INVESTIGATIONS ON NEW MEANS OF IDENTIFYING RACES OF *ASCOCALYX ABIETINA*

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SUMMARY

Results of testing isolates of *Ascocalyx abietina* (Lagerb.) Schlaepfard-Bernhard with electrophoresis (PAGE) on a large scale are presented. Using US11 as reference, characteristic bands of the 155 isolates tested were obtained that permitted separating these into two distinct groups. When methods of identification by serology and electrophoresis were compared, a 96% concordance was obtained between 129 isolates tested by the first and then by the second method, and 100% concordance in a reciprocal test using 26 other isolates. Variations of protein profiles in the group classified as North American call for further investigations. The value of PAGE for routine testing of isolates to differentiate the European race is discussed. A report is also given of the successful use of some of the specific protein bands eluted from the gels, to produce monoclonal antibodies.

INTRODUCTION

Results of our previous investigations using polyacrylamide gel electrophoresis (PAGE) as an alternate test and an easier procedure than serology for identifying races of Ascocalyx abietina, were reported at the International Symposium on Scleroderris canker of conifers held in Syracuse, 1983 (Benhamou et al.,1983). We found from the protein profiles obtained that the isolates could be classified in six categories. The first three corresponded to the known races, the so-called North American, European, and Asian, as proposed according to serology tests (Dorworth et al.,1977). The other categories were: spruce isolates from Quebec, balsam fir isolates from Quebec, and an intermediate group of isolates combining characteristics of both the European and North American races on pine. The isolate F4 was used as reference for these tests. In subsequent work isolate US11 (European race) was used as reference and techniques were slightly modified to confirm and perfect the test. Another approach for differentiating races is to obtain monoclonal antibodies against proteins that are specific to the North American and European races.

This is a brief report on the current results obtained in these two types of investigations.

MATERIAL AND METHODS

A total of 129 isolates from pine that had been classified by serology were again tested with electrophoresis to confirm the concordance between race identification by both methods. Also, 26 isolates chosen randomly from a group of over a 100 classified first by electrophoresis were then tested by serology (thanks to Mr. C. Davis, Great Lakes Forestry Centre, CFS, Sault Ste-Marie, Ontario). In addition to testing these isolates obtained from infected tissue on pine or mass isolates from conidia and ascospores, 75 monoascospore isolates (chosen randomly from a collection of 425) were tested. The monoascospore isolates originated from four apothecia collected on red pine in plantations that were affected by both the North American and European races (isolates furnished by Dr. Michel Dessurault, Faculté de foresterie et de géodésie, Université Laval, Québec).

The culture medium used was: KH_2PO_4, 0.5 g; Na_2HPO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KCl, 0.25 g; Asparagine (D-L) 2.0 g; glucose (D-) 10.0 g; $FeCl_3$, 2 mL (0.5% sol); HCl 0.1N, 4.5 mL; V-8 juice, 50 mL of supernatant after centrifugation; H_2O dist. 943.5 mL. The pH was adjusted to

7.0 with NaOH and after sterilization of the medium was around 6.2. The medium was used at the rate of 125 mL per Erlenmeyer flasks. Incubation was at 14°C for 4 to 5 weeks. Methods of protein extraction and of electrophoresis were as described in Benhamou et al. (1983). The protein reagents used were mostly the Bio-Rad protein assay dye reagent concentrate.

For monoclonal antibody production, some of the specific protein bands were eluted, ammonium sulfate precipitated, dialysed, and resuspended in buffer. A sample was run again through electrophoresis to ensure it contained the desired protein before we injected it into mice. Benhamou and Ouellette (1986) give the details of this procedure and that for hybridoma production appears in Benhamou et al. (1985).

It is to be noted that the operators were not aware of previous culture identification in conducting the present tests.

RESULTS

The electrophoresis profiles obtained in the present tests contained bands that matched those already reported in Benhamou et al. (1983). The bottom bands (Fig. 1A) (towards anode end) of the gel were, however, always more pronounced and better demarcated than those observed previously (Benhamou et al.,1983). Although differences were also observed in other bands, only these two bottom bands were used as a basis for differentiating the isolates. Thus using the bands obtained from US11 as a reference the isolates could be separated into two distinct groups, the first group similar to it, being identifiable as the European race. Isolates of the other groups were considered as of North American race. However, some isolates in this first group yielded differences in some of the other band profiles that might indicate the presence of variants in the group. All the monoascospore isolates tested were uniform, except for one, and were identified as North American.

When methods of identification by serology and electrophoresis were compared, a 96% concordance between the 129 isolates tested by the first and then by the second method was obtained, and 100% congruence in a reciprocal test with 26 randomly sampled isolates. In two cases, discrepencies were registered in a first test, but clarified in a second test. Many of the cultures identified as uncertain by serology were clearly identified as North American group in the present tests.

For the production of monoclonal antibodies the specific, bottom protein bands were eluted and treated for mouse injection. Unfortunately, uncertain results were obtained probably because molecular weight of these proteins was too low. In other tests, protein bands were taken from the middle or higher portions of the gel that were also

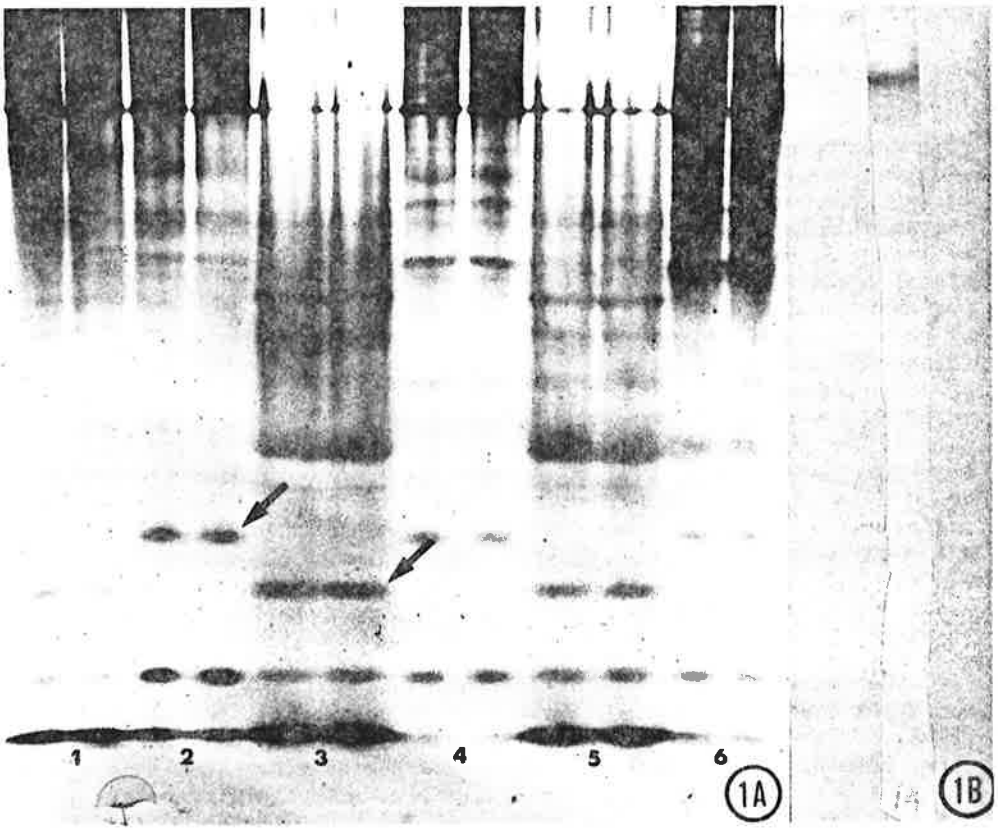

Fig. 1A. Characteristic protein profiles obtained by polyacrylamide gel electrophoresis using extracts from isolates of Ascocalyx abietina. Differences are noticeable in many of the bands, particularly in the bottom ones (arrows). Comparing the patterns of those bottom bands for six of the isolates (represented by two lanes each) isolate 1(US11), 3 and 5 are similar, and thus of the European race, whereas isolates 2, 4, 6 are different; these are of the North American race group.

Fig. 1B. Shows a specific positive reaction with Western blot (transfer of protein bands from polyacrylamide gels to nitrocellulose paper strips, treatment with antibodies and revealing reagents, see Benhamou et al.,1985) for the protein band that was used originally for monoclonal antibody production.

considered specific for either the North American or the European race. As with previous tests, the antigen solution obtained from the latter proved to be highly toxic to mice. However, as determined by ELISA tests (Ouellette and Benhamou,1986) several hybridomas were obtained for the North American race using culture filtrates as capture antigen. Some of these hybridomas were tested in western blot and found to give a strong, specific reaction (Fig. 1B).

CONCLUSION

PAGE is an easy method to sort the European race isolates of Ascocalyx abietina from other races. Except for the necessary long waiting period while cultures grow, the test is rapid, easily reproducible, and possibly less onerous than serology, at least for a large screening. We are confident therefore that this test could be advantageously used without further refinement for routine identification of cultures. However, refinements such as the use of two dimension electrophoresis, or study of comparative enzyme profiles (as is now conducted by Dr. Margarete Breitenbach, Forstliche Bundesversuchsanstalt, Vienna, Austria, Personal communication), would be greatly beneficial to further sort out the possible variants within a group, particularly, presented here as North American. Also, clarifying the status of isolates obtained from hosts other than pine, i.e. spruce, balsam fir, and larch, calls for such refinements. In attempting to establish a typical pattern for the North American race, the numerous ascopore isolates obtained would undoubtedly be a great asset.

Specific monoclonal antibodies used for race differentiation would provide an ideal method of diagnosis for A. abietina isolates. Although good progress has now been made by obtaining antibodies against the North American race, it is hampered by the difficulties in obtaining antibodies against the European race. Further efforts to settle this problem by using other European isolates as test organism are planned. In the meantime, the present monoclonal antibodies are being used to attempt following the development of the pathogen in its host.

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ZUSAMMENFASSUNG

Untersuchungen über neue Wege zur Rassen-Identifizierung bei Ascocalyx abietina

Dargestellt werden Ergebnisse aus Versuchen, in denen in großem Umfang Isolate von Ascocalyx abietina (Lagerb.) Schlaepfer-Bernhard mit Elektrophorese (PAGE) getestet wurden. Mit US11 als Referenz erhielt man bei den 155 geprüften Isolaten charakteristische Banden, die eine Trennung in zwei unterschiedliche Gruppen erlaubten. Verglich man Methoden der Identifizierung durch Serologie und Elektrophorese, so ergab sich eine 96%ige Übereinstimmung zwischen 129 mit der ersten und sodann mit der zweiten Methode getesteten Isolaten, und eine 100%ige Übereinstimmung in einem reziproken Test mit 26 anderen Isolaten. Variationen der Proteinprofile in der als nordamerikanisch klassifizierten Gruppe erfordern weitere Untersuchungen. Der Wert von PAGE für Routinetests von Isolaten zur Unterscheidung der europäischen Rasse wird diskutiert. Berichtet wird auch über die erfolgreiche Verwendung einiger spezifischer Proteinbanden nach Eluierung aus den Gelen zur Bildung von somaklonalen Antikörpern.

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