Characterization of isolates of *Phytophthora infestans* from potato and tomato in Bolivia and the Dominican Republic

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Resumen. *Phytophthora infestans* es una enfermedad que afecta la producción de papa y tomate en Bolivia y República Dominicana. En 1998, se caracterizaron 19 aislamientos recolectados de hojas de papa en cinco regiones de Bolivia para determinar la estructura genética. En 1999 en la República Dominicana se muestrearon siete regiones y se estudiaron 12 aislamientos. Los aislamientos se caracterizaron de acuerdo a determinación de tipo reproductivo, resistencia a metalaxil y marcadores moleculares (aloenzimas de glucosa-6-fosfato isomerasa, peptidasa, RG57 DNA fingerprinting y haplotipos de ADN mitocondrial). Todos los aislamientos de Bolivia presentaron el tipo reproductivo A-2, 100/100 Gpi y haplotipo mitocondrial II-a. Se observaron diferencias en genotipo al realizar la caracterización. Una masa de los aislamientos presentó un genotipo que no se ha descrito anteriormente. Los aislamientos de la República Dominicana presentaron el tipo reproductivo A-1, resistencia a metalaxil, 86/100 Gpi, 92/100 Pep, linaje US-1 o US-1.2 y haplotipo mitocondrial I-b.

Palabras claves: ADN fingerprinting, ADN mitocondrial, resistencia.

Abstract. *Phytophthora infestans* is a serious disease affecting potato and tomato production in Bolivia and Dominican Republic. In 1998, 19 isolates collected from potato leaves in 5 regions in Bolivia were characterized to determine genetic structure. In the Dominican Republic, seven tomato-producing regions were sampled in 1999, and 12 isolates were studied. Based on mating type, metalaxyl-resistance, and molecular markers (allozymes of glucose-6-phosphate isomerase, peptidase, RG57 DNA fingerprinting and mitochondrial DNA haplotype), all isolates were characterized. All Bolivian isolates were A-2 mating type, 100/100 Gpi, and presented II-a mitochondrial haplotype. Differences were observed in the genotype after RG57 fingerprinting. A mass of these isolates was a genotype that had not been described previously. The Dominican Republic isolates were A-1 mating type, resistant to metalaxyl, 86/100 Gpi, 92/100 Pep, most US-1 or US-1.2 lineage group and I-b mitochondrial haplotype.

Keywords: DNA fingerprinting, mitochondrial DNA, resistance.

INTRODUCTION

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary has become a devastating disease in potato and tomato worldwide (Bakony *et al.*, 1998; Carter *et al.*, 1990; Fry *et al.*, 1993; Goodwin *et al.*, 1998). Recently, the frequency and severity of epidemics in potato and tomato-growing areas has increased (Fraser, et al., 1999; Fry and Goodwin, 1997). Resistance of *P. infestans* isolates to metalaxyl has contributed to this increase and has been a problem affecting farmers.

In the 1980s different genotypes appeared outside Mexico, which is considered the center of origin (Fry, 1998; Fry *et al.*, 1993), as a result of a second world migration (Goodwin *et al.*, 1994). These populations were characterized by an A2 mating type (Goodwin and Drenth, 1997; Goodwin *et al.*, 1992; Spielman *et al.*, 1991). Genetic variation in populations worldwide increased due to these migrations.

Potato is the second most important food crop in Bolivia and *P. infestans* has become a great concern for potato subsistence farmers. Sucre, La Paz, Tarija and Cochabamba are the largest potato-producing regions in the country, and favorable conditions for epidemics of late blight always exist. Little information exist about the genetic structure of the *P. infestans* population in this region. However, it is known that between 1993 and

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1996, A2 mating types prevailed through potato-growing regions in Bolivia (GILB, 1999). Late blight is also a major concern for tomato production in the Dominican Republic.

The objectives of the study were (i) to characterize *P. infestans* isolates collected from Bolivia and the Dominican Republic in 1998 and 1999; (ii) determine mating type, metalaxyl sensitivity, and allozyme (Gpi and Pep) genotypes; (iii) identify changes in the population structure of *P. infestans* in this area.

MATERIALS AND METHODS

Sources of isolates

A total of 31 isolates of *P. infestans* from Bolivia and the Dominican Republic were characterized. Nineteen of them were obtained from five potato producing areas in Bolivia, and other 12 isolates were obtained from tomato plants in seven areas in the Dominican Republic. Infected leaf tissue was placed in Petri plates with moistened filter paper and incubated at 18°C for 1-3 days to induce sporulation. Isolates were then transferred and maintained in pure culture on agar media (rye B, rye A, pea agar).

Allozyme analysis

Cellulose-acetate electrophoresis was used to analyze allozyme at two loci, glucose-6-phospate isomerase (Gpi) and peptidase (Pep), as described by Goodwin *et al.* (1995). Mycelia cultivated in rye B agar was placed in 1 ml of water then ground and homogenized. Approximately 0.25 ml of each sample was loaded in a pre-soaked cellulose –acetate membrane and electrophoresis proceeded for 15 to 20 min at 175 – 200 V. A stain solution was then poured over the membrane to visualize *Gpi* and *Pep* activity.

Mating type determination

Mating type was determined by pairing the isolates with known A1 (US-1) and A2 (US-8) testers on rye B agar. Each isolate was placed in the center of two Petri plates, one containing the A1 at each side and the other the A2 tester. If production of oospores occurs on the plate paired with A2 but not A1, then that isolate was considered A1. If the opposite is seen, then the isolate was A2.

Resistance to metalaxyl

The Dominican Republic isolates were analyzed for metalaxyl sensitivity, measuring radial growth on metalaxyl-amended agar (Goodwin et al., 1996; Matuszak *et al.*, 1994; Lee *et al.*, 1999). Mycelial plugs of 8 mm in diameter taken from the edge of an actively growing colony were transferred to rye B agar amended with metalaxyl at concentrations of 5 and 100 mg/ml. Rye B plates not amended with metalaxyl were used as control. After 10-14 days of incubation at 18°C, radial growth was taken by measuring the colony in each plate.

Isolates were grouped into three categories according to their sensitivity. Isolates that grew less than 40% compared to the control, in both 5 and 100 mg/ml metalaxyl-amended medium were considered sensitive. Those isolates that grew more than 40% in the 5 mg/ml metalaxyl-amended medium and less than 40% in the 100 mg/ml were considered intermediate. Resistant isolates had a growth of over 40% of the control in 5 and 100 mg/ml metalaxyl-amended medium.

RG57 Fingerprinting

Isolates were grown on pea broth for two weeks. Mycelia was lyophilized and ground, before DNA was extracted. A 1% agarose gel with ethidium bromide was run in order to visualize DNA. Each sample of 15 ml was digested overnight with the restriction enzyme *Eco* R1 at 37°C. Samples were then loaded onto a 0.8% agarose gel and run overnight at 30-40 volts. DNA was transferred to a Hybond-N+ membrane using the Southern blot procedure (Goodwin *et al.*, 1992; Kelly, 1996). The membrane was pre-hybridized with a buffer (2X SSC, 0.5% blocking reagent, 5% dextran sulphate, 0.1% SDS) and then hybridized overnight at 65°C with the RG57 probe.

Mitochondrial DNA haplotype

Polymorphisms in mitochondrial DNA were used to distinguish between mtDNA haplotypes of *P. infestans* (Chycoski and Punja, 1996). Primers P1 and P2 were used to discriminate between haplotypes II-a and II-b (Griffith and Shaw, 1998), using restriction enzymes *Cfo*I (P1) and *Msp*I (P2).

RESULTS

Bolivia

A total of 19 isolates from five regions in Bolivia were sampled in 1998. All isolates were A2 mating type, Gpi 100/100. DNA fingerprinting in at least eight of the isolates reveals that they belong to a lineage that has not yet been described (Table 1). In this study, haplotype II-a was detected in all 19 isolates studied from Bolivia.

Dominican Republic

A total of 12 isolates from seven tomato fields from the Dominican Republic were analyzed in 1999. All isolates collected were A1 mating type, Gpi 86/100, Pep 92/100 and resistant to metalaxyl. DNA fingerprinting revealed that most isolates belong to the US-1 lineage. However, two isolates do not have band 10, corresponding to US-1.2 clonal lineage (Table 2). The mitochondrial haplotype was I-b for the tested isolates.

Table 1. Genotypes of *Phytophthora infestans* identified in Bolivia in 1999.

Isolate	Genotype	Mating Type	Allozyme genotype		RG57 Fingerprinting	Mitochondrial DNA haplotype
			Glucose-6-phosphate	Peptidase		
BO980003		A2	100/100	na[1]	10101010110011000000110111	II-a
BO980004		A2	100/100		na	II-a
BO980006		A2	100/100		na	II-a
BO980007		A2	100/100		na	II-a
BO980012		A2	100/100		na	II-a
BO980013		A2	100/100		na	II-a
BO980014		A2	100/100		na	II-a
BO980017		A2	100/100		na	II-a
BO980019		A2	100/100		na	II-a
BO980020		A2	100/100		10101010110011000000110111	II-a
BO980024		A2	100/100		10101010110011010010110011	II-a
BO980025		A2	100/100		na	II-a
BO980026		A2	100/100		10101010000011000000110011	II-a
BO980028		A2	100/100		na	II-a
BO980029		A2	100/100		10101010110011000000110111	II-a
BO980030		A2	100/100		na	II-a
BO980034		A2	100/100		10101010110011000000110111	II-a
BO980036		A2	100/100		10101010000011000000110011	II-a
BO980037		A2	100/100		10101010000011000000110011	II-a

[1] Not available

Table 2. Genotypes of *Phytophthora infestans* identified in the Dominican Republic in 1999.

Isolate	Genotype	Mating Type	Allozyme genotype		RG57 Fingerprinting	Metalaxyl sensivity	Mitochondrial DNA haplotype
			Glucose-6-phosphate	Peptidase			
DR-1		A1	86/100	92/100	na[1]	R	na
DR-1a	US-1.2	A1	86/100	92/100	10111010100011010000110011	R	I-b
DR-2		A1	86/100	92/100	na	R	na
DR-2a		A1	86/100	92/100	na	R	na
DR-3a	US-1.2	A1	86/100	92/100	10111010100011010000110011	R	I-b
DR-4a	US-1	A1	86/100	92/100	10111010110011010000110011	R	I-b
DR-4b	US-1	A1	86/100	92/100	10111010110011010000110011	R	I-b
DR-5a	US-1	A1	86/100	92/100	10111010110011010000110011	R	I-b
DR-5b	US-1	A1	86/100	92/100	10111010110011010000110011	R	I-b
DR-5c	US-1	A1	86/100	92/100	10111010110011010000110011	R	I-b
DR-6a	US-1	A1	86/100	92/100	10111010110011010000110011	R	I-b
DR-7b		A1	86/100	92/100	na	R	na

[1] Not available

DISCUSSION

Bolivia

The presence of only the A2 mating type in Bolivia suggests that there is no sexual recombination in the *P. infestans* population. In the surrounding Andean regions such as Peru and Ecuador, the presence of A1 mating type indicates that the A2 mating type reported in Bolivia is probably a result of migration (Forbes *et al.*, 1998) from areas outside the Andes.

Two genotypes were present in many of the regions of Bolivia. One genotype appears to be novel, and different from anything yet found in South America. All the isolates tested II-a for mtDNA.

Dominican Republic

During 1999, in the Dominican Republic, populations of *P. infestans* from tomato fields were composed of primarily the same genotype. All isolates presented A1 mating type, resistant to metalaxyl, 86/100 Gpi and 92/100 Pep. The populations were described as US-1 lineage, with a variation in DNA band 10, described as US-1.2 by Goodwin (1998). The *P. infestans* population in this area is basically clonal. The allozyme analysis and RG57 fingerprinting confirm this. Both analyses showed no variation among isolates. The resistance to metalaxyl presented by the Dominican Republic isolates will be a problem for chemical controls of late blight.

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