	-	-	+	+	ND	ND	-	-	+	-	Tomato
BA24-1	II-6	-	-	+	+	s	x	-	-	+	-	Tomato
BA24-2	II-6	-	-	+	+	ND	ND	-	-	+	-	Tomato
BA24-3	II-6	-	-	+	+	ND	ND	-	-	+	-	Tomato
BA24-4	II-6	-	-	+	+	ND	ND	-	-	+	-	Tomato
BA26-1	II-8	-	-	-	-	HR	n	-	-	+	+	Pepper
BA26-2	II-8	-	-	-	-	HR	n	-	-	+	+	Pepper
BA26-3	II-6	i	-	i	+	HR	n	-	-	+	+	Pepper
BA26-4	II-8	i	-	i	-	ND	ND	-	-	+	+	Pepper

Table 3--continued.

Strain	Plasmid profile	Gr Cu	Gr St	Pt ac	Sr hy	Tomato If	Tomato Cb	Pepper ECW	Pepper 10R	HR 20R	HR 30R	Host
BA27-1	II-6	-	-	+	+	s	x	-	-	+	-	Pepper
BA27-2	II-6	-	-	+	+	ND	ND	-	-	+	-	Pepper
BA27-3	II-6	-	-	+	+	ND	ND	-	-	+	-	Pepper
BA27-4	II-6	-	-	+	+	ND	ND	-	-	+	-	Pepper
BA28-1	II-6	-	-	+	+	s	x	-	-	+	-	Tomato
BA28-2	II-6	-	-	+	+	ND	ND	-	-	+	-	Tomato
BA28-3	II-6	-	-	+	+	ND	ND	-	-	+	-	Tomato
BA28-4	II-6	-	-	+	+	ND	ND	-	-	+	-	Tomato
BA29-1	I-8	i	-	+	+	s	x	-	-	+	-	Tomato
BA29-2	I-8	i	-	+	+	ND	ND	-	-	+	-	Tomato
BA29-3	I-8	i	-	+	+	ND	ND	-	-	+	-	Tomato
BA29-4	I-8	i	-	+	+	ND	ND	-	-	+	-	Tomato
BA30-1	II-1	+	-	-	-	s	p	-	-	+	-	Tomato
BA30-2	II-1	+	-	-	-	s	p	-	-	+	-	Tomato
BA30-3	II-1	+	-	-	-	s	p	-	-	+	-	Tomato
BA30-4	II-1	+	-	-	-	s	p	-	-	+	-	Tomato

Table 4. Phenotypic traits determined in strains of Xcv of the BV tomato field. All abbreviations and symbols are as in Table 1.

Strain	Plasmid profile	Gr Cu	Gr St	Pt ac	Sr hy	Tomato carb.	Pepper ECW	HR 10R	HR 20R	HR 30R
BV1-1a	I-9	-	-	+	+	x	+	+	+	+
BV1-2a	I-9	-	-	+	+	x	+	+	+	+
BV1-3a	I-9	-	-	+	+	x	+	+	+	+
BV1-3b	ND	-	-	+	+	x	+	+	+	+
BV1-4a	ND	-	-	+	+	x	+	+	+	+
BV1-5a	ND	-	-	+	+	x	+	+	+	+
BV1-5b	I-9	-	-	+	+	x	+	+	+	+
BV3-1a	III-15	+	+	+	+	x	+	+	+	+
BV3-1b	ND	+	+	+	+	x	+	+	+	+
BV3-3a	III-15	+	+	+	+	x	+	+	+	+
BV3-3b	ND	+	+	+	+	x	+	+	+	+
BV3-4	I-9	-	-	+	+	x	+	+	+	+
BV3-5	III-16	+	+	+	+	x	+	+	+	+
BV4-1a	I-9	-	-	+	+	x	+	+	+	+
BV4-1b	ND	-	-	+	+	x	+	+	+	+

Table 4--continued.

Strain	Plasmid profile	Gr Cu	Gr St	Pt ac	Sr hy	Tomato carb.	Pepper ECW	HR 10R	HR 20R	HR 30R
BV4-2a	I-9	-	-	+	+	x	+	+	+	+
BV4-2b	ND	-	-	+	+	x	+	+	+	+
BV4-3	I-9	-	-	+	+	x	+	+	+	+
BV4-4a	ND	-	-	+	+	x	+	+	+	+
BV4-4b	ND	-	-	+	+	x	+	+	+	+
BV4-5a	I-9	-	-	+	+	x	+	+	+	+
BV4-5b	ND	-	-	+	+	x	+	+	+	+
BV5-1a	III-16	+	+	+	+	x	+	+	+	+
BV5-1b	ND	+	+	+	+	x	+	+	+	+
BV5-1c	ND	+	+	+	+	x	+	+	+	+
BV5-2a	ND	+	+	+	+	x	+	+	+	+
BV5-2b	ND	+	+	+	+	x	+	+	+	+
BV5-2c	ND	+	+	+	+	x	+	+	+	+
BV5-3a	III-17	+	+	+	+	x	+	+	+	+
BV5-3b	III-17	+	+	-	+	ND	+	+	+	+
BV5-4a	III-18	+	+	+	+	x	+	+	+	+
BV5-4b	III-18	+	+	-	+	ND	+	+	+	+
BV5-5a	I-9	-	-	+	+	x	+	+	+	+
BV5-5b	ND	-	-	+	+	x	+	+	+	+
BV6-1a	I-9	-	-	+	+	x	+	+	+	+

Table 4--continued.

Strain	Plasmid profile	Gr Cu	Gr St	Pt ac	Sr hy	Tomato carb.	Pepper HR ECW	10R	20R	30R
BV6-2	I-9	-	-	+	+	x	+	+	+	+
BV6-3a	I-9	-	-	+	+	x	+	+	+	+
BV6-3b	ND	-	-	+	+	x	+	+	+	+
BV6-4a	I-9	-	-	+	+	x	+	+	+	+
BV6-4b	ND	-	-	+	+	x	+	+	+	+
BV6-5a	I-9	-	-	+	+	x	+	+	+	+
BV6-5b	ND	-	-	+	+	x	+	+	+	+
BV7-1a	III-18	+	+	+	+	x	+	+	+	+
BV7-1b	ND	+	+	+	+	x	+	+	+	+
BV7-2	III-18	+	+	+	+	x	+	+	+	+
BV7-3a	III-18	+	+	+	+	x	+	+	+	+
BV7-3b	III-18	+	+	-	+	ND	+	+	+	+
BV7-4a	III-18	+	+	+	+	x	+	+	+	+
BV7-4b	ND	+	+	+	+	x	+	+	+	+
BV7-4c	ND	ND	ND	ND	+	ND	+	+	+	+
BV7-5a	III-18	+	+	+	+	x	+	+	+	+
BV8-1a	I-9	-	-	+	+	x	+	+	+	+
BV8-1b	ND	-	-	+	+	x	+	+	+	+

Table 4--continued.

Strain	Plasmid profile	Gr Cu	Gr St	Pt ac	Sr hy	Tomato carb.	Pepper ECW	HR 10R	HR 20R	HR 30R
BV8-2	I-9	-	-	+	+	x	+	+	+	+
BV8-3	I-9	-	-	+	+	x	+	+	+	+
BV8-4a	I-9	-	-	+	+	x	+	+	+	+
BV8-4b	ND	-	-	+	+	x	+	+	+	+
BV8-5a	I-9	-	-	+	+	x	+	+	+	+
BV11-1a	III-16	+	+	+	+	x	+	+	+	+
BV11-2a	III-16	+	+	+	+	x	+	+	+	+
BV11-3a	III-16	+	+	+	+	x	+	+	+	+
BV11-4a	III-16	+	+	+	+	x	+	+	+	+
BV11-5a	III-16	+	+	+	+	x	+	+	+	+
BV11-5b	II-5	+	+	+	+	x	+	+	+	+
BV14-1a	ND	+	+	+	+	x	+	+	+	+
BV14-2a	III-16	+	+	+	+	x	+	+	+	+
BV14-2b	ND	+	+	+	+	x	+	+	+	+
BV14-2c	II-6	+	+	-	+	ND	+	+	+	+
BV14-3a	III-16	+	+	+	+	x	+	+	+	+
BV14-4a	III-16	+	+	+	+	x	+	+	+	+
BV14-4b	ND	+	+	+	+	x	+	+	+	+
BV14-4c	ND	+	+	+	+	x	+	+	+	+
BV14-4d	ND	+	+	+	+	x	+	+	+	+

Table 4--continued.

Strain	Plasmid profile	Gr Cu	Gr St	Pt ac	Sr hy	Tomato carb.	Pepper HR ECW	10R	20R	30R
BV14-5a	III-16	+	+	+	+	x	+	+	+	+
BV14-5b	ND	+	+	+	+	x	+	+	+	+
BV20-1a	ND	-	-	+	+	x	+	+	+	+
BV20-1b	ND	-	-	+	+	x	+	+	+	+
BV20-2a	I-9	-	-	+	+	x	+	+	+	+
BV20-2b	ND	-	-	+	+	x	+	+	+	+
BV20-2c	ND	-	-	+	+	x	+	+	+	+
BV20-3a	I-9	-	-	-	+	ND	+	+	+	+
BV20-3b	I-9	-	-	-	+	ND	+	+	+	+
BV20-4a	I-9	-	-	+	+	x	+	+	+	+
BV20-4b	ND	-	-	+	+	x	+	+	+	+
BV20-5a	I-9	-	-	+	+	x	+	+	+	+
BV20-5b	ND	-	-	+	+	x	+	+	+	+

Table 5. Phenotypic traits determined in strains of Xcv of the Ohio tomato field. All abbreviations and symbols are as in Table 1.

Strain	Plasmid	Gr Cu	Gr st	Pc ac	Sr hy	Pepper ECW	10R	20R	30R
Oh-1	III-30	i	+	-	-	s	1	2	s
Oh-2	II-4	i	+	-	-	s	1	2	s
Oh-4	II-5	+	+	-	-	t	1	2	t
Oh-5	II-5	+	+	-	-	t	1	2	t
Oh-6	II-5	+	+	-	-	s	1	2	s
Oh-7	II-4	i	+	-	-	s	1	2	s
Oh-8	II-5	+	+	-	-	t	1	2	t
Oh-9	III-1	+	+	-	-	t	1	2	t
Oh-10	III-1	+	+	-	-	t	1	2	t
Oh-11	II-5	+	+	-	-	s	1	2	s
Oh-12	II-5	-	+	-	-	s	1	2	s
Oh-13	II-5	+	+	-	-	t	1	2	t
Oh-14	II-4	+	+	-	-	t	1	2	t
Oh-15	II-5	i	+	-	-	s	1	2	s
Oh-16	III-30	i	+	-	-	s	1	2	s
Oh-17	ND	i	+	-	-	s	1	2	s

Table 6. Phenotypic traits determined in strains of Xcv of the Florida tomato field. All abbreviations and symbols are as in Table 1.

Strain	Plasmid profile	Gr Cu	Gr st	Pt ac	Sr hy	Pepper HR ECW	10R	20R	30R
TA-1	III-19	+	+	-	-	+	+	+	+
TA-2	III-19	+	+	-	-	+	+	+	+
TA-3	II-1	+	+	-	-	+	+	+	+
TA-4	III-19	+	+	-	-	+	+	+	+
TA-5	II-4	+	+	-	-	+	+	+	+
TA-6	II-4	+	+	-	-	+	+	+	+
TA-7	II-4	+	+	-	-	+	+	+	+
TA-8	III-19	+	+	-	-	+	+	+	+
TA-9	III-19	+	+	-	-	+	+	+	+
TA-10	III-20	+	+	-	-	+	+	+	+
TA-11	II-1	+	+	-	-	+	+	+	+
TA-12	II-1	+	+	-	-	+	+	+	+
TA-13	II-1	+	+	-	-	+	+	+	+
TA-14	III-3	+	+	-	-	+	+	+	+
TA-15	III-3	+	-	-	-	+	+	+	+

Table 6--continued.

Strain	Plasmid profile	Gr Cu	Gr st	Pt ac	Sr hy	Pepper HR ECW	10R	20R	30R
TA-16	III-3	+	-	-	-	+	+	+	+
TA-17	III-21	+	-	-	-	+	+	+	+
TA-18	III-26	+	+	-	-	+	+	+	+
TB-1	II-4	+	+	-	-	+	+	+	+
TB-3	II-4	+	+	-	-	+	+	+	+
TB-4	III-3	+	-	-	-	+	+	+	+
TB-5	II-4	+	+	-	-	+	+	+	+
TB-6	II-4	+	+	-	-	+	+	+	+
TB-7	II-4	+	+	-	-	+	+	+	+
TB-8	II-4	+	+	-	-	+	+	+	+
TB-9	III-20	+	+	-	-	+	+	+	+
TB-10	II-4	+	+	-	-	+	+	+	+
TB-11	III-17	+	+	-	-	+	+	+	+
TB-12	III-17	+	+	-	-	+	+	+	+
TB-14	III-3	+	-	-	-	+	+	+	+
TB-15	III-20	+	+	-	-	+	+	+	+
TB-16	III-20	+	+	-	-	+	+	+	+
TB-17	III-23	+	+	-	-	+	+	+	+
TB-18	II-4	+	+	-	-	+	+	+	+
TB-19	III-19	+	+	-	-	+	+	+	+

Table 6--continued.

Strain	Plasmid profile	Gr Cu	Gr st	Pt ac	Sr hy	Pepper ECW	Pepper 10R	Pepper 20R	Pepper 30R
TB-20	II-4	+	+	-	-	+	+	+	+
TB-21	II-4	+	+	-	-	+	+	+	+
TB-22	II-4	+	+	-	-	+	+	+	+
TC-1	II-4	+	+	-	-	+	+	+	+
TC-2	II-4	+	+	-	-	+	+	+	+
TC-3	III-20	+	+	-	-	+	+	+	+
TC-4	III-20	+	+	-	-	+	+	+	+
TC-5	III-17	+	+	-	-	+	+	+	+
TC-7	III-19	+	+	-	-	+	+	+	+
TC-8	II-4	+	+	-	-	+	+	+	+
TC-9	II-4	+	+	-	-	+	+	+	+
TC-10	II-4	+	+	-	-	+	+	+	+
TC-11	III-20	+	+	-	-	+	+	+	+
TC-12	III-20	+	+	-	-	+	+	+	+
TC-13	III-20	+	+	-	-	+	+	+	+
TC-15	IV-8	+	+	-	-	+	+	+	+
TC-16	III-20	+	+	-	-	+	+	+	+

Table 6--continued.

Strain	Plasmid profile	Gr Cu	Gr st	Pt ac	Sr hy	Pepper ECW	HR 10R	HR 20R	HR 30R
TD-1	III-20	+	+	-	-	+	+	+	+
TD-2	III-20	+	+	-	-	+	+	+	+
TD-3	II-4	+	+	-	-	+	+	+	+
TD-4	III-23	+	+	-	-	+	+	+	+
TD-5	III-23	+	+	-	-	+	+	+	+
TD-6	III-23	+	+	-	-	+	+	+	+
TD-7	III-20	+	+	-	-	+	+	+	+
TD-8	III-20	+	+	-	-	+	+	+	+
TD-9	II-1	+	+	-	-	+	+	+	+
TD-10	II-4	+	+	-	-	+	+	+	+
TD-11	II-4	+	+	-	-	+	+	+	+
TD-12	III-20	+	+	-	-	+	+	+	+
TD-13	II-4	+	+	-	-	+	+	+	+
TD-14	II-4	+	+	-	-	+	+	+	+
TD-15	III-20	+	+	-	-	+	+	+	+
TD-16	II-4	+	+	-	-	+	+	+	+
TE-1	III-20	+	+	-	-	+	+	+	+
TE-3	II-4	+	+	-	-	+	+	+	+

Table 6--continued.

Strain	Plasmid profile	Gr Cu	Gr st	Pt ac	Sr hy	Pepper ECW	HR 10R	HR 20R	HR 30R
TE-5	III-20	+	+	-	-	+	+	+	+
TE-6	III-1	+	-	-	-	+	+	+	+
TE-7	III-20	+	+	-	-	-	+	+	-
TE-8	II-4	+	-	-	-	+	+	+	+
TE-9	III-20	+	+	-	-	+	+	+	+
TE-10	II-4	+	+	-	-	+	+	+	+
TE-11	II-4	+	+	-	-	+	+	+	+
TE-12	II-4	+	+	-	-	+	+	+	+
TE-13	II-4	i	+	-	-	-	+	+	-
TE-14	III-20	+	+	-	-	+	+	+	+
TE-15	III-21	+	+	-	-	+	+	+	+
TE-16	II-4	+	+	-	-	+	+	+	+
TE-17	II-4	+	+	-	-	+	+	+	+
TE-18	II-1	+	+	-	-	+	+	+	+

Table 7. Phenotypic traits determined in strains of the Florida pepper field. All abbreviations and symbols are as in Table 1.

Strain	Plasmid profile	Gr Cu	Gr st	Pt ac	Sr hy	Pepper ECW	HR 10R	HR 20R	HR 30R
PA-1	IV-9	+	-	-	-	-	+	+	-
PA-3	IV-6	-	-	-	-	-	-	+	-
PA-4	IV-10	+	-	-	-	-	+	+	-
PA-5	IV-10	+	-	-	-	-	+	+	-
PA-6	IV-10	+	-	-	-	-	+	+	-
PA-7	II-16	+	-	-	-	-	+	+	-
PA-8	IV-10	+	-	-	-	-	+	+	-
PA-9	IV-10	+	-	-	-	-	+	+	-
PA-10	IV-10	+	-	-	-	-	+	+	-
PA-11	IV-9	+	-	-	-	-	+	+	-
PA-12	IV-10	+	-	-	-	-	+	+	-
PA-13	IV-10	+	-	-	-	-	+	+	-
PA-14	IV-10	+	-	-	-	-	+	+	-
PA-15	IV-10	+	-	-	-	-	+	+	-
PA-16	IV-10	+	-	-	-	-	+	+	-
PA-17	IV-9	+	-	-	-	-	+	+	-

Table 7--continued.

Strain	Plasmid profile	Gr Cu	Gr st	Pt ac	Sr hy	Pepper ECW	HR 10R	HR 20R	HR 30R
PA-18	IV-10	+	-	-	-	-	+	+	-
PA-19	IV-10	+	-	-	-	-	+	+	-
PA-20	III-19	+	-	-	-	-	+	+	-
PA-21	IV-10	+	-	-	-	-	+	+	-
PA-22	IV-6	-	-	-	-	-	-	+	-
PA-23	III-3	i	+	-	-	-	+	+	-
PA-24	IV-6	-	-	-	-	-	-	+	-
PB-2	IV-10	+	-	-	-	-	+	+	-
PB-3	II-16	+	-	-	-	-	+	+	-
PB-4	II-16	+	-	-	-	-	+	+	-
PB-5	II-16	+	-	-	-	-	+	+	-
PB-6	III-24	-	-	-	-	-	-	+	-
PB-7	II-16	+	-	-	-	-	+	+	-
PB-8	IV-10	+	-	-	-	-	+	+	-
PB-9	IV-10	+	-	-	-	-	+	+	-
PB-10	II-16	+	-	-	-	-	+	+	-
PB-11	II-16	+	-	-	-	-	+	+	-
PB-13	II-16	+	-	-	-	-	+	+	-
PB-14	IV-7	-	-	-	-	-	-	+	-
PB-15	IV-7	-	-	-	-	-	-	+	-
PB-16	IV-7	-	-	-	-	-	-	+	-

Table 7--continued.

Strain	Plasmid profile	Gr Cu	Gr st	Pt ac	Sr hy	Pepper ECW	HR 10R	HR 20R	HR 30R
PB-17	IV-10	+	-	-	-	-	+	+	-
PB-18	IV-10	+	-	-	-	-	+	+	-
PB-19	IV-10	ND	-	-	-	-	+	+	-
PB-20	IV-10	+	-	-	-	-	+	+	-
PB-21	IV-10	+	-	-	-	-	+	+	-
PB-22	V-2	-	+	-	-	-	-	+	-
PB-23	IV-10	+	-	-	-	-	+	+	-
PB-24	IV-9	+	-	-	-	-	+	+	-
PB-25	IV-10	+	-	-	-	-	+	+	-
PC-1	IV-10	+	-	-	-	-	+	+	-
PC-2	IV-10	+	-	-	-	-	+	+	-
PC-3	III-19	+	+	-	-	-	+	+	-
PC-4	IV-7	-	-	-	-	-	-	+	-
PC-5	IV-10	+	-	-	-	-	+	+	-
PC-6	IV-10	+	-	-	-	-	+	+	-
PC-7	IV-10	+	-	-	-	-	+	+	-
PC-8	IV-10	+	-	-	-	-	+	+	-
PC-9	IV-10	+	-	-	-	-	+	+	-
PC-10	V-3	+	-	-	-	-	+	+	-
PC-12	IV-10	+	-	-	-	-	+	+	-

Table 7--continued.

Strain	Plasmid profile	Gr Cu	Gr st	Pt ac	Sr hy	Pepper ECW	HR 10R	HR 20R	HR 30R
PC-13	IV-7	-	-	-	-	-	-	+	-
PC-14	IV-7	-	-	-	-	-	-	+	-
PC-17	III-19	+	+	-	-	-	+	+	-
PC-18	IV-10	+	-	-	-	-	+	+	-
PC-19	IV-10	+	-	-	-	-	+	+	-
PC-20	IV-10	+	-	-	-	-	+	+	-
PC-21	IV-10	+	-	-	-	-	+	+	-
PC-22	IV-10	+	-	-	-	-	+	+	-
PC-24	IV-10	+	-	-	-	-	+	+	-
PC-25	IV-10	+	-	-	-	-	+	+	-
PD-1	IV-10	+	-	-	-	-	+	+	-
PD-2	IV-10	+	-	-	-	-	+	+	-
PD-3	IV-10	+	-	-	-	-	+	+	-
PD-4	IV-10	+	-	-	-	-	+	+	-
PD-5	IV-10	+	-	-	-	-	+	+	-
PD-6	II-16	+	-	-	-	-	+	+	-
PD-7	IV-10	+	-	-	-	-	+	+	-
PD-8	IV-10	+	-	-	-	-	+	+	-
PD-9	IV-10	+	-	-	-	-	+	+	-
PD-10	IV-10	+	-	-	-	-	+	+	-

Table 7--continued.

Strain	Plasmid profile	Gr Cu	Gr st	Pt ac	Sr hy	Pepper ECW	HR 10R	HR 20R	HR 30R
PE-1	IV-10	+	-	-	-	-	+	+	-
PE-2	IV-10	+	-	-	-	-	+	+	-
PE-3	IV-10	+	-	-	-	-	+	+	-
PE-4	IV-10	+	-	-	-	-	+	+	-
PE-5	IV-10	+	-	-	-	-	+	+	-
PE-7	IV-10	+	-	-	-	-	+	+	-
PE-8	IV-10	+	-	-	-	-	+	+	-
PE-9	IV-10	+	-	-	-	-	+	+	-
PE-10	IV-10	+	-	-	-	-	+	+	-
PE-11	IV-10	+	-	-	-	-	+	+	-
PE-12	IV-10	+	-	-	-	-	+	+	-
PE-13	IV-10	+	-	-	-	-	+	+	-
PE-14	IV-10	+	-	-	-	-	+	+	-
PE-15	IV-10	+	-	-	-	-	+	+	-
PE-17	IV-10	+	-	-	-	-	+	+	-
PE-19	IV-10	+	-	-	-	-	+	+	-
PE-20	IV-7	+	-	-	-	-	+	+	-
PE-21	IV-10	+	-	-	-	-	+	+	-
PE-23	IV-10	+	-	-	-	-	+	+	-
PE-24	IV-10	+	-	-	-	-	+	+	-

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BIOGRAPHICAL SKETCH

Blanca Isabel (Nelly) Canteros was born in Saladas, Corrientes, Republica Argentina, on November 14, 1953. She is the daughther of Blanca Natividad Dominguez and Jose Maria Canteros.

Nelly attended the primary schools Escuela No. 145, No. 97, and No. 72 and continued her education at the Escuela Nacional Normal de Saladas, Corrientes, where she graduated in 1970 with the degree Bachiller Pedagogico. She finished elementary and high school with honors having received Medals to the highest grade point average and being selected as Abanderada of the respective schools. In 1972 she entered the National University of the Northeast, in Corrientes, and graduated in 1977 with the degree Ingeniera Agronoma. She worked for her thesis on control of citrus canker at the Bella Vista Experimental Station of the National Institute of Agricultural Technology (INTA). Upon her graduation she continued her work at INTA while serving as Coordinator in the Program for Control of Citrus Canker of the Minister of Agriculture of Corrientes province. In 1978 she joined the staff

of INTA Bella Vista to work as INTA representative in the Cooperative Project for the Study of Citrus Canker between INTA (Argentina) and IFAS (Florida). She worked during those years with Dr. R. E. Stall (UF), Dr. J. W. Miller (DPI) and Dr. D. Zagory (AREC, Lake Alfred). Upon finalization of the project she continued at INTA as a Research Plant Pathologist and in 1985 was awarded a fellowship to pursue graduate studies.

Nelly entered the University of Florida in Spring 1986, and is currently a candidate for the degree of Doctor of Philosophy. At the end of her studies she will resume her position in Argentina.

Nelly is a member of the Consejo Profesional de Ingenieros Agronomos de Argentina, Asociacion de Ingenieros Agronomos de Corrientes, Sociedad Argentina de Fitopatologia, American Phytopathological Society, American Society for Microbiology, International Society of Citriculture, and International Society of Plant Pathology.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Robert E. Stall, Chair
Professor of Plant Pathology

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Daryl R. Pring
Professor of Plant Pathology

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Jeffrey B. Jones
Associate Professor of Plant Pathology

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Mark J. Bassett
Professor of Horticultural Science

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May 1990

Jack L. Fry
Dean, College of Agriculture

Dean, Graduate School