A Novel Population of *Phytophthora*, Similar to *P. infestans*, Attacks Wild *Solanum* Species in Ecuador

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ABSTRACT

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Twenty-six isolates of a *Phytophthora* population from two wild solanaceous species, *Solanum tetrapetalum* (n = 11) and *S. brevifolium* (n = 15), were characterized morphologically, with genetic and phenotypic markers, and for pathogenicity on potato and tomato. Based on morphology, ribosomal internal transcribed spacer region 2 (ITS2) sequence, and pathogenicity, all isolates closely resembled *P. infestans* and were tentatively placed in that species. Nonetheless, this population of *Phytophthora* is novel. Its primary host is neither potato nor tomato, and all isolates had three restriction fragment length polymorphism (RFLP) bands (probe RG57) and a mitochondrial DNA haplotype that have not been reported for *P. infestans*. All the isolates were the A2 mating type when tested with a *P. infestans* A1 isolate. The A2 mating type has not been found among isolates of *P. infestans* from potato or tomato in Ecuador. Geographical substructing of the Ecuadorian A2 population was detected. The three isolates from the village of Nono, identical to the others in all other aspects, differed by three RFLP bands; those from Nono lacked bands 10 and 16, but possessed band 19. Most of the Ecuadorian A2 isolates were nonpathogenic on potato and tomato, but a few caused very small lesions with sparse sporulation on necrotic tissue. Cluster analysis of multilocus genotypes (RFLP, mating type, and two allozymes) dissociated this A2 population from genotypes representing clonally propagated populations of *P. infestans* worldwide. The current hypotheses for the historical global movements of *P. infestans* do not satisfactorily explain the origin or possible time of introduction into Ecuador of this A2 population. Assuming the population is *P. infestans*, its presence in Ecuador suggests either a hitherto unreported migration of the pathogen or an indigenous population that had not previously been detected.

Both the A1 and A2 mating types of the potato late blight pathogen *Phytophthora infestans* (Mont.) de Bary occur in relatively equal frequencies in central Mexico, the organism's purported center of origin (19,24). Only the A1 mating type was found outside Mexico until the 1980s, when the A2 mating type was detected in Europe (22). Furthermore, most evidence indicates that, until the 1980s, most populations of *P. infestans* outside North America belonged to the clonal lineage US-1, which is the A1 mating type (15). Since the 1980s, new clonal lineages and sexual populations with both the A1 and A2 mating types have been found in different potato-growing regions of the world (13).

Mutation within A1 clonal lineages was proposed as the origin of some A2 genotypes found outside Mexico (23), but subsequent genetic similarity analyses showed that these A2 genotypes were not related to the A1 genotypes with which they coexist geographically (16). The simplest interpretation of these results is that A2 genotypes occurring outside Mexico have not resulted from mutation, but have been introduced (16), probably on infected potato tubers (12) or on tomato fruits or plantlets (13).

Much discussion about the origins and migrations of *P. infestans* can be found in several recent reviews (1,12,13). This information, however, is based primarily on what is known about isolates from potato and, to a lesser extent, tomato. At this time, little is known about the genetic makeup of populations of *P. infestans* (or closely related species) that attack other solanaceous hosts, although some of these hosts such as *Solanum muricatum* Aiton and *S. betaceum* (Cav.) Sendtner (formerly in the genus *Cyphomandra*) are cultivated crops and others have been evaluated as sources of resistance to *P. infestans* (4).

Genetic characterization of the populations of *P. infestans* and related species attacking alternative hosts could give new insight into the biology and life history of this important genus of plant pathogens. For example, all seven isolates of *Phytophthora* species collected in 1995 from several wild solanaceous plants in Ecuador were designated *P. infestans*, based on morphology, and subsequently determined to be the A2 mating type (25). This was unexpected, because the A2 mating type has not been found on potato or tomato in Ecuador, even after relatively recent and extensive sampling (10,25). If the A2 mating type of *P. infestans* had been introduced into Ecuador on potato or tomato since the 1980s, it is unclear why it would have disappeared from populations attacking these crops, while remaining in high frequency in a population attacking wild hosts.

When the A2 isolates from wild hosts in Ecuador were initially reported (25), nothing was known about their genetic relatedness to populations of *P. infestans* currently found on potato and tomato in Ecuador or elsewhere. Furthermore, it was not known whether the A2 isolates were pathogenic on these commercial hosts. In this study, we used neutral markers to compare the original seven A2 isolates and 19 additional isolates subsequently collected with known genetic marker information for potato and tomato populations of *P. infestans* in Ecuador and elsewhere. We used this information to test the hypothesis that this A2 population was introduced into Ecuador by one of the previously described migrations of *P. infestans*.

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MATERIALS AND METHODS

Sampling and isolation of the pathogen. Twenty-six single-lesion isolates were collected at four locations near Quito, the capital city of Ecuador (Table 1). All four locations are within the zone of potato production and are of similar elevation (between 2,400 and 2,700 meters above sea level) and ecological conditions. Nono, Calacali, and Pululahua are all on the western slope of the Andes, several kilometers northwest of Quito. San Jose de Minas is also on the western slope, but about 40 km north of Quito. Two or three different sites at least 100 m apart were sampled within each location. The diseased plants were identified as *S. brevifolium* H. & B. ex Dun. and *S. tetrapetalum* Rusby based on published descriptions (5). These species are woody vines belonging to the series *Apendiculata* and grow under trees, often climbing up trunks, branches, and fence posts.

Collections were carried out during three consecutive years, starting in 1995. First attempts at isolating the pathogen from leaf lesions with potato tuber slices (25) were unsuccessful, because the pathogen grew very poorly on potato tuber tissue. Isolates ultimately were obtained in the following manner. A small piece of leaf tissue from the edge of a sporulating lesion was cut out and surface-sterilized for 1 to 3 min with a 0.5% hypochlorite solution, rinsed in sterile distilled water, and then plated on SEL-A1, a selective medium (21). The petri dishes were kept at 18°C in the dark until hyphal growth was visible. Agar plugs containing young hyphae were then transferred to rye B (3) or 10% clarified V8 juice agar. Mating type was determined for all isolates as described previously (25).

Phenotypic description. Isolates were compared for sporangial length and width, pedicel length, and form of the papilla. The sporangia were either washed from the plates with distilled water or a piece of approximately 1 cm^2 of agar culture was cut out and inverted several times into 0.1 ml of distilled water on a glass slide. Between 60 and 300 sporangia from 3- to 5-week-old cultures on rye B (occasionally SEL-1A medium) were measured for each isolate using light microscopy and video image analysis. Pedicel length was measured with an ocular micrometer.

Isozyme electrophoresis. Isozyme electrophoresis for the enzymes glucose-6-phosphate isomerase (*Gpi*) and peptidase (*Pep*) was done on starch gels as described previously (27) and on polyacrylamide gels (polyacrylamide gel electrophoresis [PAGE]). PAGE was done using 1-mm-thick 7.5% gels with 25 mM Tris-0.19 M glycine at pH 8.8 as a separating gel and electrode buffer. Bands were clearer when a 1-cm stacking gel (2.5% acrylamide-0.06 M Tris-HCl, pH 6.7) was used (6). PAGE gels were run with a constant current of 5 mA for 1 h, after which the current was increased to 10 mA. Voltage rose continually throughout, from about 50 to 280 V. Electrophoresis was terminated when the bromophenol blue dye reached the bottom of the gel, about 16 cm. Allozyme phenotypes were scored as described previously (27) and represent the mobilities of the enzyme alleles relative to an allele designated as 100. A US-6 isolate with a recorded 92/100 banding pattern on starch from the *P. infestans* collection at Cornell University was used for comparison.

DNA extraction. Isolates were grown for 8 to 10 days at 18°C in clear pea broth that was made by autoclaving 120 g of fresh or frozen peas in 1 liter of water. The peas were removed and the broth autoclaved a second time prior to use. The mycelium was harvested by vacuum filtration, frozen at –20°C for a few hours, and then lyophilized. Lyophilized mycelium was ground with a mortar and pestle with liquid nitrogen or by using a small amount of sand. DNA was extracted according to a miniprep version of a previously published technique (7). One milliliter of preheated (60°C) extraction buffer (0.05 M EDTA; 0.1 M Tris, pH 8.0; 0.5 M NaCl; 0.7% β-mercaptoethanol; and 0.25% sodium dodecyl sulfate) was added to 35 mg of ground lyophilized tissue and incubated for 1 h at 65°C. After that, 333 µl of 5 M potassium acetate was added, and the tubes were shaken vigorously and placed in crushed ice for 20 min. They were then centrifuged for 10 min at 16,000 × *g* (14,000 rpm) and the supernatant collected in a new tube. DNA was precipitated with 800 µl of isopropanol, left on ice for 1 h, and centrifuged at 16,000 × *g* (14,000 rpm) for 5 min. The supernatant was poured off and the pellet air-dried for 1 h. The pellet was dissolved in 100 µl of Tris-EDTA (TE; 10 mM Tris-HCl, pH 8.0, and 1 mM EDTA) and then treated with 1 µl of RNAase (10 mg/ml) for 1 h at 37°C. For restriction fragment length polymorphism (RFLP) analysis, the DNA was dissolved in 700 µl of TE, reprecipitated with 75 µl of 3 M sodium acetate and 500 µl of isopropanol, left on ice for 2 min, and then centrifuged for 5 min at a relative centrifugal force of 16,000 × *g* (14,000 rpm). The pellet was air-dried for 1 h, dissolved in TE, and then treated with 1 µl of RNAase (10 mg/ml) for 1 h at 37°C. DNA was compared visually for quality and concentration with a DNA mass ladder by electrophoresis on a 1% agarose gel with Tris-borate-EDTA buffer (TBE; 0.045 M Tris-borate and 0.001 M EDTA, pH 8.0).

Ribosomal DNA (rDNA) internal transcribed spacer region 2 (ITS2). The ITS2 of the rDNA of *P. infestans* can be amplified using polymerase chain reaction (PCR) and the specific primers ITS3 and PINF2 (28). DNA from all the A2 isolates was amplified using these primers and a template of about 10 ng of DNA as described previously (28). In addition, the ITS2 of six isolates, one each from the six separate collection events (Table 1), were cloned using the Invitrogen Original TA Cloning Kit (Invitrogen Corp., Carlsbad, CA) according to the manufacturer's instructions. The cloned DNA region was purified using the Wizard *Plus* Minipreps DNA Purification System (Promega Corp., Madison, WI) as recommended by the manufacturer. Plasmids containing inserts were digested with *Eco*RI and the products separated on a 1% agarose gel with Tris-acetate-EDTA buffer (0.04 M Tris-acetate and 0.001 M EDTA, pH 8.0). The size of the insert was compared with that of the amplified PCR product.

The cloned ITS2 of the rDNA of all six isolates was then sequenced using the dsDNA Cycle Sequencing System (Gibco BRL, Gaithersburg, MD) according to the manufacturer's instructions. The radiolabeled products were resolved on a 6% polyacrylamide denaturing gel that was then dried and visualized by autoradiography. These sequences were then aligned with the sequences of 14 other species of *Phytophthora* to identify any dissimilar nucleotides. Sequencing was done using the Clustal method of MegAlign (DNASTAR Inc., Madison, WI).

Mitochondrial DNA (mtDNA) haplotypes. DNA of each isolate was amplified using primers designed for specific regions of the mitochondrial genome of *P. infestans* (20). Digestion of the amplified regions with the restriction enzymes *Cfo*I, *Msp*I, and *Eco*RI yields band patterns by which the isolates can be classified into four different haplotypes: Ia, Ib, IIa, and IIb (2,20).

For PCR, the final concentrations of the master mix were 0.325 µM of each forward and reverse primer, 2.5 mM $MgCl_2$, 100 µM dNTPs, 1.5 units of *Taq* polymerase, and 4 ng of template DNA. PCR was performed on a PTC-200 Peltier Thermal Cycler (MJ Research, Inc., Watertown, MA) with the following temperature profile: 94°C for 1 min; 35 cycles of 92°C for 30 s, 55°C for 30 s, and 72°C for 1 min; and a final extension of 72°C for 5 min. Eight microliters of the amplified product was digested with 1 unit of *Cfo*I when ampli-

TABLE 1. Isolates of a *Phytophthora* population collected from two wild *Solanum* species in the highlands near Quito, Ecuador, between 1995 and 1997

Host	No. of isolates	Year collected	Location	RFLP[a] genotype
S. brevifolium	4	1995	Pululahua	EC-2
S. brevifolium	1	1996	Pululahua	EC-2
S. tetrapetalum	9	1996	Calacali	EC-2
S. brevifolium	7	1997	Calacali	EC-2
S. brevifolium	3	1997	Nono	EC-2.1
S. tetrapetalum	2	1997	San Jose de Minas	EC-2

[a] RFLP = restriction fragment length polymorphism.

fication was done with primer set 1, with 1 unit of *Msp*I when primer set 2 was used, and with 1 unit of *Eco*RI when primer sets 3 and 4 were used. The digested products were then run on a 2% agarose gel in TBE buffer at 10 V/cm and visualized with ethidium bromide under UV light.

RG57 DNA fingerprints. RFLP fingerprints were obtained for all isolates using the moderately repetitive probe RG57 (17). Two micrograms of DNA from each isolate was digested with *Eco*RI for 24 h and then separated on 0.7 or 0.8% agarose gels (56 V, 20 mA) for 24 to 45 h in 1× TBE. Hybridization and detection were done using the nonradioactive kit ECL (Amersham, Inc., Buckinghamshire, United Kingdom) according to the manufacturer's instructions.

Cluster analysis. *Gpi*, *Pep*, and mating type data were combined with RFLP fingerprints as described previously (11) to create multilocus genotypes. Using cluster analysis, multilocus genotypes of isolates from *S. brevifolium* and *S. tetrapetalum* were compared with published genotypes of *P. infestans* taken from a global marker database (11). Data analyses and presentation of results were as described previously (11).

Metalaxyl resistance. Isolates were tested for resistance to 5 and 100 µg of metalaxyl per ml in 10% unclarified V8 medium and classified as resistant, intermediate, or sensitive. Conditions of the test and criteria for classification were described previously (10).

Pathogenicity. Each isolate was inoculated on detached leaflets of potato, tomato, and *S. brevifolium* or *S. tetrapetalum*. An isolate from potato or tomato was included for comparison. Potato and tomato leaflets came from 8- to 12-week-old plants grown in the greenhouse, while leaflets from *S. brevifolium* or *S. tetrapetalum* were collected from plants growing near the experiment station of the International Potato Center in Quito, Ecuador. Potatoes used in these tests included four tetraploid accessions of *S. tuberosum* (cvs. Pimpernel, Alpha, Uvilla, and Yungay), which are free of known R genes, and four accessions of diploid *S. phureja* from the Ecuadorian national collection (BOM540, Phu644, SOL059, and HSO101), also free of known R genes. Isolates were inoculated on three of the following tomato cultivars: Flora Dade, FMX-93, Carib, and Heat, which were all free of known major genes for resistance (Ph genes).

Inoculum of each isolate was produced on leaflets from its primary host, although *S. brevifolium* and *S. tetrapetalum* were used interchangeably. Sporangia were washed off the leaves and left at 4°C for 1 h to induce zoospore release. One 10-µl drop of zoospore suspension (approximately 2×10^4 zoospores per ml) from each isolate was put on either side of the midrib of the abaxial surface of the leaflets to be tested. Four leaflets of each host-by-isolate combination were inoculated. The leaflets were placed in the lids of inverted petri dishes containing water agar in the base (two leaflets per dish) and incubated for 10 days at 18°C with 14 h of light per day. Symptoms were recorded after 7 and 10 days of incubation. In some cases when lesions did form, lesion diameters were measured as described previously (25) and compared with the lesions produced by the control isolates.

RESULTS

All 26 isolates from *S. brevifolium* and *S. tetrapetalum* fit the phenotypic description of the *P. infestans* A2 mating type, producing abundant oospores when paired with the A1 mating type tester, but not with the A2 tester. All 26 isolates had deciduous, semipapillate sporangia and a small (2 to 3 µm) pedicel. Sporangia had a mean length of 26.9 µm and a mean width of 16.2 µm, which, although relatively small, fall within the range for *P. infestans* (9).

The rDNA ITS2 characterization also supported the hypothesis that the A2 isolates from *S. brevifolium* and *S. tetrapetalum* are *P. infestans* or are very closely related. We obtained an amplified product of approximately 465 bp for all isolates, which corresponds to previously published results for *P. infestans* and the closely related species *P. mirabilis* and *P. phaseoli* (28). The ITS2 sequences were also identical to those reported for *P. infestans*, which is identical to that of *P. mirabilis* (C. D. Smart, *unpublished data*).

Other molecular markers indicated that this A2 Ecuadorian population is different from *P. infestans* found on potato and tomato in Ecuador. For example, all A2 isolates had the *Pep* 100 allele, but also an allele that in our analysis had a relative mobility of 76 on starch and 81 with PAGE (Fig. 1). The US-6 isolate banding pattern was approximately 92/100 on starch, as expected, but was measured as 88/100 with PAGE (Fig. 1). An allele with a mobility of 78 on starch has been described (11), but we were unable to obtain a representative isolate for comparison. Since electrophoretic running conditions may change mobilities, we do not know if the *Pep* allele we measured for the Ecuadorian A2 population is novel or is the same as that reported earlier as 78. In either case, the allele we measured does not occur in populations attacking potato or tomato in Ecuador (26).

The *Gpi* banding pattern of all A2 isolates appeared to be 100/100 on starch (pH 6.0), which differs from the predominant genotypes of *P. infestans* that attack potato and tomato in Ecuador (26).

Assessment with the RFLP probe RG57 further differentiated this population from those attacking potato and tomato in Ecuador and also indicated that it is novel relative to populations of *P. infestans* that have been reported thus far (11,13). For example, all Ecuadorian A2 isolates had three bands that have not been described for *P. infestans* (Fig. 2). We have named these bands 1a, 8a, and 20a, following the precedent of other authors (8). The three isolates from the village of Nono, which was the complete sample from that site, had the novel bands but differed from the other Ecuadorian A2 isolates at three other loci (Fig. 2). Nono isolates lacked bands 10 and 16, but possessed band 19. Bands 10, 16, and 19 have been reported for *P. infestans* (11).

Cluster analysis (Fig. 3) of a multilocus genotype consisting of RFLP, allozyme, and mating type information demonstrated that both A2 genotypes from *S. brevifolium* and *S. tetrapetalum* are quite distinct from any previously published genotypes associated with clonally propagated populations of *P. infestans* (11). The new genotypes from *S. brevifolium* and *S. tetrapetalum* have been named EC-2 for the most common one and EC-2.1 for the one from Nono, according to a proposed nomenclature (11,14).

All isolates from *S. brevifolium* and *S. tetrapetalum* were of the same mtDNA haplotype, but this haplotype is distinct from those reported for *P. infestans* (2,20). Amplification with each of the four primer sets produced a band that corresponded to published results for *P. infestans* (20). After digestion, however, differences were detected between the Ecuadorian A2 isolates and described *P. infestans* haplotypes for two of the amplification products. With primer sets 1 and 3, the Ecuadorian A2 isolates were characterized as haplotype I (a or b). Nonetheless, amplification with primer set 4 and digestion with *Eco*RI produced a novel two-band pattern that is not consistent with published results for known haplotypes of *P. infestans*. One of these bands comigrated with the 209-bp

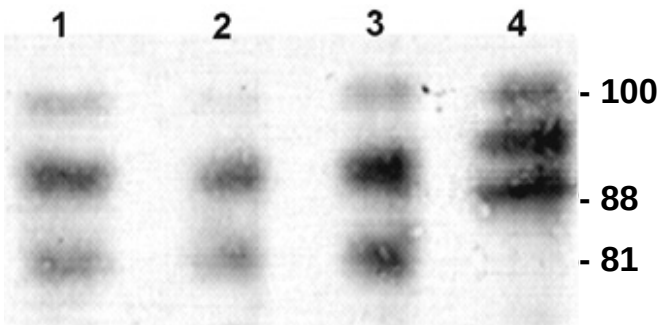

Fig. 1. Peptidase (*Pep*) profiles from polyacrylamide gel electrophoresis of Ecuadorian A2 isolates of a *Phytophthora* population isolated from *Solanum brevifolium* (lanes 1 and 2) and *S. tetrapetalum* (lane 3) and a US-6 genotype of *P. infestans* (lane 4). Relative mobilities were 81/100 for the Ecuadorian A2 isolates and 88/100 for US-6.

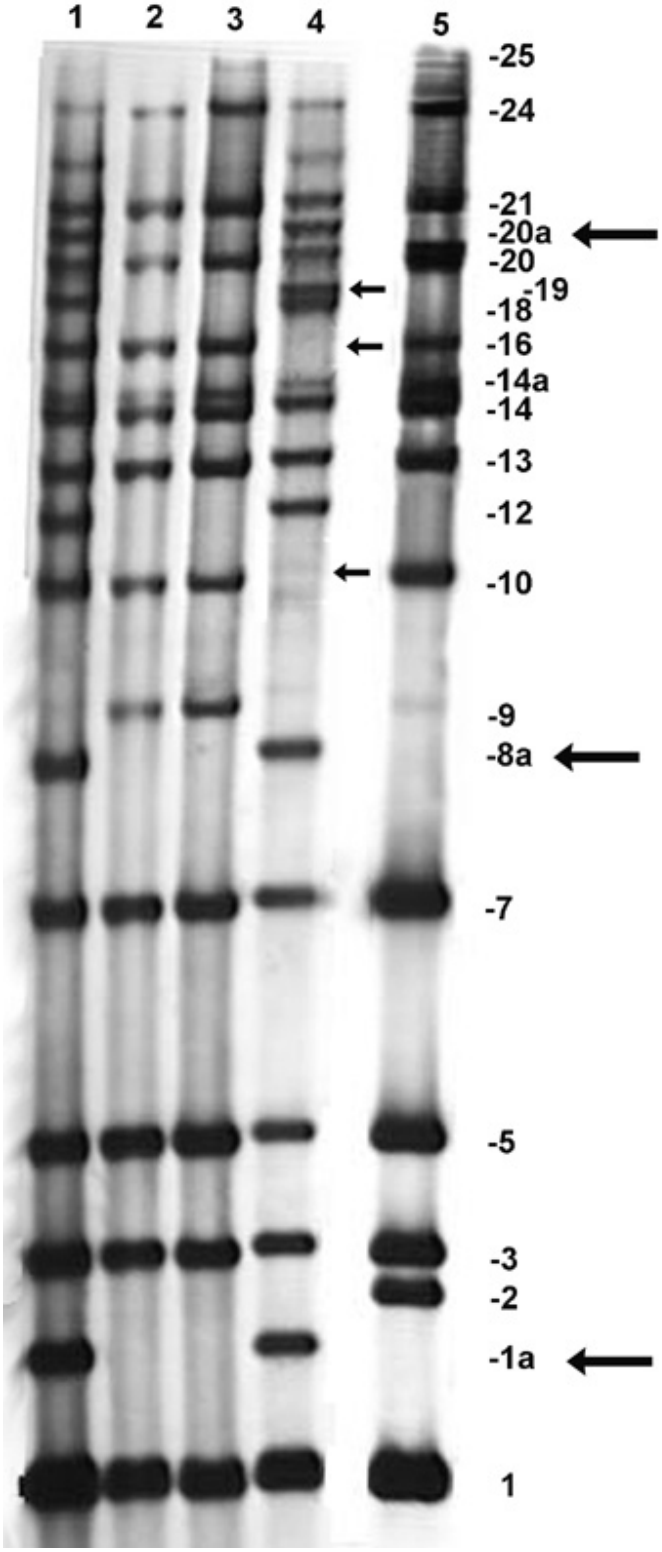

Fig. 2. Restriction fragment length polymorphism genotypes (probe RG57) characteristic of two clonal lineages of *Phytophthora infestans* (US-1 and EC-1) and a *Phytophthora* population (EC-2 and EC-2.1), all found in Ecuador. Lane 1, EC-2; lanes 2 and 3, US-1; lane 4, EC-2.1 (a genotype from the village of Nono belonging to the EC-2 clonal lineage); and lane 5, EC-1. In Ecuador, genotypes EC-2 and EC-2.1 are found on the wild hosts *Solanum brevifolium* and *S. tetrapetalum*, EC-1 is found on potato, and US-1 is found on tomato. Arrows on lane 4 identify differences between the Nono EC-2.1 genotype and the EC-2 genotype in lane 1. Arrows on numbers indicate bands found in all EC-2 and EC-2.1 isolates that have not yet been reported for *P. infestans*. The band numbering system on the right was established previously (17).

band characteristic of haplotypes Ia and Ib (20), but the other, of approximately 750 bp, appeared to be a long fragment that included the 387- and 361-bp fragments that also characterize haplotypes Ia and Ib (20). Therefore, we conclude that the Ecuadorian A2 isolates lack an *Eco*RI restriction site that characterizes haplotypes Ia and Ib. Using primer set 4 and *Eco*RI, haplotypes IIa and IIb have two bands of 596 and 361 bp (20), which is also different from Ecuadorian A2 isolates.

Primer set 2 also gave novel banding patterns. After amplification and digestion with *Msp*I, all Ecuadorian A2 isolates had a fragment that corresponds to the 720-bp fragment of haplotypes Ia, IIa, and IIb (20). However, unlike Ia, IIa, and IIb, the Ecuadorian A2 haplotype had two additional bands, a bright one with approximately 125 bp and another fainter band with approximately 100 bp (Fig. 4). Since the amplification product using primer set 2 corresponds in size (approximately 970 bp) to that published previously, and fragments of 720 and 100 bp were detected, we expected to find one or more additional fragments that would add up to approximately 250 bp. Therefore, we deduced that the bright 125-bp band probably consists of two fragments of similar size. The banding pattern of the Ecuadorian A2 isolates using primer set 2 also differs from that published for haplotype Ib (20). For these reasons, the haplotype of the Ecuadorian A2 isolates is novel and cannot easily be classified using the described system (2).

Pathogenicity on potato and tomato. The Ecuadorian A2 isolates from *S. brevifolium* and *S. tetrapetalum* generally formed small necrotic spots on potato and tomato leaflets. Some of the isolates repeatedly caused small lesions on both potato (generally *S. phureja*) and tomato, with a little sporulation occurring only on necrotic tissue. These lesions rarely expanded beyond 2 cm in diameter and contrasted strikingly with the large, heavily sporulating lesions caused by potato and tomato isolates on their respective hosts. Host specificity between potato and tomato isolates also occurs in Ecuador, but differences measured with the detached leaf test are small and quantitative (26). The A2 isolates caused actively sporulating lesions on *S. tetrapetalum* and *S. brevifolium* after 7 days. Lesions occupied the entirety of the small leaflets of these species, and abundant sporulation occurred on green tissue. Isolates from potato and tomato usually caused no visible symptoms, but occasionally resulted in necrotic flecking on either *S. tetrapetalum* or *S. brevifolium*.

Metalaxyl resistance. Most Ecuadorian A2 isolates almost covered the control plate (85 mm in diameter) after 1 week, while on the 5-µg/ml concentration, growth varied between 0 and 80% of the controls. At 100 µg/ml, none of the isolates had grown at all after 7 days of incubation. Thus, none of the isolates was resistant to metalaxyl, although 17 were classified as intermediately resistant.

DISCUSSION

The lesions and epidemics seen on *S. brevifolium* and *S. tetrapetalum* strongly resembled those of late blight on potatoes, but nevertheless, our first concern when we discovered this disease was whether it was really due to *P. infestans*. Our genetic analyses and observations regarding host range are consistent with the hypothesis that this population is more similar to *P. infestans* than to any other known species of *Phytophthora.* The population is morphologically indistinguishable from *P. infestans* and has the same ITS2 sequence as *P. infestans*, *P. mirabilis*, and *P. phaseoli.* The A2 population in Ecuador can be distinguished from *P. phaseoli* based on sporangia form and oospore formation (*P. phaseoli* is homothallic) and from *P. mirabilis* based on host range. The isolates we studied attack plants within the same genus as potato and tomato, and some isolates weakly attack both of these cultivated hosts. The primary hosts of *P. mirabilis* and *P. phaseoli* are not closely related to the genus *Solanum*. Nonetheless, the Ecuadorian A2 population differs from *P. infestans* based on RFLP fingerprint, *Pep* genotype, mtDNA haplotype, and primary host. Therefore, subsequent genetic analyses, especially quantitative assessment of gene flow,

may eventually warrant the description of a new species of *Phytophthora* for the A2 isolates of Ecuador. This type of analysis was recently used to demonstrate that *P. infestans* and *P. mirabilis* are different species (18).

Lack of sexual recombination in the A2 population from *S. brevifolium* and *S. tetrapetalum* is supported by low genetic diversity (two genotypes from 26 isolates), the apparent absence of similar genotypes with the A1 mating type, and heterozygosity at the *Pep* locus for all individuals. Therefore, if this population is *P. infestans*, it represents the third, clonally propagated, host-specific population of *P. infestans* to be identified in Ecuador to date. Earlier studies demonstrated that tomatoes in Ecuador are attacked by the clonal lineage US-1 (26) and potatoes by the clonal lineage EC-1 (10). Following a suggested nomenclature (11) and until more thorough taxonomic studies can be done, we designate the A2 population attacking *S. brevifolium* and *S. tetrapetalum* as EC-2 of the species *P. infestans*.

At this point, we included the isolates from Nono in the same clonal lineage as the other Ecuadorian A2 isolates, even though they differ by three RFLP bands. We used conventional nomenclature (11,14) to identify the Nono genotype as EC-2.1, within the clonal lineage EC-2. This was done because the Nono isolates are identical to the other A2 isolates in all other aspects including morphology, presence of three novel RFLP bands, the *Pep* 76/100 genotype, and the novel mtDNA haplotype. This approach also seems appropriate at this time because our sample size is small (n = 26) and, therefore, nothing is known about the genetic structure of this population outside our sampling area. Both hosts are fairly common in the Andes, and we assume that this pathogen population exists in other parts of Ecuador and perhaps even in other countries. Further information could lead to a reconsideration of the classification of the Nono subpopulation.

Our studies provide no evidence for gene flow between this A2 population and the A1 populations of *P. infestans* on potato and tomato in Ecuador. Several neutral markers (three RFLP bands, the A2 mating type allele, the *Pep* 76 allele, and the mtDNA haplotype) and sensitivity to metalaxyl differentiate the A2 clonal lineage from populations attacking potato and tomato (10,26). Absence of a common host in nature could provide the principal barrier to gene flow, but low sexual compatibility may also be involved. We paired some A2 isolates with A1 isolates from potato and tomato and then visually examined about 200 oospores per cross with light microscopy. Approximately 25% of the oospores appeared well developed in the crosses with A1 isolates from potato. Oospores were less abundant, and less than 10% were well developed in the crosses with A1 isolates from tomato. We do not know if oospores that appear viable would produce viable progeny or if these progeny would be pathogenic on the hosts of the parent isolates. Nonetheless, the potential for gene flow between this A2 population and A1 populations on potato and tomato deserves further examination. Transfer of the A2 allele to potato or tomato populations could have major epidemiological consequences.

It is difficult to speculate on the origin of the Ecuadorian A2 population. It is unique and dissimilar to other known populations of *P. infestans*, but, as we mentioned earlier, studies done to date have focused on the potato and tomato populations of *P. infestans* and not populations attacking alternative hosts. Therefore, the unique character of the Ecuadorian A2 population may be due, at least in part, to sampling bias. Examination of non-tuber-bearing species of *Solanum* in other parts of the world may lead to the discovery of new genotypes of *P. infestans* or closely related *Phytophthora* species. These could provide links between the Ecuadorian A2 population and the well-studied populations of *P. infestans* that attack potato and tomato.

In spite of sampling problems, it appears unlikely that the Ecuadorian A2 population arrived in Ecuador as part of either one of the two main migrations of *P. infestans* that have been described to date (13). These two migrations are well documented and the

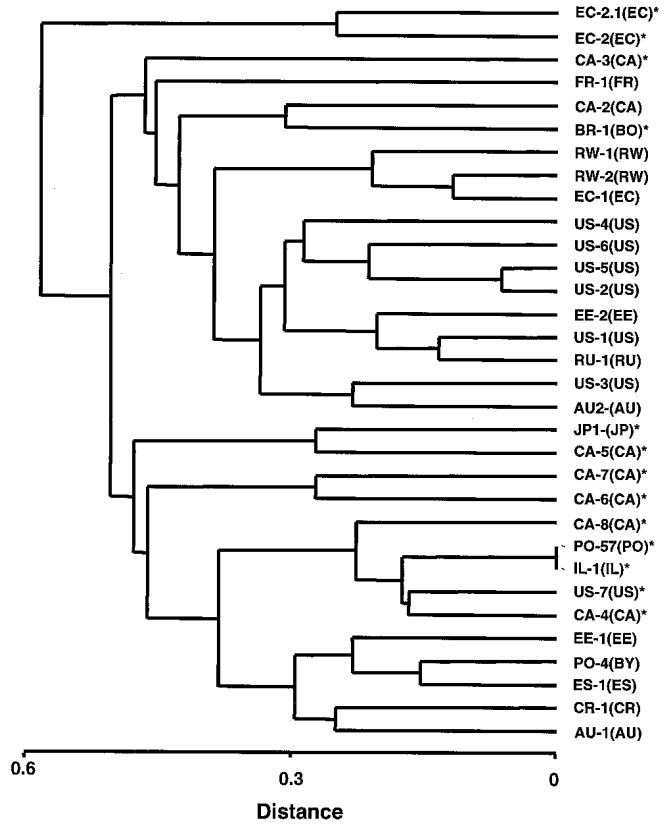

Fig. 3. Cluster analysis of genotypes of *Phytophthora infestans* coming from a published database representing putatively clonal populations worldwide (11) and two *Phytophthora* genotypes (EC-2 and EC-2.1) coming from two Ecuadorian wild hosts, *Solanum tetrapetalum* and *S. brevifolium*. The analysis is based on a distance coefficient (Jaccard) for multilocus genotypes consisting of restriction fragment length polymorphism fingerprint with the RG57 probe, mating type, and dilocus alloenzyme genotype. Genotype labels are the International Organization for Standardization (ISO) two-letter country code plus a unique number (11). Those labels followed by an asterisk indicate the A2 mating type.

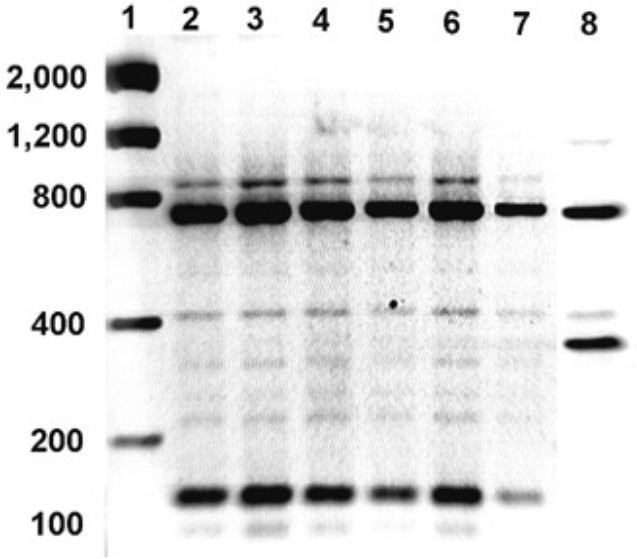

Fig. 4. Pattern of bands produced after polymerase chain reaction amplification with primer set 2 and digestion with *Msp*I for previously described mitochondrial DNA (mtDNA) haplotypes of *Phytophthora infestans* (20). Lane 1, DNA ladder (numbers are the approximate size in base pairs); lanes 2 to 7, *Phytophthora* isolates from *Solanum brevifolium* and *S. tetrapetalum*; and lane 8, mtDNA haplotype Ia or IIb of *P. infestans*.

genotypes involved are not related to the Ecuadorian A2 genotypes. Thus, if the Ecuadorian A2 population did originate in Mexico, it must have come to Ecuador via another migration. How and when this may have happened is unclear. Therefore, the possibility that the A2 population is indigenous to Ecuador cannot be eliminated at this time.

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