CHARACTERIZATION OF *Phytophthora infestans* POPULATIONS IN ANTIOQUIA, COLOMBIA

CARACTERIZACIÓN DE LAS POBLACIONES DE *Phytophthora infestan* EN ANTIOQUIA, COLOMBIA

Elizabeth Gilchrist Ramelli[1]; Sonia Jaramillo Villegas[2]; Lucia Afanador Kafuri[3] and Rafael Eduardo Arango Isaza[4]

Abstract. *From the* ***Phytophthora infestans*** *collection of the Universidad Nacional de Colombia, the isolates collected in different locations in Antioquia, Colombia between 1994 and 2000 were evaluated. These isolates were obtained from late blight lessons in different hosts. In 2000, these isolates were characterized by mating type, mitochondrial haplotype and virulence races. All isolates were of the A1 mating type and two mitochondrial haplotypes were identified: IIa, present in isolates from all the hosts tested, and Ib present only in isolates from tomato and water cucumber (****Solanum muricatum****). The Antioquia population of* ***P. infestans*** *showed a large complexity of virulence factors (10 out 11), especially those isolates collected from potato, while the tomato population was less complex. The A1 mating type and the mitochondrial haplotype IIa has been associated with the EC1 population that possibly is replacing the US1 population.*

Key words: Late blight, clonal lineage, South America, haplotypes, mating type.

Resumen. *De la colección de* ***Phytophthora infestans*** *de la Universidad Nacional de Colombia, se evaluaron aquellos aislamientos provenientes de diferentes localidades de Antioquia, Colombia entre 1994 y 2000. Dichos aislamientos fueron obtenidos de lesiones de tizón tardío en diferentes hospederos. En el año 2000 se caracterizaron por el tipo de apareamiento, haplotipo mitocondrial y razas de virulencia. Todos los aislamientos correspondieron al tipo de apareamiento A1 y se presentaron dos haplotipos mitocondriales: IIa, en aislamientos de todos los hospederos evaluados, y Ib solamente en aislamientos colectados de tomate y pepino de agua (****Solanum muricatum****). La población antioqueña de* ***P. infestans*** *presenta una amplia complejidad de factores de virulencia (10 de 11), especialmente para los aislamientos colectados de papa, mientras que la población de tomate fue menos compleja. El tipo de apareamiento A1 y el haplotipo mitocondrial IIa han sido asociados a la población EC1 que posiblemente está desplazando la población US1.*

Palabras claves: Tizón tardío, linaje clonal, Sur América, haplotipos, grupo de compatibilidad.

The oomycete *Phytophthora infestans* (Mont.) de Bary is the causal agent of several economically important diseases in potato and tomato. In tropical countries the pathogen infects other crops like water cucumber (*Solanum muricatum*), tamarillo (*S. betaceum*) and lulo (*Solanum quitoense*). The population of *P. infestans* is mostly composed of clonal lineages with distinct genotypes that can be characterized by RLFP's (*Restriction Fragment Length Polymorphism*), isozimes and mitochondrial haplotypes (Oyarzun *et al*., 1997; Pérez *et al*., 2001).

P. infestans reproduces sexually if the two mating types A1 and A2 are present. Sexual reproduction is present in the entire world. It has been reported in Mexico (Forbes *et al*., 1998; Garry *et al.,* 2005), Europe (Drenth *et al.,* 1994) and Asia (Ghimire *et al.,* 2003). It is also present in South América, specially in Venezuela (Briceño *et al.,* 2009) and in Colombia (Vargas *et al.,* 2009). The evidence confirms the asexual reproduction of *P. infestans*, since only one isolate of 840 collected in Venezuela presented fertility with A1. In Colombia, Vargas *et al.* (2009) found the A2 mating type in one isolate collected from *Physalis peruviana* L. However, this isolate generates oospores with the A1 mating type reference isolate, but this oospores are not infective (Restrepo Silvia, Universidad de los Andes, personal communication). In Ecuador, the US-1 genotype has been replaced by the EC-1 lineage, which is more pathogenic and less sensitive to the systemic fungicide metalaxyl (Pérez *et al*., 2001; Forbes *et al*., 1996). In Colombia, US-1 and EC-1 clonal lineages have been reported (Griffith and Shaw, 1998; Forbes *et al*., 1998; Vargas *et al.,* 2009).

The populations of *P. infestans* can be also studied in terms of their physiological races which are determined by the presence of one or more of the

[1] Ingeniera Agrónoma, D.Sc. Universidad Nacional de Colombia, Sede Medellín. Facultad de Ciencias Agropecuarias. A.A. 1779. Medellín, Colombia. <elygilchrist@hotmail.com>
[2] Profesora Asociada. Universidad Nacional de Colombia, Sede Medellín. Facultad de Ciencias Agropecuarias. A.A. 1779. Medellín, Colombia. <sjaramal@unalmed.edu.co>
[3] Profesora Asociada. Universidad Nacional de Colombia, Sede Medellín. Facultad de Ciencias Agropecuarias. A.A. 1779. Medellín, Colombia. <lafanado@unal.edu.co>
[4] Profesor Asociado. Universidad Nacional de Colombia, Sede Medellín. Facultad de Ciencias Agropecuarias. A.A. 1779. Medellín, Colombia

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11 known virulence genes. No correlation between physiological race and genotype has been found so far and several studies indicate that the complexity of virulence factors in the oomycete has increased with time (Tooley *et al.,* 1986). At the time of this research (1994-2000), there was a small amount of information available concerning the Colombian populations of *P. infestans* and the strategic geographical position of this country justifies a study on the structure of the population of this pathogen. Forty isolates of *P. infestans* collected from different hosts and locations in the north-central Colombian Andes (Antioquia department) were characterized by mating type, mitochondrial haplotype and physiological races. This work, carried out in 2000, enriches the study of populations of the pathogen in the South American continent and the rest of the world.

MATERIALS AND METHODS

Sources of *Phytophthora infestans* isolates. Between 1994 and 2000, 40 isolates of *P. infestans* were collected from affected plants of commercial growing fields and house garden of potato *Solanum tuberosum* cultivars (ICA Puracé, ICA Nevada, ICA Cumanday and Diacol Capiro), *Solanum phureja,* tomato and water cucumber. Isolates were taken from infected leaf tissue and collected in 14 different locations of the Antioquia department. Geographical origin (First letter of each isolate, A: Antioquia), year (last two numbers of each isolate), and host of each isolate were recorded (Table 1; Figure 1).

Media and culture conditions. All isolates of *P. infestans* were kept at 15-18 ºC on Petri dishes containing rye agar (25 g of rye powder, 20 g of table sugar and 18 g of agar per liter). For the sexual compatibility assay the media was complemented with 0.05 g/l of β-sitosterol. For DNA extraction, mycelia were grown for 8 days in pea water broth (30 g of frozen peas and 20 g of table sugar per liter), harvested by filtration and stored at –20 ºC.

Mating type. To avoid the introduction to the country of unknown mating type, at the date of this research, only the mating type A1 was used as a control. Mating type was determined by paring each isolate with a known A1 culture on rye-agar medium (Deahl *et al.,* 1991). Plates were incubated at 15 ºC in the dark, during 30 days. Analysis consisted on checking each pairing for the formation of oospores under macro and microscopic examinations of micelia formed in the area of interaction between the isolates paired. A self-paring of each isolate was used as a control.

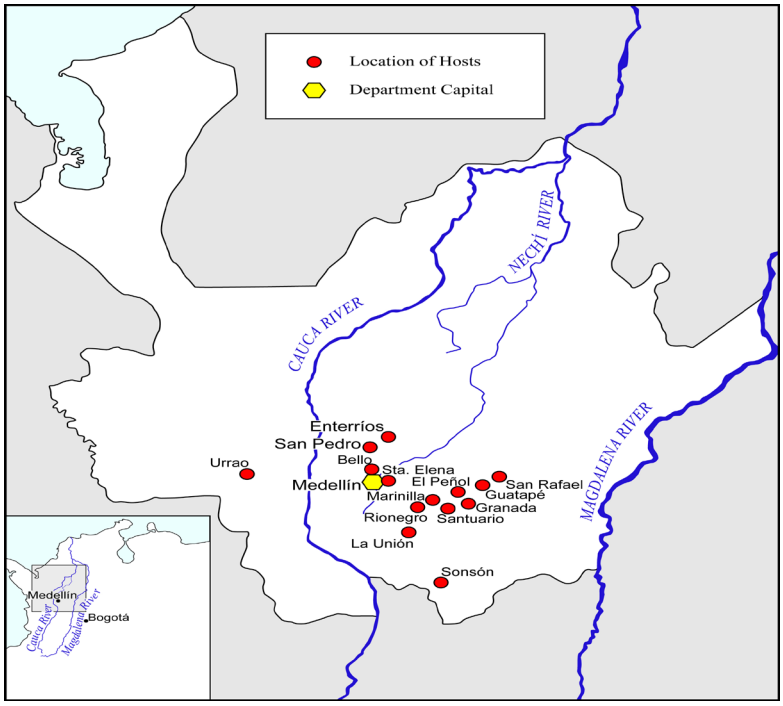

Figure 1. Map of Antioquia-Colombia, showing detailed locations where isolates of *Phytophthora infestans* were collected.

Table 1. Localization of *Phytophthora infestans* isolates from Antioquia-Colombia used in this study. Location from wich isolates were collected. Mating type, virulence factors, mitochondrial haplotype and clonal lineage for each isolate are indicated.

Isolate	Location (Host)*	Mating type	Virulence+	MtDNA type	Probable clonal lineage
A0394	La Union (P)	A1	1.2.3.4.7.8.10.11	IIa	EC-1
A3196	La Union (P)	A1	NT	IIa.	EC-1
A11500	La Union (P)	A1	1.2.3.4.7.8.10.11	IIa	EC-1
A11700	La Union (P)	A1	1.2.3.4.7.8.9.10.11	IIa	EC-1
A1195	Sonson (P)	A1	1.3.4.7.10.11	IIa	EC-1
A1496	Sonson (P)	A1	NT	IIa	EC-1
A1596	Sonson (P)	A1	NT	IIa	EC-1
A1696	Sonson (P)	A1	NT	IIa	EC-1
A2296	Sonson (P)	A1	NT	IIa	EC-1
A0894	San Pedro (P)	A1	1.2.3.4.7.10.11	IIa	EC-1
A0994	Entrerríos (P)	A1	1.2.3.4.7.10.11	IIa	EC-1
A6797	Santuario (P)	A1	1.3.4.7.8.9	IIa	EC-1
A6897	Santuario (P)	A1	1.2.3.4.7.10.11	IIa	EC-1
A7497	Santuario (P)	A1	1.2.3.4.7.10.11	IIa	EC-1
A8497	Santuario (P)	A1	3.4.6.7.8.9.10	IIa	EC-1
A9597	Santuario (P)	A1	1.2.3.4.6.7.8.9.10	IIa	EC-1
A7097	Marinilla (P)	A1	1.2.3.4.7.8.9.10	IIa	EC-1
A7197	Marinilla (P)	A1	1.2.3.4.6.7.8.9.10.11	IIa	EC-1
A8597	Marinilla (P)	A1	1.2.3.4.6.7.8.9.10.11	IIa	EC-1
A8097	Granada (P)	A1	1.2.3.4.6.7.8.9.10.11	IIa	EC-1
A8197	Granada (P)	A1	1.2.3.4.6.7.8.10.11	IIa	EC-1
A8297	Granada (P)	A1	3.4.7.10.11	IIa	EC-1
A9497	Granada (P)	A1	1.3.4.8	IIa	EC-1
A9097	Guatape (P)	A1	1.2.3.4.7.8.10.11	IIa	EC-1
A3897	Rionegro (P)	A1	NT	IIa	EC-1
A2796	Sta Elena (P)	A1	1.6.9.10	IIa	EC-1
A2896	Sta Elena (P)	A1	NT	IIa	EC-1
A10099	Sta Elena (P)	A1	1.2.3.4.6.7.8.9.10.11	IIa	EC-1
A0694	Bello (P)	A1	1.2.3.4.7.10.11	IIa	EC-1
A1295	Rionegro (WC)	A1	NT	Ib	US-1
A3796	Santuario (WC)	A1	NT	IIa	EC-1
A8797	Marinilla (WC)	A1	1.3.4.6.7.8.9.10.11	IIa	EC-1
A9197	El Peñol (WC)	A1	1.2.3.4.6.7.8.9.10.11	IIa	EC-1
A2696	Marinilla (T)	A1	3.4.6.7.10	Ib	US-1
A8997	El Peñol (T)	A1	3.8	Ib	US-1
A9397	El Peñol (T)	A1	0	Ib	US-1
A8697	San Rafael(T)	A1	3.7.10.11	Ib	US-1
A3496	NT (T)	A1	NT	Ib	US-1
A3594	NT (T)	A1	NT	IIa	EC-1
A5197	Urrao (T)	A1	NT	IIa	EC-1

*Host plant from which the isolate was obtained. P=potato, T=tomato, WC= water cucumber, NT= not tested
+Virulence, as assayed by the ability to grow on potatoes carrying the appropriated resistance genes (Black *et al.,* 1953).

DNA extraction. DNA was extracted using the method of Afanador *et al.* (1993). Briefly, 100 μL of mycelium powder was incubated in a microcentrifuge tube with 400 μL of CTAB extraction buffer (CTAB 2% w/v, 100 mM Tris HCl, pH 8.0, 1.4 M NaCl, 20 mM EDTA pH 8.0), for 30 min at 65 ºC, inverting the tubes every 8 min. Additional 400 μL of cloroform:isoamyl alcohol (24:1) were added, mixed by inversion during 15 min, followed by centrifugation at 20.000 CRF (Centrifugal Relative Force) during 10 min. This step was repeated, and followed by precipitation with isopropanol and resuspention in TE buffer.

Mitochondrial haplotypes. Mitochondrial haplotypes were determined by PCR-RFLP using primer pairs P1 (P1 forward and P1 reverse), P2 (P2 forward and P2 reverse) and P4 (P4 forward and P4 reverse) as described by Griffith and Shaw (1998). PCR (PTC-100™ MJ Research, Inc.) amplification conditions were as follows for all primer combinations: deoxynucleotide triphosphates dNTP´s (Promega), 200 μM each, PCR buffer 1X (1 M KCl, 1 M Tris HCl pH 8.3, 1 M $MgCl_2$), primers (Operon Technologies, Inc.) 0.34 μM each, bovine serum albumin, 160 μg/mL, *Taq* DNA polymerase (Promega) 0.2 μL (1U) and 2 a 10 ng of total DNA in 0.2 mL microcentrifuge tubes (final volume, 25 μL). PCR cycles were as follows: 1 cycle of 94 ºC for 90 s; 40 cycles of 94 ºC for 40s, 55 ºC for 60s and 72 ºC for 120s.

Five microliters of the amplified product was digested with the following restriction enzymes (Promega): P1 reactions with *Cfo*I, P2 with *Msp*I and P4 with *Eco*RI. Digested DNA (10 μL) was mixed with 5 μL of loading buffer and loaded into a 2% agarose gel in 1 X TBE buffer (containing 0.1 μg/mL of ethidium bromide). The gel was run at 80 vol. during 4 h. Restriction patterns were visualized with an UV transilluminator and the images were recorded by a gel documentation system (BIO-RAD, Gel Doc 1000).

Physiological races. The virulence specificity of a subset of 28 isolates was determined by inoculation of detached leaflets from the international set of differential potato cultivars provided by the Centro Internacional de la Papa (CIP), carrying the 11 known mayor (R) resistance genes. Briefly, leaflets collected from the middle part of each cultivar were inoculated in a moist chamber with 20 μL of sporangial suspension (approx. 5.000 sporangia/mL) placed on the abaxial surface of each leaflet. Each test included the susceptible cultivar Alfa (α) (containing no know R genes) as a control. The virulence assays were repeated at least twice.

RESULTS

Mating type. In all tests only mating type A1 was detected as indicated by the absence of oospores in the growing area between the isolates on all pairings.

Mitochondrial haplotypes. PCR-RFLP analysis revealed the presence of two mitochondrial haplotypes in the Antioquian population of *P. infestans*: haplotypes IIa and Ib (Table 1). The mitochondrial haplotype IIa was present in all potato isolates (29 samples), in 2 isolates from tomato and in 3 isolates from water cucumber, while the haplotype Ib was only present in 5 isolates from tomato and in one isolate from water cucumber (Figure 2).

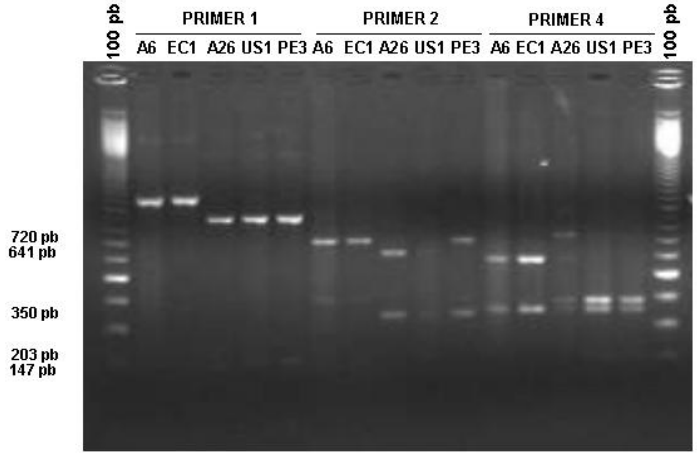

Figure 2. Mitochondrial haplotypes of *Phytophthora infestans*, as analyzed by primer pairs P1, P2 y P4. From left to right 100 pb weight marker, A6, EC1, A26, US1, Pe3 samples amplified with P1, P2 and P4 primers, 100 pb weight marker.

***Physiological races*.** Eighteen pathotypes were identified among 28 isolates tested, showing variation in the level of complexity of their races. The most common pathotype was presented on 21.42 % of the isolates, and infected ten of the eleven potato cultivars (1.2.3.4.6.7.8.9.10.11). One of the isolates coming from tomato had no virulence genes (Race 0). Virulence factor No. 5 was not present in any isolate. Furthermore, the isolates collected after 1997, infected the R9 cultivar, which indicates an increment in the pathogen complexity. The R3 cultivar was the most affected by the tested isolates, by a 26/28 proportion. To calculate the Shannon (H_S) and Gleason (H_G) diversity index, isolates were divided in two groups by date of collection: one was from 1994 to 1996, and the other from 1997 to 2000. This analysis showed an increase on diversity factors from HG =2.5 and H_S=1.47 to H_G =4.26 and H_S=2.09.

DISCUSSION

Forty isolates of *P. infestans* were analyzed in this work, which were collected from different *Solanum* hosts and locations of a north-central Colombian Andean region of the Antioquia department, South America. The isolates were tested for mating type, mitochondrial haplotype and virulence factors.

All isolates tested showed to be of the mating type A1 suggesting that the current population of *P. infestans* in Antioquia is clonal with asexual reproduction. To 2000, the presence of the A2 mating type of *P. infestans* in Colombia cannot be ruled out considering that is has been reported in Ecuador, in association with wild species of *Solanum* such as *S. brevifolium* y *S. tetrapetalum* (Ordoñez *et al*., 2000), in potato in Uruguay, Brazil, Bolivia and Argentina (Brommonschenkel 1988; Deahl *et al.*, 2003), and in Mexico and the United States (Deahl *et al.,* 1991; Goodwin *et al.,* 1992). In recent studies the A2 mating type has been reported in Colombia in *P. peruviana* (Vargas *et al*., 2009).

These results suggest that it might be important to use a different sampling strategy to continue monitoring the Colombian population of *P. infestans,* increasing the range of hosts for sampling. This approach, combined with regulations on the importation of asexual seeds and the control of other host species, may avoid or retard the entrance of the infective A2 mating type and the probability to produce infective oospores with the A2 isolates currently present.

Two mitochondrial haplotypes of *P. infestans* are present in the population studied, the IIa haplotype which is related with the clonal lineage EC-1 (Oyarzun *et al*., 1997), and the Ib related with the US-1 (Griffith and Shaw, 1998). This finding is consistent with what has been found in Peru, where EC-1 populations have been replaced by US-1. This change in the population is probably due to the fact that EC-1 is resistant to the systemic fungicide metalaxyl, whereas US-1 is sensitive to it (Pérez *et al.,* 1998). In Marruecos, Sedegui *et al*. (2000) found changes on *P. infestans* population in potato, but not in tomato, which is in agreement with the results obtained in this study.

As seen in Table 1 the complexity of the *P. infestans* population in potato is higher that the one in tomato, and this could be associated with clonal lineage US-1 being less complex than EC-1 as has been found in Ecuador (Forbes *et al.*, 1997) and in Peru (Pérez *et al.,* 2001). Several works worldwide have shown that the isolates that correspond to the so called "new" (EC-1) population are more aggressive on their original host (Goodwin *et al.,* 1995; Lebreton *et al.*, 1999; Sedegui *et al.*, 2000), indicating that the pathogen is coevolving with its host and adapting to the agricultural practices that farmers are using in each region, such as the type of chemical control employed and the genetic make up of the cultivars. Furthermore, extensive potato crops with a single variety or a very narrow genetic base are highly susceptible to any adaptation in the microorganism.

From this study we can conclude that the *P. infestans* population in Antioquia-Colombia up to 2000, was A1 mating type, composed mainly of the two mitochondrial haplotypes, IIa and Ib which are associated with the clonal lineages EC-1 and US-1, respectively. The clonal lineages present were very diverse on its virulence factors, specially the EC-1 population, which is more complex than the US-1 lineage. Our work contributes to the worldwide effort to characterize *P. infestans* and it specially gives some information on the pathogen populations in a place located in a strategic region in the South American continent, since Colombia is the crosspoint between South and Central America.

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