Genetic Control of Basidiospore Formation by Isolates of Lenzites trabea

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ABSTRACT

A model is proposed to account for the formation of fertile basidiocarps by monobasidiospore isolates of *Lenzites trabea*. It involves two assumptions. (i) Such isolates are derived from binucleate basidiospores whose nuclei are homozygous for sexual incompatibility but heterozygous at other loci. They do not produce clamp-connections even though they are heterokaryotic. (ii) Fusion of dissimilar nuclei in the basidia is followed by meiosis, giving rise to recombinant nuclei and hence variation in such traits as capacity to cause decay.

Variation in capacity to cause decay of wood and to produce cellulolytic enzymes among selected monobasidiospore and mono-oidial isolates indicate that (i) fruiting monobasidiospore isolates have two nuclear types that are homozygous at the locus governing sexual incompatibility but are heterozygous at other loci; and (ii) genetically controlled variation among the secondary and tertiary homokaryons obtained from a given source appears to result both from mutations and genetic recombination during formation of basidiospores. Phytopathology 60:951-954.

The genetics of wood-destroying fungi is a neglected aspect of studies on wood deterioration and its control. Only *Schizophyllum commune, Collybia velutipes,* and *Polyporus betulinus* have been studied intensively from a genetic standpoint. We need to know much more about the genetic control of the enzymatic degradation of wood.

Lenzites trabea (Pers. ex Fries) was selected for these studies because of its economic importance, its susceptibility to de-dikaryotization by preservative chemicals, the wide variation known to exist among isolates of this fungus in their capacity to cause decay, and its suitability for genetic analysis.

An intriguing feature of *L. trabea* is the capacity of some monobasidiospore isolates to produce fertile basidiocarps in culture. These isolates have been assumed to be homokaryotic (1, 3) because they lacked clamp connections, whereas dikaryotic isolates have clamp-connections, and successive generations of monobasidiospore isolates all carry the same factor for sexual incompatibility as the parent monobasidiospore isolate. Fruiting of monobasidiospore isolates has been observed in other basically heterothallic species (4, 8), but no satisfactory explanation of the genetic mechanisms involved has been advanced.

A model is proposed to account for this phenomenon. It involves two assumptions. (i) Such isolates are derived from binucleate basidiospores whose nuclei are homozygous for sexual incompatibility but heterozygous at other loci. They do not produce clamp-connections even though they are heterokaryotic. (ii) Fusion of dissimilar nuclei in the basidia is followed by meiosis, giving rise to recombinant nuclei and hence variation in such traits as capacity to cause decay.

When formation of basidiospores by monobasidiospore isolates is preceded by a meiotic process, as suggested by the model, variation in any quantitative physiological or morphological trait should be greater among monobasidiospore isolates obtained from a given, presumably homokaryotic, isolate than if a mitotic process is involved. Also, since oidia are produced asexually by hyphal fragmentation, and since the component nuclei of a heterokaryon can be separated by this method (5), variation among oidial isolates from a given presumed homokaryon can be used to indicate whether different nuclear types are present within monobasidiospore isolates having the capacity to fruit in culture.

This investigation was undertaken to evaluate this model by determining the pattern of variation among selected monobasidiospore and mono-oidial isolates of *L. trabea*. Capacity to cause decay of wood and capacity to produce cellulolytic enzymes were selected as the phenotypic traits to be studied.

MATERIALS AND METHODS.—Two wild-type dikaryotic isolates obtained from angiospermous wood that decayed in nature were used: isolate 1 (Madison 5031) in Missouri in 1951 and isolate 19 [Madison 5060(2)] in Wisconsin in 1952. They were provided by the Forest Disease Laboratory, Forest Service, USDA, Laurel, Maryland.

Three successive generations of homokaryons were obtained from these two dikaryons. Primary homokaryons were obtained from the dikaryons as monobasidiospore isolates. Secondary homokaryons were obtained from primary homokaryons that produced fertile basidiocarps and released viable basidiospores; tertiary homokaryons were obtained in a like manner from secondary homokaryons. Comparisons also were made among 10 mono-oidial isolates obtained from each of

four primary homokaryons, two which produced fertile basidiocarps in culture and two which did not.

All monobasidiospore isolates were obtained from basidiospores produced in culture. Spore suspensions were streaked on 1% malt-extract agar and incubated at 30 C for 12-24 hr. Isolated germinating spores were transferred to 2% malt-extract agar. These cultures were examined microscopically; those without clamp-connections were assumed to be homokaryons from single germinating basidiospores.

Oidial suspensions were obtained using the method of Day (6) and streaked on petri dishes containing water agar. After incubation for 12 hr at 30 C, isolated germinating oidia were transferred to 2% malt-extract agar.

Decay tests.—Soil-block decay chambers were prepared according to the ASTM standard (2) with modifications as discussed below. The chambers were incubated in continuous light at 30 C for 10 weeks. Chambers for a given test were randomized in the incubator, but were equidistant from the light to minimize differences in decay due to the influence of light (7).

Cylindrical test blocks of loblolly pine sapwood (*Pinus taeda* L.) were cut from the third to the tenth annual rings counting from the cambium of a single tree. They were 2.5 cm in diam, were 0.9 cm in the direction of the grain, and were randomized before use. The average per cent wt loss of five blocks was the measure of decay capacity of each isolate. The least significant difference (LSD) between averages was calculated for 95% probability limits. The components of variation due to genetic influences among related isolates (s_g) and to environmental influences and error (s_e) were calculated. The genetic component of variation (s_g) was expressed as a standard deviation for each group of isolates obtained from a given parent.

Results and Discussion.—*Variation among monobasidiospore isolates.*—Variation in decay capacity (DC.) was observed among secondary and tertiary homokaryons (Fig. 1). Among both types of isolates, some caused less while others caused more decay than their parent monobasidiospore isolates. The variation due to genetic influences (s_g) was 24.9% and 15.9% for secondary homokaryons and 19.0% for the tertiary homokaryons (Fig. 1).

Part of the variability among isolates could be due to mutations that occurred during the 2 to 3 years these cultures had been maintained in culture. To determine the extent of such changes in culture, a new series of secondary homokaryons was isolated from primary homokaryon 1-33, and a new series of tertiary homokaryons from secondary homokaryon 1-33 S-1. Their DC.'s were determined immediately after isolation and compared with the results for the series obtained from similar isolates but maintained in culture for 2-3 years. Also, the DC.'s of the nine secondary homokaryons previously obtained from primary homokaryon 1-9 were redetermined after a total of 4-5 years in culture.

The amount of variation observed among the recently isolated homokaryons was much less ($s_g = 4.7\%$ for secondary and 5.7% for tertiary homokaryons, Fig. 2-B, C) than among similar isolates maintained in culture for 2-3 years ($s_g = 15.9\%$ for secondary and 19.0% for tertiary homokaryons, Fig. 1). Furthermore, the amount of variation observed among secondary homokaryons after 4 to 5 years in culture remained high ($s_g = 20.2\%$, Fig. 2-A).

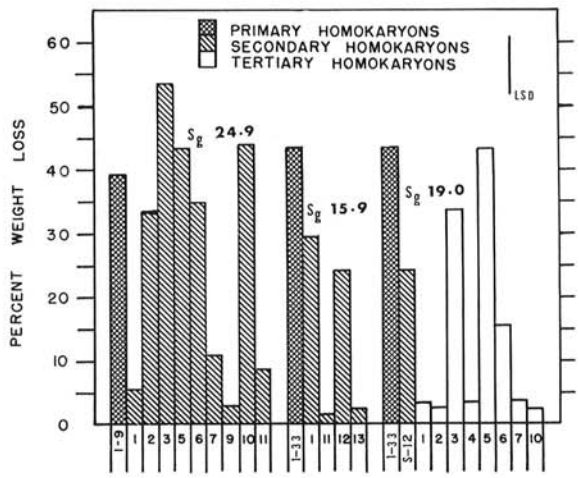

Fig. 1. Per cent wt losses due to decay by primary, secondary, and tertiary homokaryons. Each bar represents the average for five replicate blocks exposed to each isolate.

A loss in capacity to cause decay due to mutation apparently occurred in both of the primary homokaryons during the 2 years between the decay tests. The average wt loss (AWL) due to decay changed from 38.5% to 1.9% for isolate 1-9 and 43.2% to 1.2% for isolate 1-33. Similarly, mutations for decreased DC. occurred in the secondary homokaryons from 1-9 (Fig. 1, 2-A); AWL changed from 33.5% to 1.4% for S-2, 44.0% to 27.2% for S-5, and 11.4% to 0.7% for S-7. One of the nine isolates showed an increase in capacity to cause decay during the additional 2 years in culture. AWL increased from 7.8% to 45.6% for isolate S-11.

There also was some variation among the isolates whose DC.'s were determined immediately after isolation. Secondary homokaryon S-17 (Fig. 2-B) as well as tertiary homokaryon 10 (Fig. 2-C) were significantly different from the average of their respective populations. The large amount of variability observed among isolates from the same parent homokaryons probably resulted from (i) mutations in capacity to cause decay that apparently accumulated during the several years the isolates were maintained in culture; or (ii) a meiotic process resulting in differences in the nature of recombinant basidiospore nuclei.

The large number of isolates that apparently mutated is significant. This implies a degree of instability in primary and secondary homokaryons having the capacity to form fertile basidiocarps in culture. This could be the result of unstable heterokaryotic associations. As the nuclear types of a heterokaryon approach homozygosity, it is possible that the heterokaryon

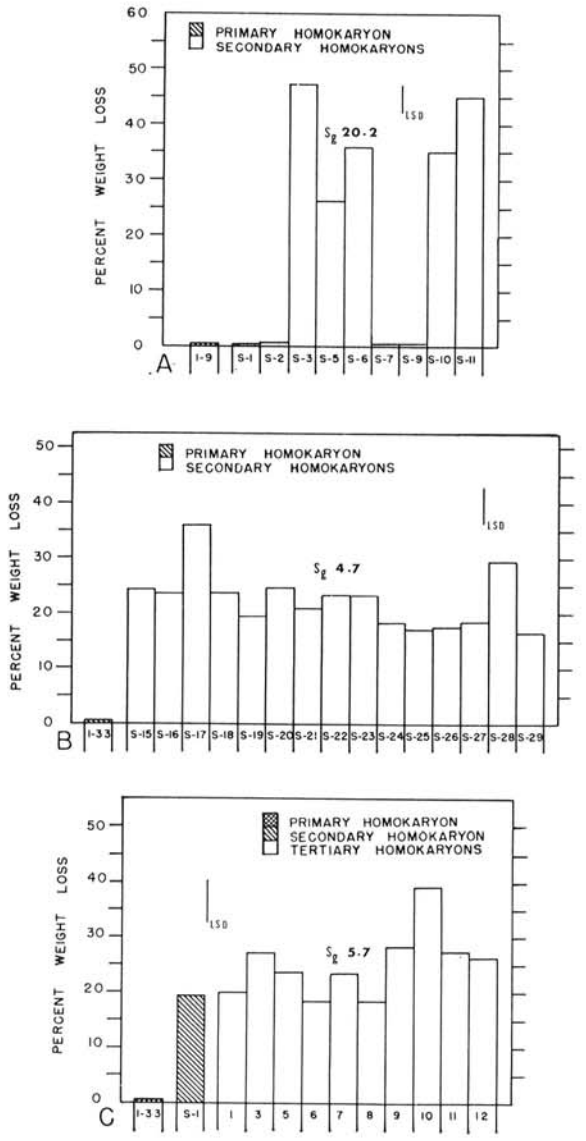

Fig. 2. Per cent wt losses due to decay by primary, secondary, and tertiary homokaryons. Each bar represents the average for five replicate blocks exposed to each isolate.

tends to dissociate into its component nuclear types. Shortly after isolation, all secondary and tertiary homokaryons thus far obtained formed basidiocarps in culture. Some of these isolates apparently lost their capacity to form basidiocarps when maintained in culture for a period of time. This is also true of some primary homokaryons that fruited in culture shortly after being isolated. Apparently loss in ability to fruit in culture accompanied the reduction in DC in primary homokaryon 1-9, whereas this was not the case with isolate 1-33. Isolate 1-33, however, showed a decrease in the number of basidiocarps produced.

Variation among mono-oidial isolates.—Oidial isolates from primary homokaryons that did not fruit in culture (19-1 and 1-3) were very similar in colony morphology and showed little variation in capacity to cause decay (Fig. 3-A). Oidial isolates from primary homokaryons that produced fertile basidiocarps (1-1 and 1-33) showed greater variation in both colony morphology and DC. than did those from isolates 19-1 and 1-3. Oidial isolates from 1-1 (an isolate which fruited shortly after being isolated but apparently lost this capacity) were all similar in colony morphology but had greater variability in DC. than did those from either 19-1 or 1-3. Oidial isolates from 1-33 (an isolate which fruited in culture after isolation and retained this capacity), however, germinated to form two morphologically distinct types of colonies, spreading (S) and compact (C). All oidial isolates of each colony type had similar capacities to cause decay, but the AWL caused by the two groups differed considerably (AWL = 2.6% and 19.0%, Fig. 3-B). None of the mono-oidial isolates obtained from 19-1, 1-3, or 1-1 fruited in culture.

One observation is not consistent with the model. In previous decay tests, isolate 1-33 caused an AWL of 43%, whereas the AWL in the present study was only 3% (Fig. 1, 3-B). This may indicate that a mu-

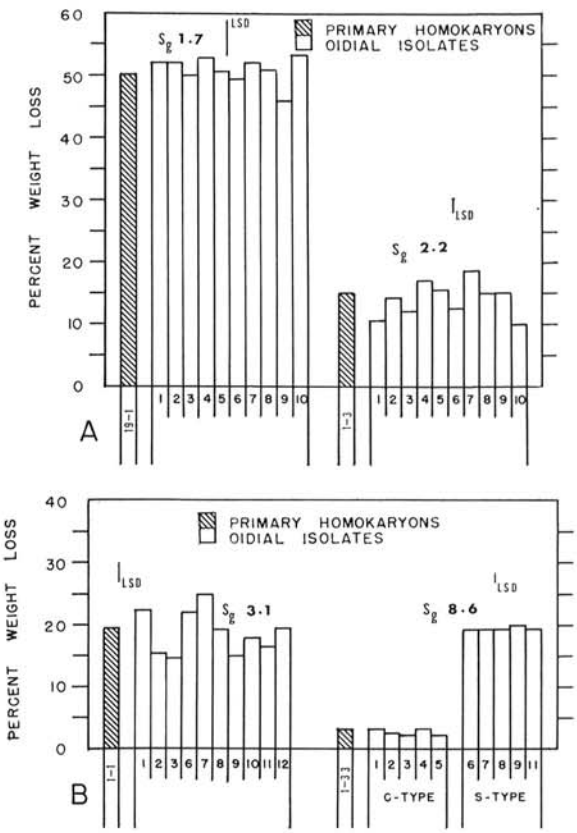

Fig. 3. Per cent wt losses due to decay by **A)** primary homokaryons that have not fruited in culture and mono-oidial isolates obtained from them; **B)** primary homokaryons that have fruited in culture and mono-oidial isolates obtained from them. Each bar represents the average for five replicate blocks exposed to each isolate.

tation occurred in this isolate, and that the mutant nuclei have been maintained in the mycelium with the "normal" nuclei. The above situation could also exist if 1-33 originally had two nuclear types and a mutation occurred in one of the nuclear types and was maintained and eventually displaced the "normal" nuclear type from which it arose. Thus, the C-type oidial isolates may contain the mutant nuclear type, whereas the S-type isolates may carry either the other nuclear type or both nuclear types in association. If the fruiting of monobasidiospore isolates is dependent upon the binucleate condition as postulated in the model, the S-type oidial isolates must carry both nuclear types, as all of them fruited in culture.

Fifty-four mono-oidial isolates were obtained from 1-33 0-9 (Fig. 3-B); all of them fruited in culture. This was similar to the situation found with secondary and tertiary monobasidiospore isolates. The fact that all of the secondary and tertiary monobasidiospore isolates, as well as all of the mono-oidial isolates obtained from 1-33 0-9, fruited in culture is not necessarily inconsistent with the model. A genetic mechanism may be operating in these isolates assuring that a large portion of the basidiospores or oidia will be binucleate; it is also possible that only the binucleate basidiospores and oidia are viable in these isolates.

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