Relationships Between Genetic Polymorphisms and Fungicide Resistance Within *Alternaria alternata*

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This work was supported by grants-in-aid from the Ministry of Education, Sciences and Cultures of Japan and from the Ministry of Agriculture, Forestry and Fisheries of Japan.
This paper is dedicated to the late Syoyo Nishimura.
We thank H. Saito, N. Doke, K. Kawakita, and H. Yoshioka for valuable suggestions and discussions.
Accepted for publication 24 July 1996.

ABSTRACT

Adachi, Y., Watanabe, H., and Tsuge, T. 1996. Relationships between genetic polymorphisms and fungicide resistance within *Alternaria alternata*. Phytopathology 86:1248-1254.

We cloned four kinds of nuclear, moderately repetitive DNA sequences from the Japanese pear pathotype of *Alternaria alternata*. Three of the four sequences were dispersed on several chromosomes of the fungus. The three dispersed repetitive sequences were used as DNA fingerprinting probes for comparison of the genetic structure between subpopulations of the Japanese pear pathotype that are sensitive and resistant to the fungicide polyoxin. Among 112 isolates collected from 15 sites in Tottori Prefecture, Japan, during July 1988, 4 and 29 isolates were identified as highly and moderately resistant, respectively; the remaining 79 were sensitive. DNA fingerprinting with the three repetitive sequences revealed a great deal of genetic variability in the pathogen population and could not differentiate polyoxin-resistant and -sensitive subpopulations. All 112 isolates were placed in 83 fingerprint types by pooling results of fingerprinting with three probes. A dendrogram of 83 types was constructed based on cluster analysis of similarity coefficients, using the unweighted pair group method with arithmetic average. The dendrogram clearly showed that subpopulations could not be differentiated in correlation with the level of polyoxin sensitivity. Similar variability also was observed in restriction fragment length polymorphisms of nuclear ribosomal RNA genes (rDNA). Polyoxin-resistant and -sensitive subpopulations carried multiple rDNA variants and shared similar variation. These results suggest that the two levels of polyoxin resistance evolved many times, resulting in a random distribution of resistance gene(s) within the genotypes.

Additional keywords: AK-toxin, black spot of Japanese pear, host-specific toxin.

One of the consequences of increasing pesticide use is the development of resistant pest populations, as has occurred in fungi (11), insects (10), and weeds (24). Development of fungicide resistance in phytopathogenic fungal populations is one of the most serious problems in agriculture. Substantial changes in the populations of several major plant pathogens in their sensitivity to fungicides have been observed, leading frequently to significant crop damage and discontinuation or modification in the use of important chemicals (11,45). Because the evolution of resistance involves changes in the frequencies of genes controlling resistance in pathogen populations, an understanding of genetic changes at the population level is important in the development of adequate and effective methods of disease management.

The Japanese pear pathotype of *Alternaria alternata* (Fr.:Fr.) Keissl., which produces host-specific AK-toxins, is the causal agent of black spot of some Japanese pear (*Pyrus serotina* Rehder var. *culta* Rehder) cultivars, including the commercially important cv. Nijisseiki (29,37,48). This disease occurred in the 1900s, just after cultivation of the Nijisseiki pear was initiated, and has been the most critical problem in pear cultivation (32,48). Polyoxin resistance in the Japanese pear pathotype was the first report of field emergence of fungicide-resistant isolates of fungal pathogens in Japan (35).

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Publication no. P-1996-0903-03R

When polyoxin, an antibiotic fungicide, was introduced in 1967, it provided nearly complete control of this disease. Polyoxin-resistant isolates were detected first in 1971 in Tottori Prefecture, Japan, and caused severe losses in pear fruit production (33–35). Polyoxin resistance in *Alternaria* species now has been reported at many locations in Japan, as have resistant isolates of other fungal genera that also were controlled with polyoxin (18).

Polyoxin selectively inhibits the synthesis of cell wall chitin in some fungi (8,12,41). Hori et al. (16,17) reported that the primary effect of polyoxin on *A. alternata* is the competitive inhibition of chitin synthase. *A. alternata* isolates could be classified either as sensitive to polyoxin or as having one of two distinct levels of resistance (33,34). Polyoxin resistance in *A. alternata* involves reduced permeability of the cell membrane to the drug (16,17). On the basis of differences in sensitivity, Georgopoulos (11) hypothesized that polyoxin resistance involves major genes. Defined genetic tests of this hypothesis, however, have not been made, because this fungus lacks a known sexual cycle.

The prevalence of polyoxin-resistant isolates led to the discontinuation of this antibiotic for use in disease management in agricultural fields. In 1976, however, the use of polyoxin to control black spot of Japanese pear was resumed in Tottori Prefecture, because the highly resistant population was very locally distributed (34,52). In contrast, isolates that express intermediate levels of resistance to polyoxin have been widely distributed in the prefecture, so the pathogen population still has the potential to cause serious problems (34,52).

Fungicide resistance is one of the important questions about population genetics of phytopathogenic fungi, because it provides

insight into how the pathogen population reacts to particular selection pressure. There are, however, relatively few studies of resistant populations in phytopathogenic fungi. It is expected that comparison of the genetic structure of fungicide-sensitive and -resistant subpopulations within the same species could show the processes of rapid evolution of fungicide resistance. The development of restriction fragment length polymorphisms (RFLPs) has advanced considerably the study of fungal population biology and genetics (5,25,27). In addition, highly polymorphic, moderately repetitive DNA sequences have been used to provide DNA fingerprints in a variety of organisms (5,6,25,27). The important applications in population genetics include identifying individuals and assessing genetic similarity within and between populations (6,25, 27).

Previously, we used RFLPs in nuclear ribosomal RNA genes (rDNA) to estimate genetic variability in *A. alternata* (1,2,23,47). We also isolated three dispersed repetitive DNA sequences, AAR sequences, from a genomic library of the Japanese pear pathotype as DNA fingerprinting probes. The objectives of this study were to describe the genetic variability within populations of the Japanese pear pathotype and to compare the genetic structure of polyoxin-resistant and -sensitive subpopulations by rDNA RFLP analysis and AAR DNA fingerprinting.

MATERIALS AND METHODS

Fungal isolates. Laboratory strain 15A of the Japanese pear pathotype of *A. alternata* was used to select nuclear repetitive DNA sequences. Field isolates of the Japanese pear pathotype were collected from 15 sites (15 orchards) in Tottori Prefecture (~3,500 km^2), located in western Japan, during July 1988 (Fig. 1) (2). Tottori Prefecture has many widely distributed orchards of pear cv. Nijisseiki, a susceptible host. The efforts to control black spot of pear have been conducted uniformly around the prefecture and have been based on annual guidelines, which include the frequency and timing of polyoxin application. The areas of the pear orchards used were 200 to 300 m^2, and the orchards contained 10 to 30 pear plants that were 20 to 30 years old. All diseased leaves were taken from different trees in each field on the same day. A single sample was removed from each leaf, even if the leaf had more than one discrete lesion. Lesions on the leaves were cut into ~4-mm^2 pieces with a sterile blade, wetted by immersion in 70% ethanol for 15 s, and surface-sterilized by immersion in 1% sodium hypochlorite for 3 min. After they were rinsed with sterile distilled water, the leaf pieces were placed on potato sucrose agar (PSA) in petri dishes and incubated at 25°C for 2 days. The growing fungi were identified by light microscopy, and their pathogenicity for pear was tested as described previously (2). A total of 112 isolates, named T88-series isolates, were maintained on PSA. Single-spore isolates were made by the method described previously (1).

Assay for sensitivity to polyoxin. Polyoxin sensitivity was tested by the spore-germination assay of Nishimura et al. (34). Isolates were cultured statically in 30 ml of potato sucrose broth (PSB) in 100-ml Erlenmeyer flasks at 25°C for 10 days. Spores were prepared from the resulting mycelial mats (15). The principal fungicide used was polyoxin B, formulated as wettable powder with 10% active ingredient. The presence of polyoxin at 1 ppm does not inhibit spore germination but causes abnormal germination of spores, which is observed as the tip swelling of germ tubes (34). Spores were suspended in distilled water with or without 1 ppm of polyoxin to give a concentration of ~10^5 spores per ml, and a 100-µl portion was placed on a glass slide. After incubation in a moist chamber at 25°C for 24 h, the percentages of normally germinating spores were determined by counting at least 200 spores. Spores of all isolates used germinated normally in the absence of polyoxin: the percentage of normally germinating spores was more than 90%. Polyoxin sensitivity was classified into three distinct levels based on the percentage of normal germination in the presence of polyoxin: up to 30% normal germination was termed sensitive, 30 to 70% was moderately resistant, and more than 70% was highly resistant (34).

DNA extraction. Fungal isolates were grown in 50 ml of PSB in 100-ml Erlenmeyer flasks at 25°C for 3 days on an orbital shaker (120 rpm). Total DNA was prepared from the resulting mycelia, as described previously (2). Plasmid DNA was extracted by the alkaline lysis method (40). Recombinant λ phage DNA was isolated by the plate lysate method (40).

Genomic library construction and screening. A genomic library of strain 15A of the Japanese pear pathotype was constructed in the λ replacement vector, λ FixII (Stratagene, La Jolla, CA) (2). The library was subjected to plaque hybridization screening with ^{32}P-labeled total DNA and rDNA clone Alt1 of strain 15A (50). Repetitive DNA clones were selected as described for *Magnaporthe grisea* by Hamer et al. (14).

Preparation and fractionation of intact chromosome-sized DNA. Protoplasts were prepared from *A. alternata* mycelia as described previously (51). Chromosome-sized DNA molecules were prepared from the fungal protoplasts by the method of Orbach et al. (36). Pulsed-field gel electrophoresis was performed with a contour-clamped homogeneous electric field (CHEF) apparatus (CHEF-DRII, Bio-Rad Laboratories, Hercules, CA). Gel contain-

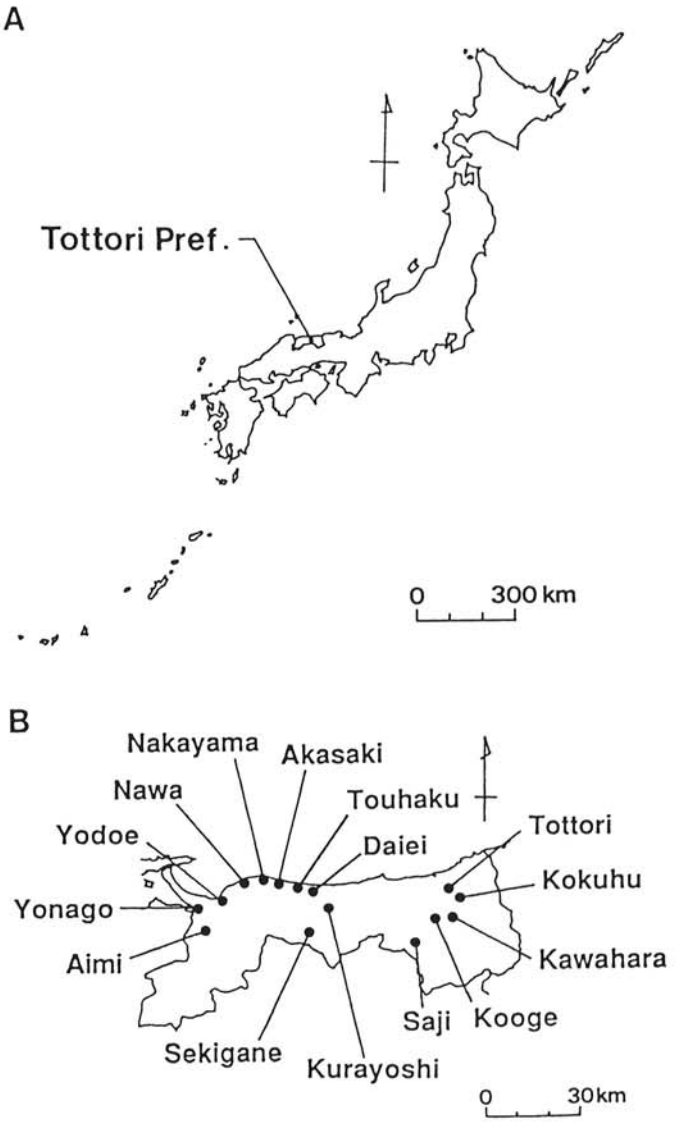

Fig. 1. Geographic origin of field isolates of the Japanese pear pathotype of *Alternaria alternata* used in this study. **A,** Location of Tottori Prefecture in Japan. **B,** Distribution of the 15 collection sites in Tottori Prefecture.

ing 0.8% Ultra PURE agarose (Gibco BRL, Gaithersburg, MD) was run in 0.5× TBE (45 mM Tris-base, 45 mM boric acid, 1 mM EDTA) at 14°C at 3 V/cm with a 120-s switching interval for 24 h, a 240-s switching interval for 24 h, followed by a 600-s switching interval for 90 h.

Hybridization. Fungal DNA was digested to completion by restriction endonucleases and electrophoresed in 0.8% agarose gel by standard protocols (40). The fractionated DNA was transferred to Hybond N+ nylon membrane (Amersham, Arlington Heights, IL) by the alkaline transfer method (39). Chromosome-sized DNA molecules separated by pulsed-field gel electrophoresis were blotted to Hybond N+ membrane as described by Orbach et al. (36).

DNA probes were produced by labeling with [α-^{32}P]dCTP (ICN Biochemicals, Inc., Costa Mesa, CA) by the random-primer method (9). Hybridization was carried out in 5× SSPE (1× SSPE = 180 mM NaCl, 10 mM sodium phosphate, pH 7.7, 1 mM EDTA) (40), containing 5× Denhardt's solution (40), 0.5% sodium dodecyl sulfate (SDS), 100 μg of sonicated salmon sperm DNA per ml, and 50% formamide at 42°C. Hybridized blots were washed at 65°C, with the final wash in 1× SSPE and 0.1% SDS.

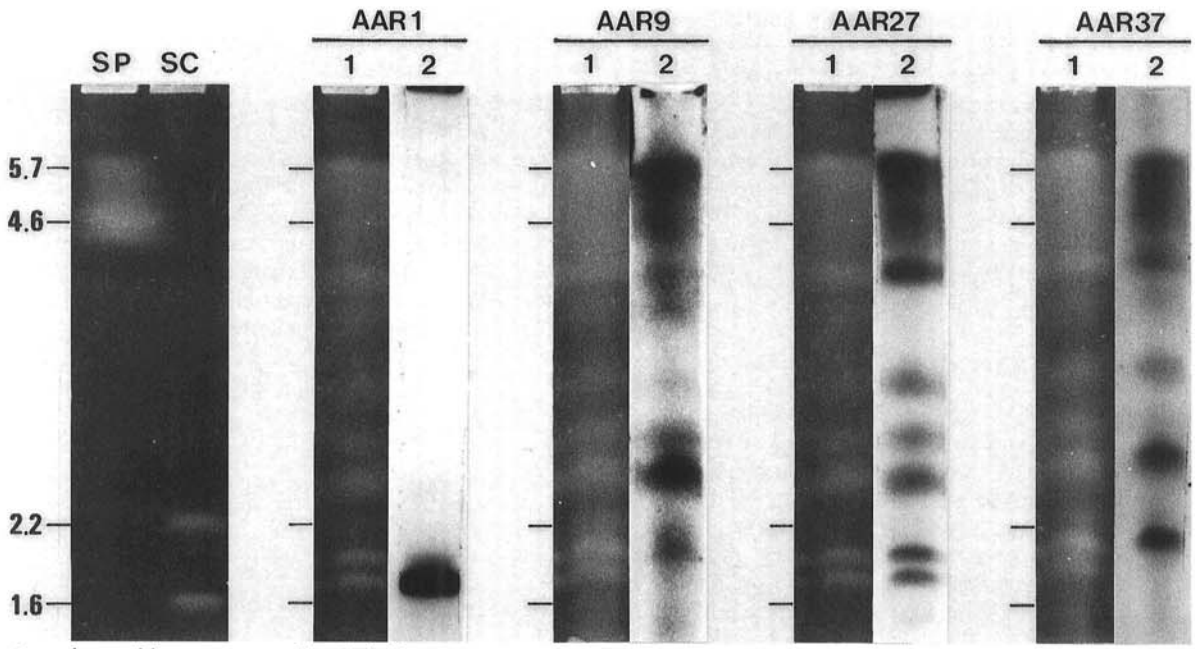

Fig. 2. Contour-clamped homogeneous electric field gel electrophoresis and hybridization of chromosome-sized DNA molecules of the Japanese pear pathotype of *Alternaria alternata*, strain 15A, with AAR1, AAR9, AAR27, and AAR37 probes. Lane 1, chromosome-sized DNA electrophoresed in 0.8% agarose gel and stained with ethidium bromide. Lane 2, Southern blot hybridized with ^{32}P-labeled AAR1, AAR9, AAR27, or AAR37 DNA. The sizes (in megabases) of marker DNA molecules (chromosomal DNA of *Saccharomyces cerevisiae* [SC] and *Schizosaccharomyces pombe* [SP]) are indicated on the left.

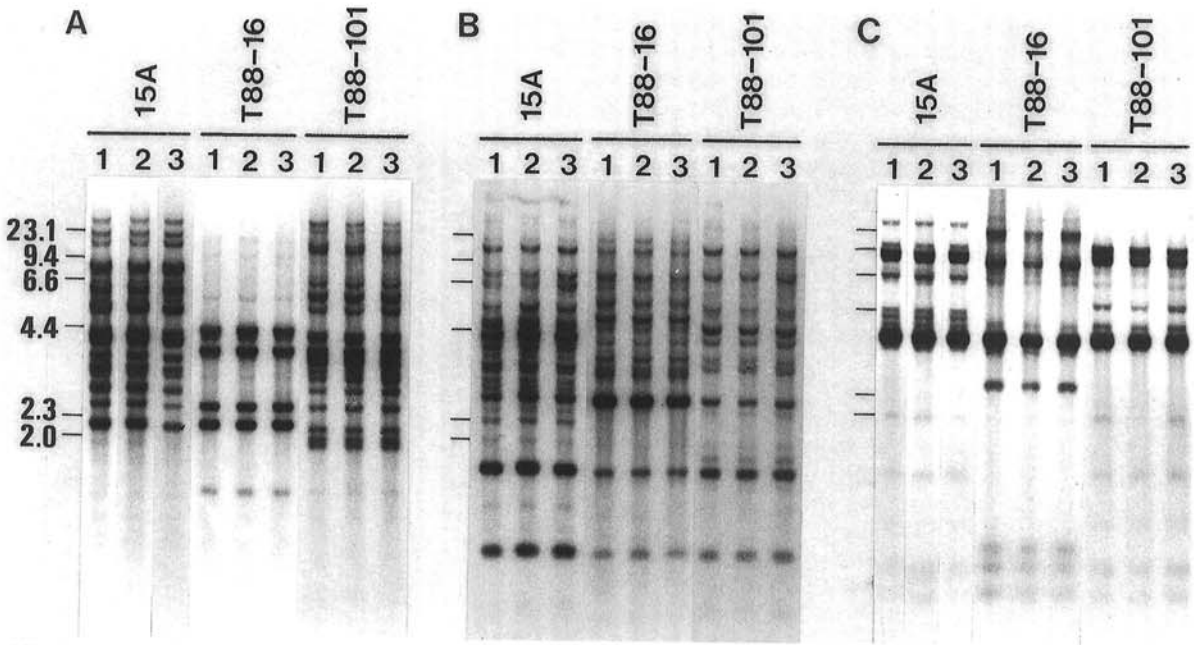

Fig. 3. Hybridization of total DNA from strains 15A, T88-16, and T88-101 of the Japanese pear pathotype of *Alternaria alternata* with **A,** pAR274, **B,** AAR9, and **C,** AAR37 probes. Potato sucrose agar cultures of the strains after three and six serial transfers were subjected to hybridization of total DNA with AAR probes. Total DNA was digested with **A,** *Sal*I, **B,** *Pst*I, or **C,** *Hin*dIII and fractionated in 0.8% agarose gel. The Southern blot was hybridized with ^{32}P-labeled AAR probes. The sizes (in kilobases) of marker DNA fragments (*Hin*dIII-digested λ DNA) are indicated on the left. Lane 1, original culture; lane 2, culture after three serial transfers; and lane 3, culture after six serial transfers.

Data analysis. The genetic similarity of isolates was assessed based on DNA fingerprints by the similarity coefficient of Nei and Li (31). The restriction fragment sizes and polymorphisms were determined from developed autoradiographs. The relative intensity of bands was ignored. The presence or absence of comigrating restriction fragments was scored for all isolates. A dendrogram was constructed from the similarity coefficient data by the unweighted pair group method with arithmetic mean average clustering (44).

RESULTS

Cloning of repetitive DNA sequences. Recombinant clones containing repetitive DNA sequences were identified in a genomic library of strain 15A of the Japanese pear pathotype of *A. alternata*. Of 3,500 plaques containing recombinant phage DNA, 39 hybridized intensely to genomic DNA but not to rDNA. The presence of repeated DNA sequences was confirmed in 27 of the clones by slot blot hybridization of recombinant phage DNA with a total genomic DNA probe. Four independent clones that did not share homology were selected by cross-hybridization experiments: AAR1, AAR9, AAR27, and AAR37.

AAR sequences were hybridized to chromosome-sized DNA molecules separated by CHEF electrophoresis. At least nine chromosome-sized DNAs of strain 15A were resolved by CHEF electrophoresis (Fig. 2). AAR9, AAR27, and AAR37 hybridized to multiple bands: AAR9 and AAR27 hybridized to eight bands, and AAR37 hybridized to seven (Fig. 2). In contrast, only one band was hybridized with AAR1 (Fig. 2). These results indicate that AAR9, AAR27, and AAR37 carry nuclear repetitive DNA sequences dispersed in the fungal chromosomes.

Hybridization of restriction fragments of AAR DNA with genomic DNA showed that AAR27 carried a relatively small repetitive sequence and that other clones carried larger sequences (data not shown). A 2.2-kb *Sal*I segment within AAR27 that contained repetitive sequences was identified and subcloned in plasmid vector pSP72 (Promega Corporation, Madison, WI). The subclone was named pAR274 and was used for DNA fingerprinting.

Total DNA of strain 15A was digested with *Bam*HI, *Eco*RI, *Hin*dIII, *Pst*I, *Sal*I, or *Xba*I and probed with ^{32}P-labeled pAR274, AAR9, and AAR37 DNA. Each probe hybridized to multiple restriction fragments. To verify banding pattern stability during vegetative growth, six serial transfers of plate cultures with strains 15A, T88-16, and T88-101 were conducted. Each culture was done on PSA at 25°C for 10 days. No changes in fingerprint pattern were observed during serial transfers (Fig. 3). Additionally, 24 single-spore isolates from each strain were subjected to DNA fingerprinting. All single-spore isolates had hybridization patterns identical to their parents. These results suggest that the AAR fingerprints are mitotically stable and that the AAR sequences are useful as DNA fingerprinting probes for population studies of the pathogen.

rDNA variation within polyoxin-resistant and -sensitive subpopulations. Polyoxin sensitivity of 112 isolates sampled in Tottori Prefecture during July 1988 could be classified into three distinct levels that were termed sensitive, moderately resistant, and highly resistant. The percentages of normal germination in 1 ppm of polyoxin were less than 20% in 79 sensitive isolates, 30 to 50% in 29 moderately resistant isolates, and more than 70% in 4 highly resistant isolates (Fig. 4). Polyoxin-resistant isolates were detected in 12 of 15 sites in Tottori Prefecture, showing that this trait is widely distributed in the prefecture (Fig. 4).

To assess the genetic relatedness between polyoxin-resistant and -sensitive subpopulations, the distribution of rDNA variation was compared between the subpopulations based on polymorphisms in the *Xba*I-digestion patterns. Altogether, five rDNA variants were detected and identified as types A2, A4, A5, B1, and B2, which have been characterized in our previous study (Table 1) (2). These polymorphisms were explained on the basis of the length and presence or absence of *Xba*I sites in the intergenic spacer region (2). Highly resistant, moderately resistant, and sensitive subpopulations contained three, four, and five rDNA variants, respectively (Table 1). Types A2, A4, and B2 were found in all of the subpopulations. Thus, *Xba*I RFLPs in rDNA could not differentiate subpopulations from one another.

Genetic similarity among polyoxin-resistant and -sensitive isolates. Probe-enzyme combinations pAR274-*Sal*I, AAR9-*Pst*I, and AAR37-*Hin*dIII were used to characterize 112 isolates, because they provided the most desirable resolution of fragment distribution in strain 15A. Examples of DNA fingerprints are shown in Figure 5. pAR274, AAR9, and AAR37 hybridized to 3 to 17 *Sal*I fragments, 1 to 16 *Pst*I fragments, and 2 to 14 *Hin*dIII fragments, respectively, in all isolates.

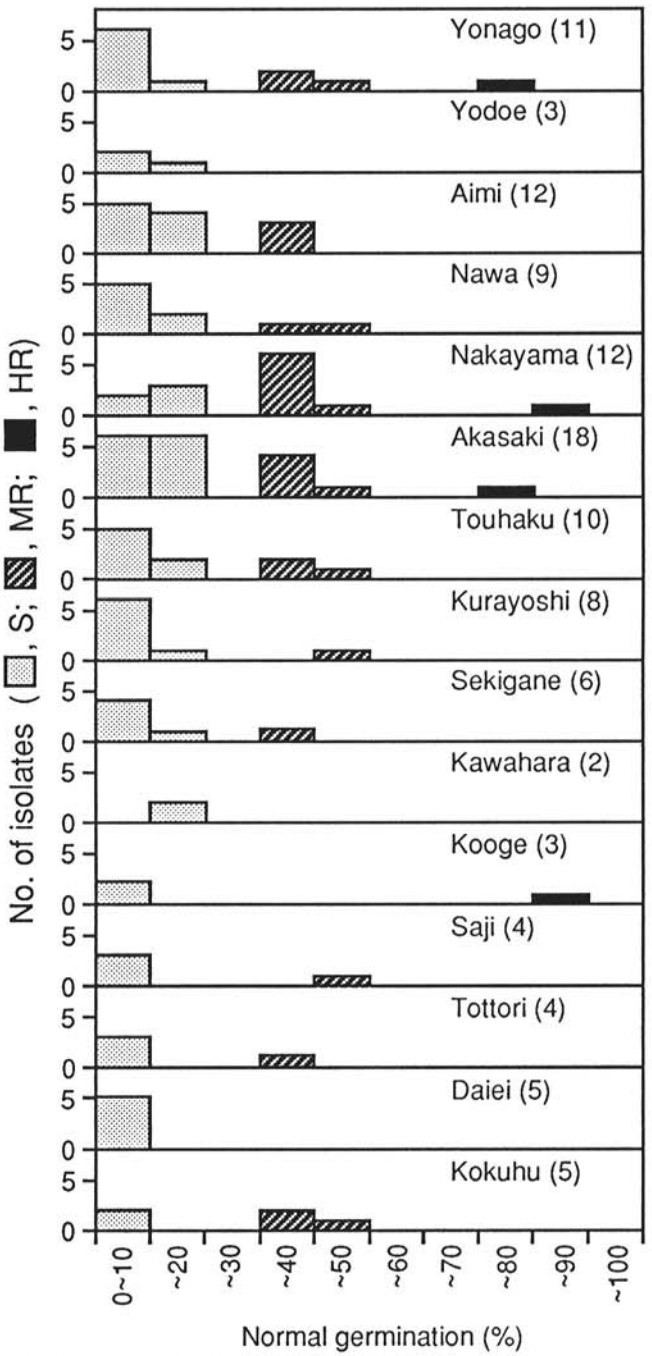

Fig. 4. Distribution of polyoxin-resistant isolates in the Japanese pear pathotype of *Alternaria alternata* collected from 15 sites in Tottori Prefecture, Japan, during 1988 (Fig. 1). The numbers of isolates tested are shown in parentheses following site names. Polyoxin sensitivity was determined by the spore-germination assay described by Nishimura et al. (35). S = sensitive; MR = moderately resistant; and HR = highly resistant.

The pAR274, AAR9, and AAR37 probes produced 42, 51, and 33 fingerprint types, respectively, and all 112 isolates could be placed into 83 types by pooling results of three probes. Genetic similarity between 83 types was calculated by similarity coefficient, and a dendrogram was constructed (Fig. 6). Of 83 types, 68 were isolate specific, and 15 were found in two or more isolates; 55 types were detected only in the sensitive subpopulation; and 23 types were detected only in resistant subpopulations: 3 and 20 types were from highly and moderately resistant subpopulations, respectively (Fig. 6). The remaining five types, 4, 12, 32, 34, and 72, were identified in both polyoxin-resistant and -sensitive isolates (Fig. 6).

The dendrogram identified three major and four minor genetic groups at a similarity level of more than 70% among the 72 fingerprint types. These groups, which contained approximately 90% of the isolates, correlated with rDNA types B1, B2, A2, and A4 (Fig. 6). The remaining 11 types appeared to be genetically diverse in DNA fingerprints and rDNA types. Polyoxin-resistant isolates were distributed in 27 fingerprint types and did not cluster into distinct genetic groups. Thus, the cluster analysis based on AAR DNA fingerprinting could not differentiate polyoxin-resistant and -sensitive subpopulations.

TABLE 1. Distribution of nuclear ribosomal RNA gene (rDNA) variants in polyoxin-resistant and -sensitive subpopulations of the Japanese pear pathotype of *Alternaria alternata* collected from Tottori Prefecture, Japan, during 1988

	No. of isolates					
	rDNA type[b]					
Subpopulation[a]	A2	A4	A5	B1	B2	Total
Highly resistant	1	2	0	0	1	4
Moderately resistant	5	4	0	15	5	29
Sensitive	11	14	3	30	21	79
Total	17	20	3	45	27	112

[a] Polyoxin sensitivity was determined by the spore germination assay described by Nishimura et al. (35).

[b] rDNA type designation based on the *Xba*I-digestion patterns described by Adachi et al. (2).

DISCUSSION

Polyoxin-resistant isolates of the Japanese pear pathotype of *A. alternata* emerged initially in 1971 in Tottori Prefecture. Highly resistant populations dominated Nijisseiki pear orchards in 1971 and declined gradually once use of polyoxin was stopped (34,52). Practical use of polyoxin resumed in 1976; however, growers cultivating Nijisseiki pear overcame polyoxin resistant *A. alternata* through the application of structurally unrelated fungicides either in rotation or in combination. In 1981, highly resistant isolates were not common in the orchards, but moderately resistant isolates had increased and often dominated (34,52). Our results showed that polyoxin-resistant, especially moderately resistant, isolates were widely distributed in the prefecture in 1988.

We previously analyzed rDNA RFLPs of 322 isolates of the Japanese pear pathotype of *A. alternata* collected from different locations and detected 8 rDNA variants within the pathogen population (2). To compare the genetic structure of polyoxin-resistant and -sensitive subpopulations, the presence and distribution of rDNA variation was investigated. The sensitive, moderately resistant, and highly resistant subpopulations all contained multiple rDNA variants, and some variants were found in more than two subpopulations. Thus, these subpopulations cannot be differentiated from one another at the rDNA level. The rDNA RFLPs may be correlated with genetic distance within and between fungal species (5). Because *A. alternata* is a haploid imperfect fungus, rDNA polymorphisms were assumed to be correlated closely with genetic relatedness among individual isolates (2). Thus, distribution of the common polymorphisms in rDNA among the three subpopulations probably reflects their close similarity in population structures.

We have cloned four moderately repetitive DNA sequences from strain 15A of the Japanese pear pathotype and named AAR clones. Three of the four sequences were dispersed on several chromosomes. The fourth was found only on one chromosome of strain 15A. Dispersed repetitive DNA sequences have been used in filamentous fungi to identify genetic subpopulations and to differentiate individual isolates within a species by means of DNA fingerprinting (5,6,27). DNA fingerprinting has been used to examine genetic variability within a population and to measure genetic sim-

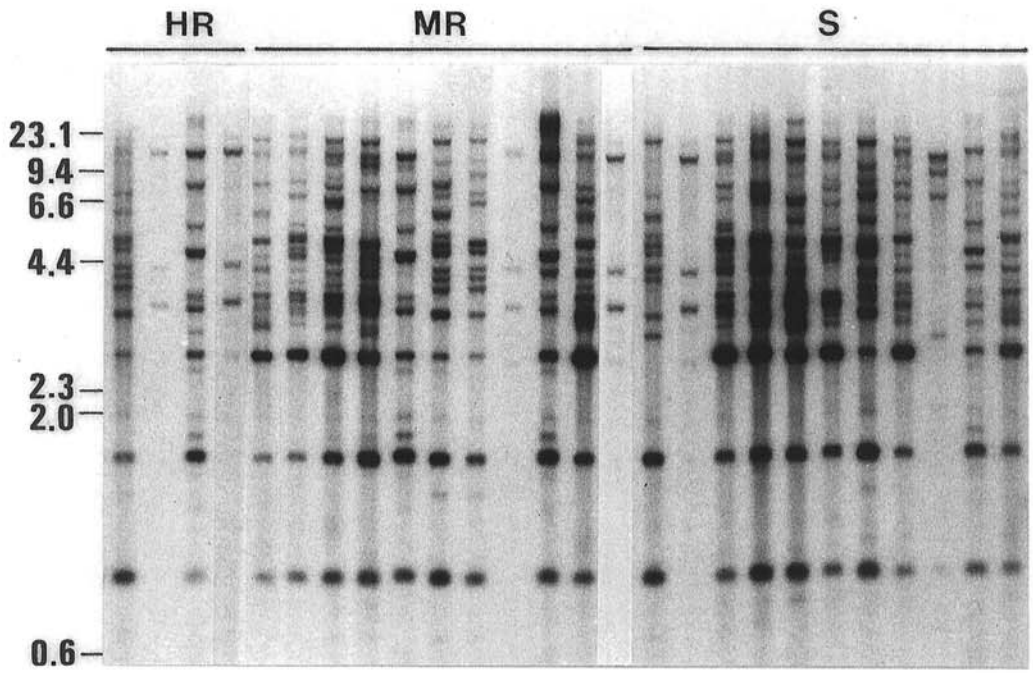

Fig. 5. AAR9 DNA fingerprints of representative isolates from polyoxin-resistant and -sensitive subpopulations of the Japanese pear pathotype of *Alternaria alternata*. Total DNA was digested with *Pst*I and fractionated in 0.8% agarose gels. The blot was hybridized with ^{32}P-labeled AAR9 DNA. The sizes (in kilobases) of marker DNA fragments (*Hin*dIII-digested λ DNA) are indicated on the left. HR = highly resistant; MR = moderately resistant; and S = sensitive.

ilarity between populations that are partitioned in time and space or defined by pathogenic specialization (3,4,13,21,22,26,28,30).

We used the three dispersed sequences as DNA fingerprinting probes for comparison of genetic structure of polyoxin-sensitive and -resistant subpopulations. The hybridization patterns showed a great deal of genetic variability in our sample and could not differentiate sensitive, moderately resistant, and highly resistant subpopulations. All 112 isolates were placed into 84 fingerprint types. The dendrogram based on cluster analysis of the fingerprinting data clearly showed that the subpopulations could not be differentiated. We detected five clonal lines that contained different isolates identical in rDNA RFLPs and DNA fingerprints but not in polyoxin sensitivity. These results suggest that the two levels of polyoxin resistance evolved many times, resulting in a random distribution of resistance gene(s) within the different genotypes.

Brown et al. (4) proposed a hypothesis concerning development of fungicide resistance and its genetic background in *Erysiphe graminis* f. sp. *hordei* on the basis of the genetic structure of the pathogen subpopulations, which were sensitive and resistant to the triazole fungicide triadimenol. If fungicide resistance was under polygenic control, isolates with the same level of resistance would be genetically similar. On the other hand, if resistance was under monogenic or oligogenic control, different clones could carry independent mutations to the same level of fungicide resistance; isolates with the same level of resistance might be genetically diverse, if, following this hypothesis, the two levels of polyoxin resistance are controlled by one gene or a few genes. However, there has been no defined genetic analysis of polyoxin resistance in *A. alternata*, which lacks a known sexual cycle. To assess the molecular basis of polyoxin resistance in *A. alternata,* we have established gene manipulation techniques for the fungus and are attempting to clone the genes involved in polyoxin resistance (19,42,51).

It has been reported that the genetic variability of resistant populations of insects and weeds selected by insecticides and herbicides, respectively, is generally reduced because of temporary, marked reduction of population size, followed by an increase of resistant populations that escape from selection by drugs (10,53). In contrast, Chauvel and Gasquez (7) reported that there was no genetic difference between herbicide-resistant and -sensitive subpopulations in the gramineous weed *Alopecurus myosuroides* based on isozyme analysis. They concluded that the genetic identity of the two subpopulations could be explained by continuous genetic exchange between resistant and sensitive plants because this species has an outcrossing mating system. Although the data, which are available for comparison among several organisms, are limited, these results may reflect the differences in strategies of adaptation among organisms.

Nuclear rDNA RFLPs and AAR DNA fingerprinting revealed that there is a great deal of variability in the Japanese pear pathotype population of *A. alternata.* Petrunak and Christ (38) found considerable genetic variation in an *A. alternata* population based on isozyme analysis. They believe that such variation occurs through recombination, whether asexual or sexual. Although it is unknown whether parasexual recombination occurs commonly in *A. alternata* in the field, heterokaryon formation has been reported in this fungus in laboratory experiments (46,49). Although no sexual cycle is known in *A. alternata*, teleomorphs for several other *Alternaria* species have been discovered (43). In addition, repetitive DNA sequences could act as a source of genetic variability in the fungal population (20). Genetic changes in fungi could involve transposition of transposons and unique crossovers mediated by dispersed repetitive DNA sequences. We have no evidence for any of these mechanisms in *A. alternata* at this time. Molecular characterization of AAR sequences may provide more information on these matters, however.

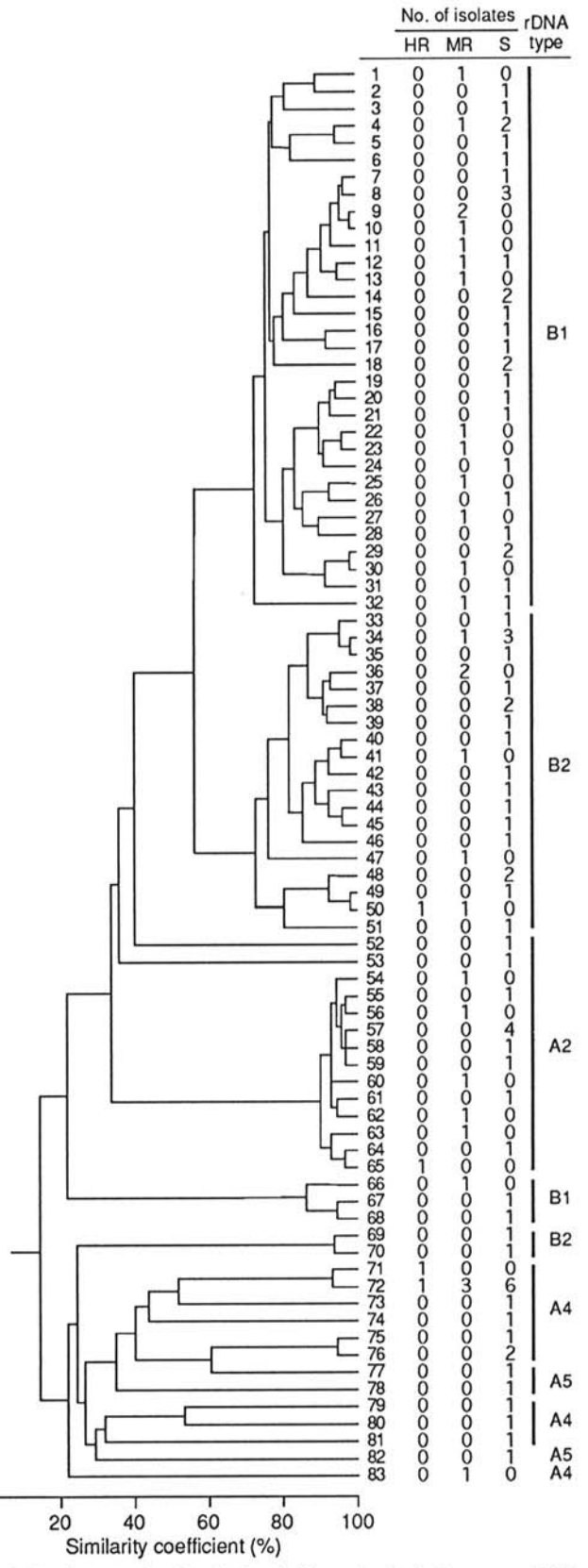

Fig. 6. Dendrogram showing the level of genetic similarity among 112 isolates of the Japanese pear pathotype of *Alternaria alternata* collected from Tottori Prefecture, Japan, during 1988. The 112 isolates were divided into 83 fingerprint types, which were determined from pooling hybridization data produced by pAR274, AAR9, and AAR37 probes. A dendrogram was constructed from similarity coefficients by the unweighted pair group arithmetic mean (45). Nuclear ribosomal RNA genes (rDNA) types were based on *Xba*I-digestion patterns. HR = highly resistant; MR = moderately resistant; and S = sensitive.

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