DNA Fingerprint Probe from *Mycosphaerella graminicola* Identifies an Active Transposable Element

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ABSTRACT

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DNA fingerprinting has been used extensively to characterize populations of *Mycosphaerella graminicola*, the Septoria tritici blotch pathogen of wheat. The highly polymorphic DNA fingerprints of *Mycosphaerella graminicola* were assumed to reflect the action of transposable elements. However, there was no direct evidence to support that conclusion. To test the transposable element hypothesis, the DNA fingerprint probe pSTL70 was sequenced, along with three other clones from a subgenomic library that hybridized with pSTL70. Analysis of these sequences revealed that pSTL70 contains the 3′ end of a reverse transcriptase sequence plus 29- and 79-bp direct repeats. These are characteristics of transposable elements identified in other organisms. Southern analyses indicated that either the direct-repeat or reverse-transcriptase sequences by themselves essentially duplicated the original DNA fingerprint pattern, but other portions of pSTL70 contained single-copy DNA. Analysis of 60 progeny from a sexual cross between two Dutch isolates of *Mycosphaerella graminicola* identified several new bands that were not present in the parents. Thus, the putative transposable element probably is active during meiosis. Tests of single-spore isolates revealed gains or losses of one or more DNA fingerprint bands in 4 out of 10 asexual lines derived from isolate IPO94269. Therefore, DNA fingerprint patterns produced by the putative transposable element were capable of changes during asexual reproduction of this isolate. Probe pSTL70 did not hybridize at high stringency to genomic DNAs from other fungi related to *Septoria* and *Mycosphaerella*. These results indicate that the DNA fingerprint probe pSTL70 most likely identifies a transposable element in *Mycosphaerella graminicola* that may have been acquired recently, and appears to be active during both sexual and asexual reproduction.

Additional keywords: *Sln*1, transposition, transposon.

DNA fingerprinting is a powerful tool for analyzing the genetic structure of fungal populations. Several fingerprinting strategies have been employed. Some techniques are based on restriction fragment length polymorphism (RFLP) analysis, others use the polymerase chain reaction (PCR), while still others are based on a combination of both approaches (e.g., amplified fragment length polymorphisms [AFLP]). All identify genetic variation at a large number of polymorphic loci. Ability to assay variation simultaneously at a large number of loci increases the level of resolution and is what distinguishes DNA fingerprinting from other methods of genetic analysis.

The most widely used DNA fingerprinting approach is RFLP analysis using a small piece of repetitive genomic DNA as a probe. This technique has been used extensively to analyze the population biology of the ascomycetes *Magnaporthe grisea* (13) and *Mycosphaerella graminicola* (21), and the oomycete *Phytophthora infestans* (10). Thousands of isolates of each species have been analyzed. In most cases, the DNA fingerprint probes were identified originally by screening genomic libraries for clones that produced highly polymorphic, multiple-banded patterns when hybridized to restriction-enzyme digested genomic DNA in Southern analyses. Most DNA fingerprint probes identify 20 or more bands per isolate, many of which are polymorphic. These bands typically segregate plus/minus in genetic analyses (10,27,30), depending on whether the repetitive sequence recognized by the probe is present or absent at each locus.

Even though DNA fingerprinting has been used extensively in population genetics analyses, the reason for the patterns produced by most probes is not known. Due to their high copy numbers, plus/minus genetic segregations, and high levels of polymorphism, it seems likely that many DNA fingerprint probes might contain portions of transposable elements. Transposable elements are small pieces of DNA that are capable of movement within the genome, often by generating a new copy through an RNA intermediate or directly by DNA–DNA interactions (7). Many transposable elements have been discovered in fungi (17), although few have been associated with DNA fingerprints. One exception is the *Magnaporthe grisea* repeat (MGR) 586 probe (13), which has been used extensively for DNA fingerprint analysis and contains part of an inverted-repeat transposon (5). However, the nature of the repeating elements in the *Mycosphaerella graminicola* pSTL70 (3,23) and *P. infestans* RG57 (10) probes has not been determined.

The pSTL70 probe has proven extremely useful for analyzing the population genetics of *Mycosphaerella graminicola*, the cause of Septoria tritici leaf blotch of wheat. This probe reveals 20 bands, on average, per isolate (4). However, isolates from Israel had significantly fewer bands compared with those from other populations worldwide (23). This was interpreted as a possible amplification of repetitive DNA in the genome of *Mycosphaerella graminicola* as populations dispersed from their presumed center of origin in the Middle East. The cause of this amplification of repetitive DNA is not known for certain, but was hypothesized by McDonald et al. (23) to result from the movement of a transposable element.

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The purpose of this study was to test the hypothesis that the DNA fingerprint probe pSTL70 from *Mycosphaerella graminicola* contains part of a transposable element. Secondary goals were to test whether the putative transposable element was capable of movement during sexual and asexual reproduction, and to characterize the type of transposon responsible for the DNA fingerprint pattern.

MATERIALS AND METHODS

Identification of additional clones hybridizing to pSTL70. Subgenomic libraries were constructed from isolates IPO323 and IPO94269, the parents of the *Mycosphaerella graminicola* mapping population (16). Approximately 2 µg of genomic DNA from each isolate were digested to completion with the restriction enzyme *Pst*I and separated on 1% agarose gels. DNA fragments from 0.5 to 3 kb and from 3 to 9 kb for each isolate were excised from gels and purified with the Wizard PCR Prep (Promega Corp., Madison, WI) according to the manufacturer's instructions. Two libraries were made for each isolate because preliminary analyses indicated that both parents had different *Pst*I DNA fingerprint fragments in those size ranges. The DNA fragments were ligated into pBluescript vector, transformed into competent cells of *Escherichia coli* strain INVαF′ and spread onto 9 cm LBA (10 g of tryptone, 5 g of yeast extract, 10 g of NaCl, and 15 g of agar per liter, pH 7.0) plates containing ampicillin at 50 µg/ml and 40 µl of X-Gal at 40 mg/ml per plate for blue-white color selection. White colonies were transferred into 200 µl of LB+amp medium (LBA minus the agar, with ampicillin at 50 µg/ml added after autoclaving) in 96-well Microtest (Becton Dickinson, Franklin Lakes, NJ) tissue culture plates and grown at 37°C overnight. The 96 cultures from each plate were transferred onto large (15 cm) LB+amp agar plates with a replica plater and incubated upside down at 37°C overnight. Colonies were transferred onto pieces (8 × 12 cm) of Zeta Probe (BioRad, Hercules, CA) membranes by briefly laying the membrane pieces on top of the 96 colonies and lifting to pick up the bacteria. Membranes were placed colony side up onto blotting paper soaked with 10% sodium dodecyl sulfate for 3 min, then placed in 0.5 M NaOH/1.5 M NaCl for 5 min, 0.5 M Tris, pH 8.0/1.5 M NaCl for 5 min, and 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) for 5 min. Finally, a UV Stratalinker (Stratagene, La Jolla, CA) was used to crosslink the DNA to the membrane. For Southern analysis by the chemiluminescence technique, pSTL70 DNA was labeled using the Random Primer Fluorescein/antifluorescein-HRP labeling kit (DuPont NEN, Pittsburgh, PA) and hybridized according to the manufacturer's instructions. Approximately 3,900 clones (1,920 from IPO323 and 2,016 from IPO94269) were screened. Clones that hybridized in the initial screen were retested by digesting with *Pst*I to release the insert, separating the fragments on agarose gels, blotting, and probing as described previously. Ability of each clone to reproduce the original DNA fingerprint pattern was tested by Southern analysis of genomic DNA digested with *Pst*I.

DNA sequence analysis of pSTL70 and related clones. The original pSTL70 plasmid plus three clones that hybridized after two rounds of screening were sequenced on an ALFexpress automated DNA sequencer (Amersham Pharmacia Biotech, Piscataway, NJ) as described previously (11,12). After each round of sequencing, new primers were made, and the remaining unsequenced portion of the clone was amplified by PCR and cloned as described previously (11,12). This process continued until the complete sequence was obtained. Several PCR clones were sequenced at each step to minimize errors introduced through imperfect amplification. Therefore, each region was sequenced a minimum of three to five times. The individual sequence pieces were assembled

TABLE 1. Summary information about the *Mycosphaerella graminicola* DNA fingerprint probe pSTL70 and three additional clones that hybridized to pSTL70 in Southern analyses[a]

Clone	Size (bp)	Overlap with pSTL70[b]	Blastx results	Expected value	Characteristics
pSTL70	2,860	NA	*Sln*1 homologue[c]	6e-40	29- and 79-bp repeats
2E11	3,073	1–1,279	*Sln*1 homologue	3e-34	Coding sequence
9A5	2,640	1,351–2,441	…	…	29-bp repeats
11E8	1,636	2,439–2,860	Reverse transcriptase	0.012	Coding sequence

[a] NA = not applicable and … = no matches.
[b] Indicates the range of bases on the pSTL70 clone that had high similarity with the other clones.
[c] *Sln*1 is an osmosensing histidine kinase that forms part of a two-component regulatory system in the yeast *Saccharomyces cerevisiae* (29).

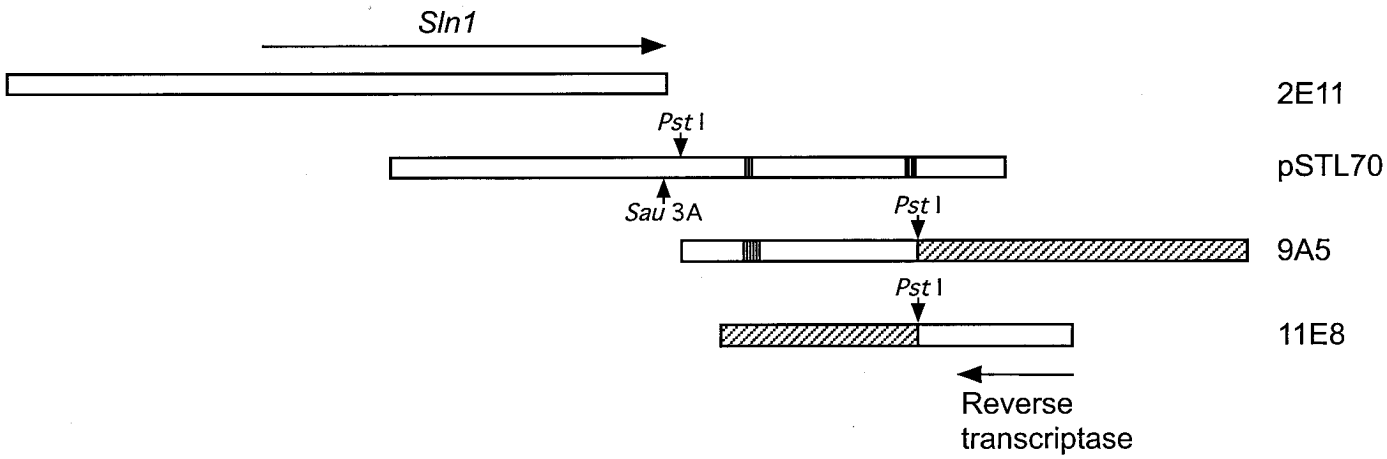

Fig. 1. Relationships between the DNA fingerprint probe pSTL70 from *Mycosphaerella graminicola* and three other clones that hybridized to pSTL70 in Southern analyses. Clone names are indicated on the right. Horizontal lines above and below clones 2E11 and 11E8, respectively, indicate coding sequences. Vertical arrows pointing downward indicate recognition sites for the restriction enzyme *Pst*I. The upward-pointing vertical arrow indicates a *Sau*3A site. Narrow vertical bars indicate the locations of 29-bp repeats in clones pSTL70 and 9A5. Slightly wider vertical bars on pSTL70 show the locations of the two 79-bp repeats. Clones 9A5 and 11E8 are chimeras; hatch marks indicate the portions of the chimeras that do not correspond to any part of pSTL70. Clone pSTL70 also probably is a chimera at the indicated *Sau*3A site.

into the complete sequence with the Contig Manager of MacDNASIS (Hitachi Software, San Francisco).

Sequences were checked for similarity with others in GenBank with the blastn and blastx programs (1). Pairwise comparisons between sequences were made with the BLAST 2 sequences option on the National Center for Biotechnology Information web site. Restriction enzyme recognition sites within each sequence were predicted with MacDNASIS.

Culturing of fungal isolates, DNA extraction, and Southern analysis. Fungal isolates were cultured on yeast-malt agar (YMA) plates (4 g of yeast extract, 4 g of malt extract, 4 g of sucrose, 15 g of agar per liter, with kanamycin to 50 µg/ml added after autoclaving) at room temperature. Tissue for DNA extraction was grown for up to 2 weeks in yeast-malt broth (YMA without the agar) at room temperature on a shaking platform at 150 rpm, harvested by vacuum filtration, lyophilized 24 to 30 h, and stored at –80°C. Long-term storage of cultures was on lyophilized filter-paper disks at –80°C.

DNA was extracted essentially as described by Ossanna and Mischke (28), except without the final chloroform extraction, and was quantified with a Hoefer DyNAQuant 2000 fluorometer (Hoefer Scientific Instruments, San Francisco). Southern analysis was with alkaline transfer (32) and chemiluminescence as described previously. Exposures ranged typically between 5 and 30 min, occasionally overnight.

Testing the stability of DNA fingerprint patterns during sexual and asexual reproduction. To test the stability of the DNA fingerprint bands during sexual reproduction, DNA from both parents plus 60 progeny isolates of the *Mycosphaerella graminicola* mapping population (16) was digested with the restriction enzyme *Pst*I, fixed to Zeta-Probe membranes by Southern transfer, and hybridized with the reverse transcriptase portion of the 11E8 clone (probe 11E8rt).

To test for stability of the DNA fingerprint patterns during asexual reproduction, single-spore cultures were started from each of the two parents of the mapping population (isolates IPO323 and IPO94269) by streaking conidia onto YMA plates. After 3 to 6 days, conidia from 10 single-spore colonies from each "parent" isolate were streaked onto fresh YMA plates. The 10 single-spore lines from each isolate were then carried for nine additional single-spore transfers, yielding 20 A_{10} lines (10 from each parent), each the result of 10 asexual transfers. Assaying these 20 lines was expected to reveal any changes that occurred during 200 asexual transfers (100 transfers from each parent). Genomic DNA from the parents and all 20 A_{10} lines was extracted, digested with *Pst*I, and analyzed by Southern hybridization with the 11E8rt probe as described above.

To test for differences in methylation, DNA from the parents of the mapping population and representatives of five asexual lines that showed changes in DNA fingerprint pattern were digested with the methylation-sensitive and -insensitive isoschizomers *Mbo*I and *Sau*3AI, blotted and hybridized with the 11E8rt probe. *Mbo*I cleaves sites containing 5-methylcytosine but fails to cut at those with 6-methyladenosine, whereas *Sau*3AI is the opposite: it does cleave sites containing 6-methyladenosine but fails to cleave those with 5-methylcytosine.

To test the evolutionary conservation of the sequence responsible for the fingerprint patterns, DNA from four species of *Cercospora* (*C. sorghi*, *C. sorghi* var. *maydis*, *C. zeae-maydis* Group I, and *C. zeae-maydis* Group II) and three isolates of *Septoria passerinii* (a very close relative of *Mycosphaerella graminicola*) also were digested with *Pst*I, blotted, and probed with 11E8rt as described previously.

RESULTS

Additional clones hybridizing to pSTL70. Among 3,936 clones screened, 5 hybridized strongly to pSTL70. Four of these clones (2E11, 9A5, 11E8, and IC1) were from the small-insert library of isolate IPO323, while the remaining clone, 16D7, was from isolate IPO94269. Complete sequences were obtained for the original pSTL70 clone and three others. Clones 2E11, 9A5, and 11E8 each had large regions of near identity with pSTL70 (Table 1, Fig. 1). However, the three clones shared no similarity with each other. Database searches of the putative translation products with the blastx program identified a number of GenBank accessions with similarity to clones pSTL70, 2E11, and 11E8 (Table 1). The original pSTL70 clone had high similarity to a two-component regulator gene from yeast, *Sln*1 (29), and similar genes from *Emericella* (anamorph *Aspergillus*) *nidulans* and *Candida albicans* (26). Clone 2E11 had high similarity to the same genes (Table 1). This clone overlapped and extended the sequence at the 5′ end of pSTL70 (Fig. 1).

Clone 9A5 had no similarity to any sequences in GenBank. The first 1,103 bases of this clone were virtually identical to bases 1,351 to 2,441 of pSTL70, but the final 1,537 bases were unrelated (Fig. 1). These two pieces of clone 9A5 were joined at a *Pst*I recognition site. Therefore, this clone most likely was a chimera composed of two unrelated inserts. Clones pSTL70 and 9A5 both contained a 29-bp sequence that was tandemly repeated three times in pSTL70 and six times in 9A5 (Figs. 1 and 2). In addition, pSTL70 had a 79-bp tandem repeat 554 bases downstream from the 29-bp repeats (Figs. 1 and 2).

Clone 11E8 corresponded to and extended the 3′ end of pSTL70 beginning just downstream from the 79-bp repeats (Fig. 1). The sequence of clone 11E8 was similar to those for putative reverse transcriptase genes in plants (*Arabidopsis*), several insects, and the horseshoe crab *Limulus polyphemus*, although the E-values were relatively low (Table 1). Clone 11E8 had no similarity with any fungal sequences in the database. The final 239 bases of pSTL70 contained part of the putative reverse transcriptase coding sequence, but not enough of the gene to obtain positive matches in

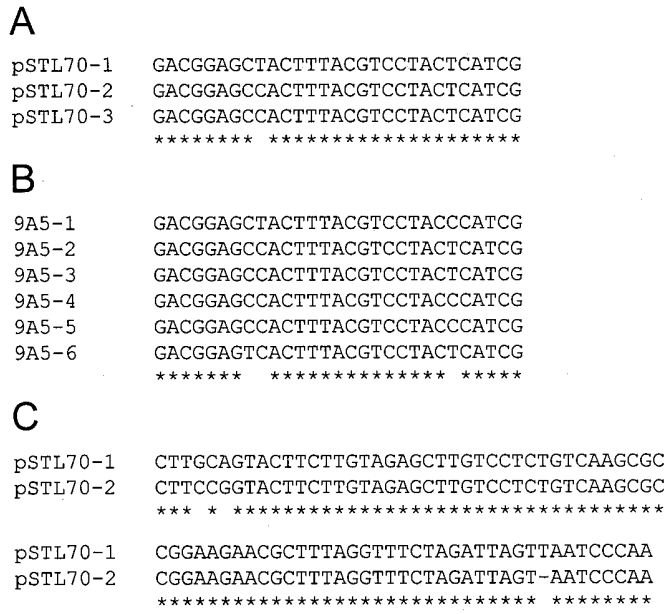

Fig. 2. Alignment of the tandem repeat sequences in clones pSTL70 and 9A5 from *Mycosphaerella graminicola*. A star under a nucleotide indicates that all sequences are identical at that position. **A,** The 29-bp repeats in clone pSTL70. All three repeats are identical except for a single C:T transition in repeat 1. **B,** The 29-bp repeats in clone 9A5. All six repeats are identical except for three C:T or T:C transitions. One of the transitions in clone 9A5 occurred at the same position as the transition in the pSTL70 29-bp repeats. **C,** The 79-bp repeats in clone pSTL70. Both repeats are identical except for a G:C transversion, an A:G transition, and a single T deletion in repeat 2 compared to repeat 1.

GenBank. The first 914 bases of the 11E8 clone were not related to the sequence of pSTL70. A *Pst*I site beginning at base 915 of 11E8 indicated that this clone also was a chimera. For the two chimeric clones 9A5 and 11E8, the regions corresponding to parts of pSTL70 were subcloned. These subclones were designated 9A5-a and 11E8rt and were used for all subsequent analyses. Sequences of clones pSTL70, 2E11, 9A5, 9A5-a, 11E8, and 11E8rt were deposited as GenBank Accession Nos. AF347057 to AF347062.

Southern analyses of genomic DNA from the parents and several progeny isolates revealed that clone 2E11 contained single-copy DNA with an obvious polymorphism that segregated among the progeny of the *Mycosphaerella graminicola* mapping population (Fig. 3A). Clone 9A5-a duplicated the original fingerprint pattern of pSTL70, but without the single-copy bands produced by clone 2E11 (Fig. 3B and C). Thus, the pattern revealed by pSTL70 was simply the summation of those produced by 2E11 and 9A5-a. Clone 11E8rt gave a fingerprint containing a subset of the bands revealed by clones pSTL70 and 9A5-a (Fig. 3D). The fingerprint patterns produced by clones 11E8rt and 9A5-a were identical except that four of the bands revealed by 9A5-a were absent when the same blot was probed with 11E8rt. Two of these missing bands had a lower intensity on blots probed with 9A5-a compared with other bands in the same lanes (Fig. 3C).

Partial sequences were obtained for the two remaining clones, 16D7 and IC1. Clone IC1 had an internal *Pst*I site and appeared to be a chimera composed of two inserts of 2.4 and 1.7 kb. Only the 1.7-kb insert hybridized to pSTL70 in Southern analyses (data not shown). This fragment was subcloned. Sequences at the ends of the subclone had high similarity to the sequences for 11E8 and 9A5.

Clone 16D7 was approximately 3 kb. This clone did not contain an internal *Pst*I site and, therefore, was not a chimera. Sequences of approximately 0.8 kb from each end had no similarity to any of the other clones analyzed. Clone 16D7 gave the same DNA fingerprint pattern as pSTL70 when used as a probe to genomic DNAs in Southern analyses (data not shown).

Changes in DNA fingerprint patterns during sexual and asexual reproduction. Among the 60 progeny of the mapping population tested, 31 had one or more bands that were not present in either of the parents (Fig. 4). In total, five new bands appeared among the progeny isolates. Two progeny had two new bands, but the rest only had one each. Different progeny often had new bands of approximately the same size; 16 progeny isolates had a 2.9-kb band that was absent from both parents. However, it was not possible to determine whether new bands of the same size in different progeny isolates resulted from independent events or were derived from a single change that occurred prior to meiosis.

Both gains and losses of DNA fingerprint bands were observed in some of the asexual progeny. All of the 10, 10-transfer single-spore lines had the same DNA fingerprint pattern as their parent isolate IPO323. However, four of the A_{10} lines from parent

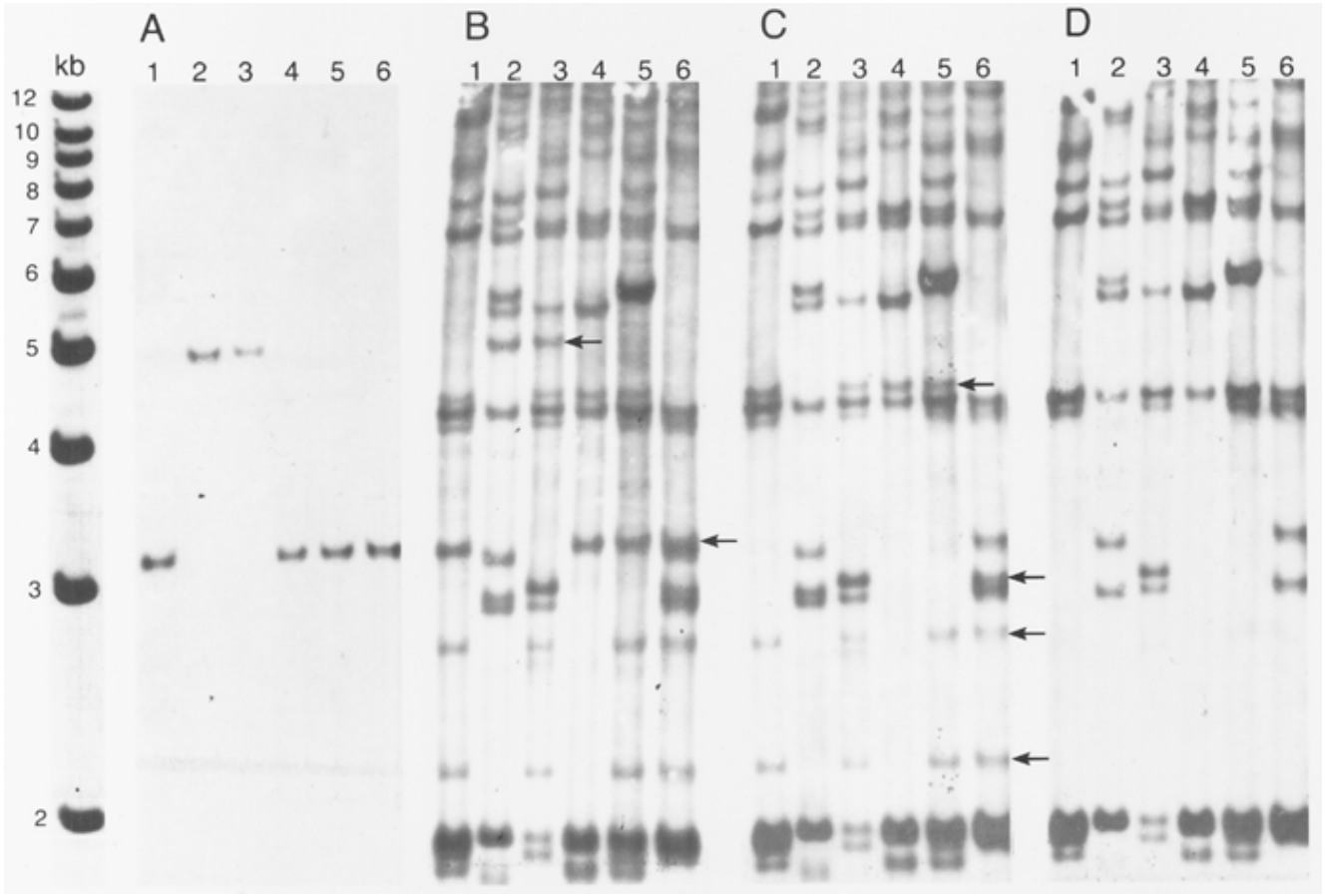

Fig. 3. Hybridization patterns produced by different fragments of the pSTL70 DNA fingerprint probe from *Mycosphaerella graminicola.* The same blot is shown in each panel. Lane 1, parent isolate IPO323; lane 2, parent isolate IPO94269; lanes 3 to 6, progeny isolates 139, 166, 184, and 194, respectively, of the cross between isolates IPO323 and IPO94269. Size markers for a 1-kb ladder from Stratagene are indicated on the left. **A,** Blot probed with clone 2E11, containing the single-copy *Sln1* gene homologue from *Mycosphaerella graminicola.* **B,** Blot probed with the original pSTL70 DNA fingerprint probe from *Mycosphaerella graminicola.* This pattern is identical to that produced by clone 9A5-a (panel C) with the addition of the bands from clone 2E11 (panel A). Arrows indicate bands produced by the single-copy portion of the probe that contains the *Sln*1 gene sequence. **C,** Blot probed with clone 9A5-a, containing the direct repeats that probably correspond to one end of the putative transposable element. Arrows indicate bands identified by the direct repeats that are not revealed by the reverse-transcriptase sequence (panel D) alone. **D,** Blot probed with clone 11E8rt, containing the reverse-transcriptase sequence of the putative transposable element. This probe gives a DNA fingerprint pattern with a subset of the bands seen for probes pSTL70 and 9A5-a.

IPO94269 had gains or losses of one or more fingerprint bands (Fig. 5). Isolate IPO94269 A_{10} line number 2 had four additional faint bands compared with its parent, line 3 had one faint (1.7-kb) and two major (5.5- and 7.0-kb) bands missing, line number 4 gained a 3.8-kb band, and line 5 lost two bands of 4.1 and 6.7 kb (Fig. 5). To test the consistency of these changes, five additional isolates from the tenth transfer of IPO94269 lines 2, 3, 4, and 5 were checked for DNA fingerprint pattern. All five additional isolates had the same fingerprint patterns as the original A_{10} isolates and confirmed the changes in lines 4 and 5 (Fig. 6). The four faint bands in line 2 were not present in the five additional A_{10} isolates from this line (Fig. 6). Therefore, these bands probably were from incomplete digestion of the original DNA sample. The 5.5-kb band that was missing in the original line 3 A_{10} isolate also was missing in the five additional isolates. However, the 7.0-kb band that was missing in the original isolate (Fig. 5) was present in the additional five isolates (Fig. 6). This may represent a change that occurred in the ninth-generation single-spore isolate that was "segregating" in isolates derived from the tenth transfer.

Evolutionary conservation and effect of methylation. When clone 11E8rt was used as a probe, no hybridization was detected with any of the *Cercospora* or *Septoria* species tested under high-stringency conditions (data not shown). Possible hybridization at low stringency was not tested.

No isoschizomers of *Pst*I are available commercially; therefore, it was not possible to compare the fingerprint patterns of methylation-sensitive and -insensitive isoschizomers of this enzyme directly. However, tests with the restriction enzymes *Mbo*I (insensitive to 5-methylcytosine, sensitive to 6-methyladenosine) and *Sau*3AI (insensitive to 6-methyladenosine, sensitive to 5-methylcytosine) revealed no differences in the RFLP patterns (Fig. 7). Unfortunately, these enzymes only yield four resolvable bands in Southern analyses with clone 11E8rt as a probe; therefore, it was not possible to assay the full range of fingerprint variation. However, it seems unlikely that changes in the DNA fingerprint patterns are caused by differences in methylation.

DISCUSSION

These results indicate that the DNA fingerprint probe pSTL70 from *Mycosphaerella graminicola* (21) contains part of a transposable element. The 29- and 79-bp repeats and coding sequence for a reverse transcriptase gene are characteristics of transposable elements from other organisms (7). Furthermore, subclones containing only the repeats or reverse transcriptase sequences duplicated most of the original DNA fingerprint bands, indicating that both of these sequences occur in multiple copies, and adjacent to each other, in the genome of *Mycosphaerella graminicola.* The rest of the pSTL70 clone was single copy, and contained part of the open reading frame for a homologue of the yeast two-component regulator gene *Sln*1. This gene has been shown to function as an osmosensing histidine kinase in yeast (29) and *Candida albicans* (26), and a presumed homologue was found in the filamentous ascomycete *Emericella* (*Aspergillus*) *nidulans* (GenBank Accession No. AB036054). Although *Sln*1 appears to be involved in osmotic regulation in these other species, the function of this sequence in *Mycosphaerella graminicola* remains unknown. The presumed *Sln*1 gene sequence ends at a *Sau*3A site in pSTL70. Because this clone was derived from a library made from a partial digest of genomic DNA with *Sau*3A (3), it seems likely that pSTL70 may be a chimera composed of two inserts, one part containing the *Sln*1 gene and the other containing the partial transposable element. Another possibility is that the transposable element is inserted into the *Sln*1 coding region. Obtaining the complete sequence of the transposable element is required to test this hypothesis.

This is only the second transposable element identified from any species related to the genus *Mycosphaerella* or in the order *Dothideales* (17). The only other transposable element identified from a species in the *Dothideales* is a retrotransposon found in the tomato pathogen *Cladosporium fulvum* (24). Although the teleomorph of *C. fulvum* is not known, this species was shown to cluster with *Mycosphaerella* in a recent phylogenetic analysis (11). The putative *Mycosphaerella graminicola* transposable element may be different from others identified previously in fungi, because there were no matches with any fungal sequences in GenBank. Instead, the closest matches were to reverse transcriptase sequences in *Arabidopsis*, horseshoe crabs, and various species of insects (springtails, fruit flies, and Japanese beetles). Because it was not present in the other *Mycosphaerella* relatives tested, including the very closely related *Septoria passerinii* (12), the *Mycosphaerella graminicola* transposable element may have been acquired recently, as hypothesized by McDonald et al. (23).

The analysis of sexual and asexual progeny isolates indicated that the *Mycosphaerella graminicola* transposable element probably is active during mitotic and meiotic reproduction. More than half of the sexual progeny had one or more bands that were not present in the parents, indicating a relatively high rate of movement possibly stimulated by meiosis. In contrast, no evidence of instability was observed at the AFLP and random amplified polymorphic DNA loci used to construct a genetic map with the same set of progeny (16; S. B. Goodwin and J. R. Cavaletto, *unpublished data*). Amount of movement of the putative transposable element in this analysis may be an underestimate, because only new bands could be identified; a band that was lost would be scored as normal plus/minus segregation. The appearance of new bands of approximately the same size in different isolates might indicate targeted insertion of the transposable element at the same

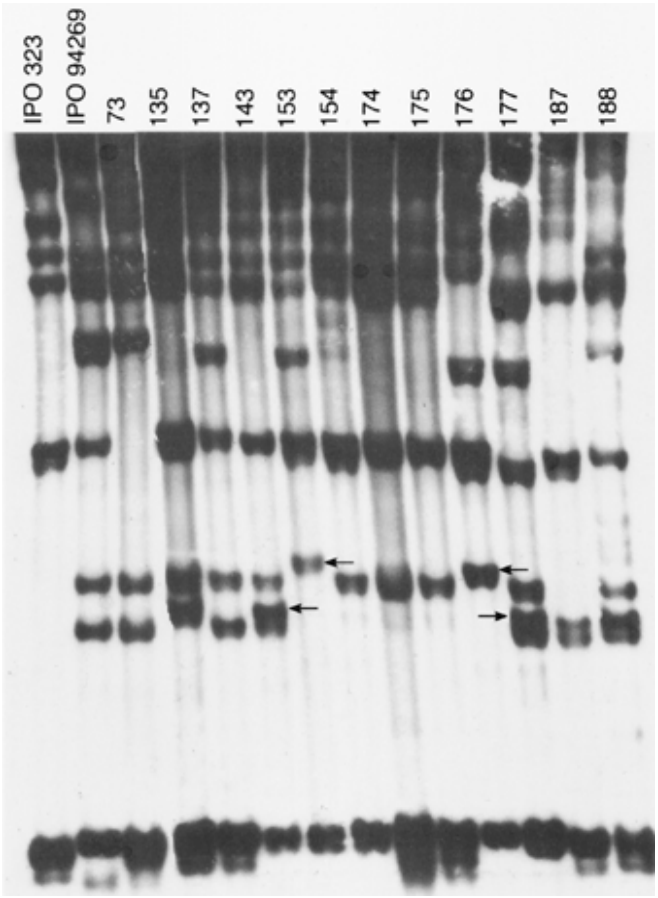

Fig. 4. DNA fingerprint patterns of the parents and 12 progeny isolates of a cross between isolates IPO323 and IPO94269 of *Mycosphaerella graminicola.* Progeny are indicated by numbers 73 through 188. Arrows indicate bands present in one or more progeny isolates that were not present in either parent.

site in multiple progeny. Similar appearances of new alleles occurred in single-copy regions of the *Magnaporthe grisea* genome near insertion sites of the retrotransposon MAGGY (27,34). Another explanation could be that transposition occurred prior to meiosis. Additional experiments are needed to test these hypotheses thoroughly.

Movement of the putative transposable element during asexual reproduction was somewhat surprising, because previous analyses showed that RFLP patterns identified with another DNA fingerprint probe, pSTL40, were stable (3). Most of the changes during asexual reproduction were seen as losses of DNA fingerprint bands. These presumably could arise by excision of the transposable element. They also could originate as mutations in *Pst*I sites or by rearrangements of the DNA near the transposable element. However, mutations and rearrangements probably would occur less frequently than movement of a transposable element; therefore, transposition is the more likely explanation. The gain of a band in IPO94269 A_{10} line 4 occurred without noticeable changes to the other DNA fingerprint bands. Therefore, the new band probably originated by transposition into a new location rather than by changes to an existing copy of the transposable element. Movement of transposons during asexual reproduction also has been shown in other fungi (25,34). Methylation seems very unlikely as the cause of these changes because digestion with enzymes that differ in their ability to cleave methylated sites revealed no changes in the resulting banding patterns.

A high rate of transposition during asexual reproduction could complicate the use of pSTL70 as a DNA fingerprint probe. However, the magnitude of this problem is still not known. Movement was observed at a high rate in only one of the two parents tested; no changes were observed in the DNA fingerprint pattern of the second parent through 100 single-spore transfers. One possible explanation for this is that the element is only active in certain genetic backgrounds. This *Mycosphaerella graminicola* transposable element could be part of a system similar to the *Ac/Ds* elements in maize (6), where the dissociation (*Ds*) element can only move in the presence of an activator (*Ac*). That could reconcile the lack of movement observed in previous studies (3) and in isolate IPO323 with the high rate of movement seen in isolate IPO94269. The types of changes observed during asexual transfers of isolate IPO94269 were very similar to those seen within clonal lineages of *Magnaporthe grisea* (19,20,34) and *Phytophthora infestans* (8,9). Therefore, DNA fingerprint patterns in *Mycosphaerella graminicola* must be interpreted cautiously; isolates that differ by only a low number of DNA fingerprint bands may be representatives of the same clonal lineage.

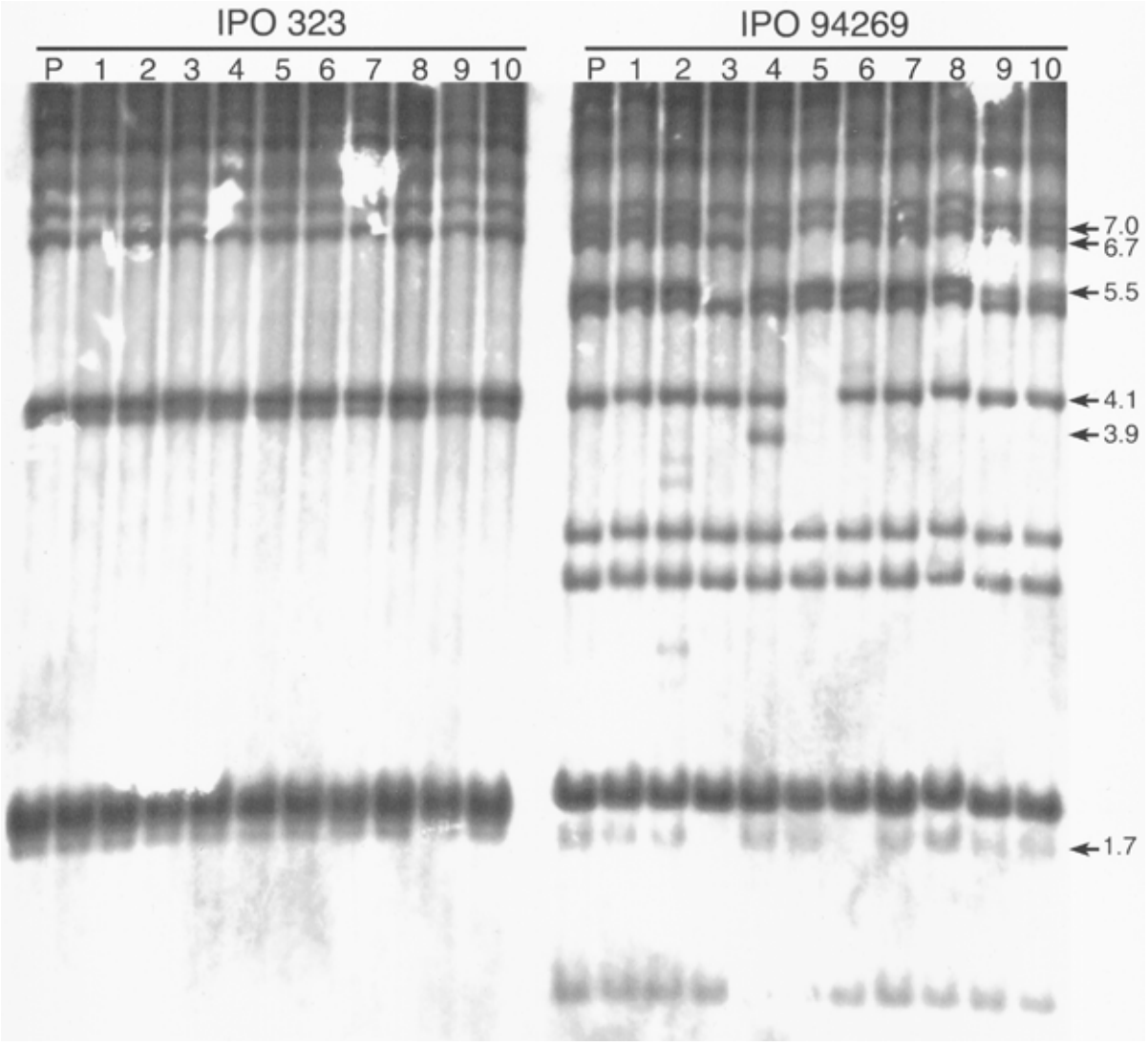

Fig. 5. Changes in pSTL70 DNA fingerprint patterns in two isolates of *Mycosphaerella graminicola* after 200 single-spore transfers (100 transfers from each parent). The two parent isolates are IPO323 and IPO94269. Parental DNA fingerprint patterns are indicated by P. Lanes 1 through 10 indicate the DNA fingerprint patterns of 10 A_{10} lines, each the result of 10 asexual single-spore transfers from the original parent isolate. Arrows indicate the locations and approximate sizes in kilobases of bands that changed in one or more of the A_{10} lines from isolate IPO94269.

An active transposable element could increase the rate of genetic change within populations of *Mycosphaerella graminicola.* Insertions, excisions, or both within coding regions could alter gene expression. Furthermore, inserted sequences could be sites for increased recombination during meiosis. Recombination, rather than transposition, could be the origin of some of the new bands seen in progeny isolates. High rates of genetic change, including duplications and recombinations, were associated with transposable elements in *Fusarium oxysporum* (14). Similar phenomena could explain the duplications and other rearrangements noted at RFLP loci in *Mycosphaerella graminicola* (22). Additional experiments are needed to test whether transposable elements cause phenotypic changes in the Septoria tritici blotch pathogen.

Transposable elements can be divided into two general types (7). Class I transposons replicate through an RNA intermediate and require a reverse transcriptase for successful transposition. Movement of class I transposable elements results in replication and amplification of transposon DNA. Class II elements transpose by DNA–DNA interactions, often by direct excision and movement of an existing transposon. Movement of class II elements may or may not increase the number of copies of the transposon (7,18). The copy number of class I transposable elements (typically 10 to 100 or more copies per genome) is usually higher than that for class II elements (with 20 or fewer copies per genome on average) (17). Other characteristics of class I elements include long terminal repeats of 200 to 600 bp flanking the sequences for several genes, including the reverse transcriptase gene required for transposition (31). Class II transposons consist typically of a transposase gene flanked by terminal inverted repeats of 10 to 100 bp. The inverted repeats of some class II elements, such as the Foldback (FB) family of transposons of *Drosophila melanogaster*, are composed of shorter direct repeats (2). Both classes occur commonly in a wide range of fungi (17).

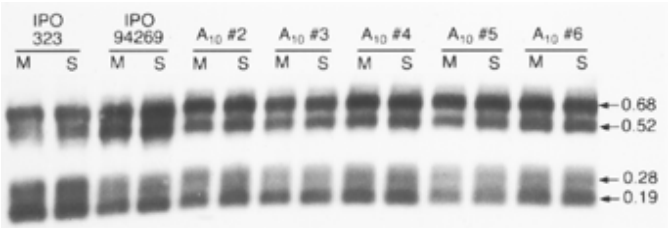

Fig. 7. Effect of methylation on restriction enzyme digestion patterns of *Mycosphaerella graminicola* revealed by probe pSTL70 for parent isolates were IPO323 and IPO94269, plus isolates from five A_{10} lines from isolate IPO94269. For each isolate, M indicates DNA digested with the restriction enzyme *Mbo*I and S indicates DNA digested with *Sau*3A. *Mbo*I and *Sau*3AI differ in their ability to cleave DNA containing methylated nucleotides: *Mbo*I cleaves sites containing 5-methylcytosine but fails to cut at those with 6-methyladenosine; *Sau*3AI cleaves sites containing 6-methyladenosine but fails to cleave those with 5-methylcytosine. These enzymes only reveal four indistinct bands in Southern analyses so it was not possible to assay the full range of fingerprint variation. Approximate sizes of the bands in kilobases are indicated on the right.

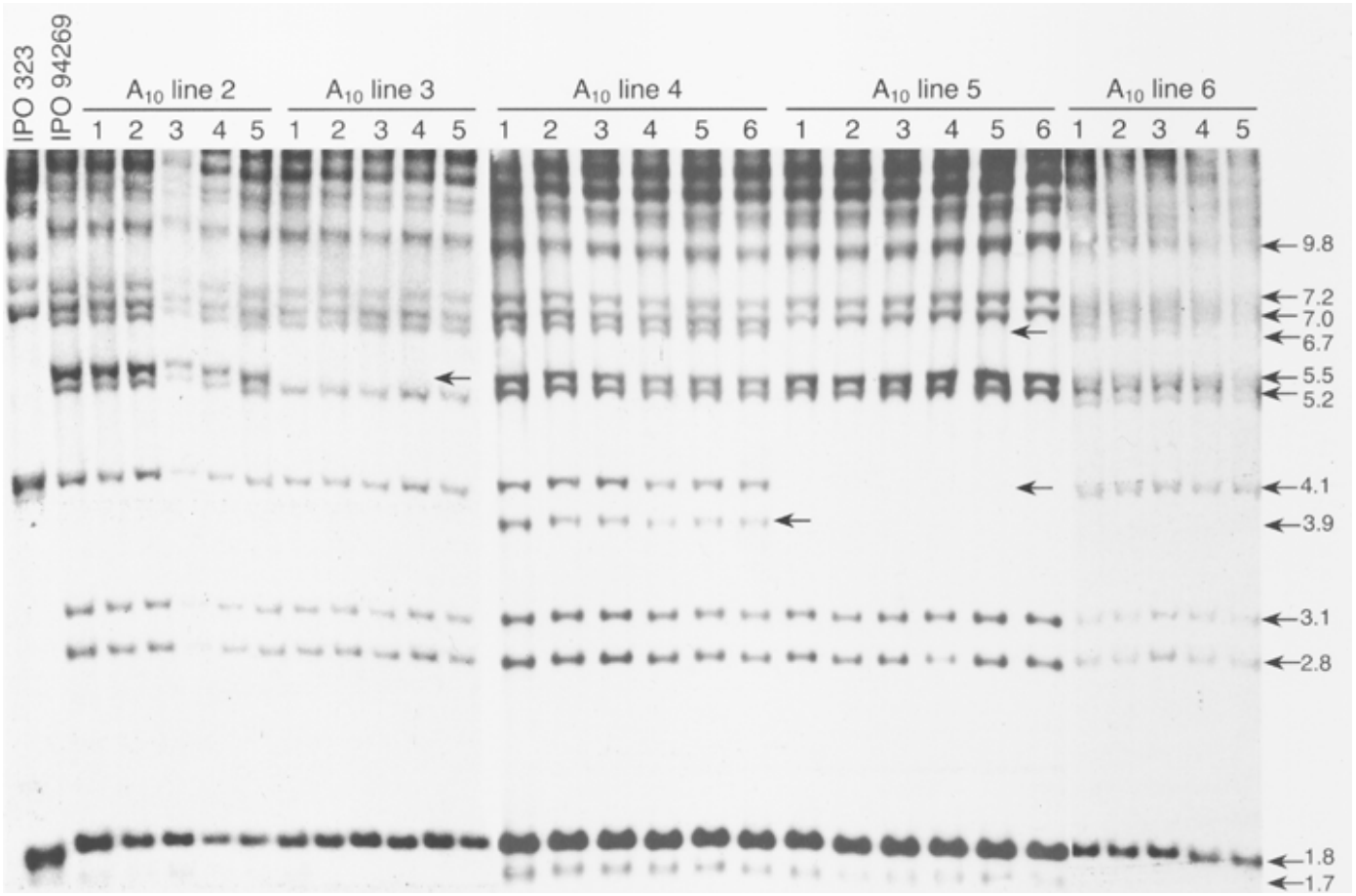

Fig. 6. Confirmation of changes in pSTL70 DNA fingerprint patterns of A_{10} lines of *Mycosphaerella graminicola* isolate IPO94269. For each A_{10} line that showed a change in the initial test, five additional isolates were taken from the A_{10} generation and assayed for DNA fingerprint pattern. For IPO94269 A_{10} lines 2, 3, and 6, only the DNA fingerprint patterns of the additional five isolates are shown. For A_{10} lines 4 and 5, DNA fingerprints of the original A_{10} isolate plus the five additional isolates are shown. Approximate sizes of the DNA fingerprint bands between 1.7 and 9.8 kb are indicated on the right. Arrows indicate bands that were changed in the original A_{10} line plus all five additional A_{10} isolates of lines 3, 4, and 5. Faint bands that were present in the original A_{10} line 2 were not present in any of the five additional line 2 A_{10} isolates. The 1.7-kb band missing from A_{10} line 6 was missing in all five additional A_{10} line 6 isolates. The 7.0-kb band that was absent in the original line 3 A_{10} isolate was present in all five additional A_{10} isolates from this line.

The class of the putative *Mycosphaerella graminicola* transposon is not clear from the data available. The reverse transcriptase coding sequence is a characteristic of class I elements. However, the 29- and 79-bp repeats are similar to the 10-, 20-, and 31-bp direct repeats within the terminal inverted repeats of the *Drosophila* FB element (2), a well-known class II transposon. Loss of bands during asexual reproduction may represent the excision and loss of a class II element. Movement in only certain genetic backgrounds also may indicate a class II element with a defective transposase gene, analogous to the *Ds* elements in maize (6). The copy number of the *Mycosphaerella graminicola* transposable element is at the low end of that typically observed for class I elements but at the high end for class II elements. A more intriguing possibility is that the 29- and 79-bp repeats might be from two different transposable elements, in which an element with 79-bp repeats transposed into a previous element with 29-bp repeats. This type of phenomenon occurs commonly in maize and may be the source of much of the repetitive DNA observed in many organisms (33). Insertion of multiple transposable elements into the same site also has been observed in *Magnaporthe grisea* (15,27). The MGR586 probe of *Magnaporthe grisea* has been shown to contain a probable class II transposable element flanked by 16-bp tandem and 9-bp inverted repeat sequences (5). If pSTL70 contains a transposable element, only the 3′ end of the reverse-transcriptase coding sequence is present. Cloning and analysis of the remaining portion of the *Mycosphaerella graminicola* transposable element will be necessary to test whether it is flanked by direct or inverted repeats, and to determine whether it is a class I or class II transposon.

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