Theor. Appl. Genet. 63, 279–281 (1982)

Two Morphological Markers Indicating Dikaryosis in *Schizophyllum commune*

F. Meinhardt, F. Saleh and K. Esser
Lehrstuhl für Allgemeine Botanik, Ruhr-Universität, Bochum (Federal Republic of Germany)

Summary. In the wood destroying basidiomycete *Schizophyllum commune* a method is described to recognize the onset of dikaryosis rapidly in using recessive genetic markers. The gene ai^+/ai causes in its mutant recessive allele (*ai*) the production of dark coloured fruit bodies. This can be made use of to evaluate macroscopically the formation of a dikaryon. Another useful marker is the gene rd^+/rd. The recessive allele (*rd*) causes phenotypically the formation of a round looking mycelium instead of the fringed looking mycelium, the wild type. This genetic marker which is closely linked to the A-incompatibility factor is therefore also qualified to detect the onset of dikaryosis without much effort.

Key words: Dikaryon formation – *Schizophyllum* – Fruit bodies – Incompatibility

Introduction

In higher basidiomycetes dikaryosis is in general the essential prerequisite for fruit body morphogenesis. Since fruiting is mostly correlated with drastic alterations of metabolism, such as formation of antibiotics or fungal aromas, it becomes evident that dikaryosis in general terms may be considered as prerequisite for productivity and therewith of biotechnological importance (Chang and Hayes 1978).

Despite the fact that in many basidiomycetes monokaryons are also able to form fruit bodies or comparable structures asexually (Stahl and Esser 1976), the productivity of these monokaryotic fruiters is considerably lower than in dikaryotic (sexual) fruiters (Meinhardt 1980; Meinhardt and Esser 1981).

This points to the need to recognize the onset of dikaryosis as quickly as possible, especially in breeding experiments. In many higher basidiomycetes dikaryosis becomes obvious by the occurrence of clamp connections. But in those fungi which like *Agaricus bisporus* do not form clamps, dikaryosis is indicated by fruiting only. However, it was recognized only quite recently that fruiting depends on the function of a series of "fruiting genes" and may even become suppressed by a single gene mutation (Esser 1981; Saleh 1981). In this case a time consuming microscopic observation is required to decide whether the nonfruiting mycelium is a monokaryon or a dikaryon. If the latter is true, the conclusion may be drawn, that the loss of fruiting and therewith the imperfect status is obtained by mutation of the fruiting genes.

In order to overcome this sometimes complicated analysis of dikaryosis, we make advantage of the fact that in a dikaryon as in a heterozygous diploid dominance and recessiveness between alleles occur. A method is described to use recessive morphogenetic genes as markers to indicate rather rapidly the onset of dikaryosis.

Materials and Methods

Strains

For these experiments a wild isolate from Recife (Brazil) and its monokaryotic progeny was used. The strain was kindly furnished by M. Semerdzieva (Prague/CSSR).

Media and Culture Conditions

Cultures were grown on complete medium (Raper and Hoffman 1974) or alternatively on Moser-B-medium (Moser 1958). Culture conditions and isolation of spores are as described previously (Esser et al. 1979).

Results and Discussion

During the experimental work to elucidate the genetics of fruiting in *S. commune*, a variant occurred spontaneously which produced monokaryotic fruit bodies of an almost black colour. This strain called "*aida*" may also

0040-5752/82/0063/0279/$ 01.00

be distinguished by the form of its hymenium from the light coloured wild strain, as may be seen from Fig. 1 a, b.

The genetic analysis of this variant revealed that the aida phenotype differs from the wild type by a single allele, as may be seen from the following data: From 7 sequentially performed backcrosses with wild strain of compatible mating type altogether 268 monokaryotic mycelia were obtained. A macroscopical evaluation showed: 129 wild types and 139 *aida* phenotypes. This is in good agreement with a 1 : 1 segregation (Chi^2-test gives $P > 0.5$). The dark phenotype is therefore caused by a monogenic mutation; gene symbols are: *aida* = *ai* and wild type = ai^+.

Genetic analysis of the aida mutant has revealed another interesting phenomenon: All dikaryons heterokaryotic for the aida gene ($ai^+ + ai$) and independent of the mating types produce fruit bodies exclusively of the wild type. This shows the dominance of the ai^+ and the recessiveness of the mutant *ai* gene. Dikaryons homokaryotic for the mutant gene ($ai + ai$) form dark fruit bodies as expected.

The gene *aida* therefore can be used as a marker for monokaryons to indicate macroscopically the establishment of a dikaryon in a uncomplicated way, provided one partner is a monokaryotic fruiter. In nonfruiters the expression of aida is not as obvious, because it causes only a slightly darker mycelium.

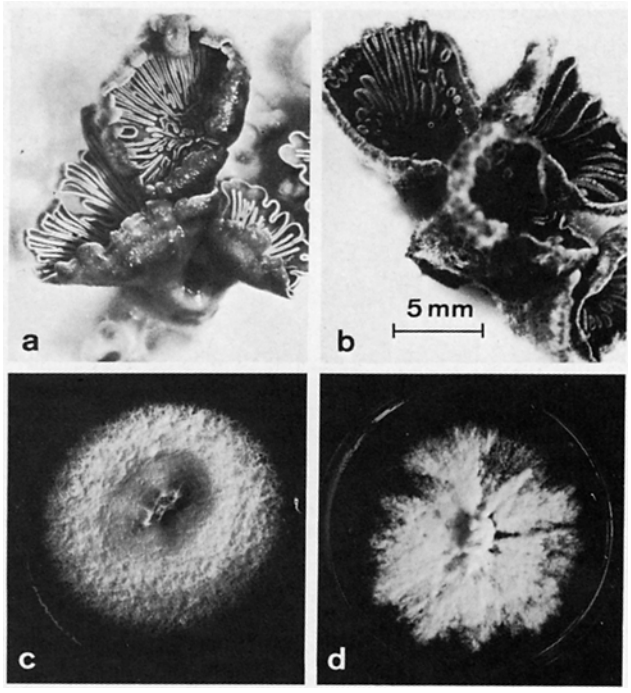

Fig. 1 a–d. Fruit body (*a, b*) and mycelial morphology (*c, d*) of monokaryons of *Schizophyllum commune*. **a** wild type, dominant (ai^+); **b** black mutant, recessive (*ai*); **c** round mycelium, recessive (*rd*); **d** fringed mycelium, dominant (rd^+)

In these cases, if the occurrence of dikaryosis has to be proved, in nonfruiters another recessive morphological mutant is much more suitable, because its phenotype concerns the mycelial habit. This mutant was called round (*rd*) and causes colonial type mycelial growth (Fig. 1 c), whereas its dominant allele rd^+ produced colonies with a fringed phenotype (Fig. 1 d). In heterokaryotic dikaryons the fringed phenotype always dominates over the round phenotype. This allows a very rapid identification of a dikaryon, because it is not necessary to wait for fruit bodies as in using the marker *aida*.

The monogenic difference between these two strains was revealed by the analysis of a cross $rd^+ \times rd$. In the offspring of 184 monokaryons 90 round and 94 fringed strains were found. In the Chi^2-test gives this a $P > 0.5$ for 1 : 1 segregation.

The genetic analysis showed further that the rd^+/rd gene is closely linked with the mating type factor A, because in the above quoted offspring no recombination between A and rd^+/rd was found.

Conclusions

The findings described in this publication have implication for both fundamental and applied research, because mutants like those described offer the advantage of recognizing dikaryosis macroscopically. In fundamental research this results mainly in a saving of time, but in applied or biotechnological work mutants like these offer the additional advantage of performing dikaryotization without great laboratory effort and might therefore be well suited for breeding fungi in the mushroom industry.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (Bonn-Bad Godesberg). The experimental data originated from a PhD thesis of F. Saleh, whose sojourn in Bochum was supported by a grant of the Deutscher Akademischer Austauschdienst (DAAD, Bonn-Bad Godesberg).

Literature

Chang, S.T.; Hayes, W.A. (eds.) (1978): Biology and cultivation of Edible Mushrooms. New York, San Francisco, London: Acad. Press

Esser, K. (1981): Impact of basic research on the practical application of fungal processes. In: Soc. Industr. Microbiol., Developments in Industrial Microbiology, Vol. **22** Arlington/Va.

Esser, K.; Saleh, F.; Meinhardt, F. (1979): Genetics of fruit body formation in higher basidiomycetes. II. Monokaryotic and dikaryotic fruiting in *Schizophyllum commune*. Curr. Genet. **1**, 85–88

Meinhardt, F. (1980): Untersuchungen zur Genetik des Fortpflanzungsverhaltens und der Fruchtkörper- und Antibiotikabildung des Basidiomyceten *Agrocybe aegerita.* Bibl. Mycol. **75,** Vaduz: J. Cramer

Meinhardt, F.; Esser, K. (1981): Genetic studies of the basidiomycete *Agrocybe aegerita.* II. Genetic control of fruit body formation and its practical implications. Theor. Appl. Genet. **60,** 265–268

Moser, M. (1958): Die künstliche Mykorrhizabeimpfung an Forstpflanzen. Forstwiss. Z. (Wien) **77,** 32–40; 271–278

Raper, J.R.; Hoffman, R.M. (1974). *Schizophyllum commune.* In: Handbook of genetics, Vol. **1** (ed. King, R.C.), pp. 597–626. New York, London: Plenum Press

Saleh, F. (1981): Genetische Untersuchungen zur Morphogenese des Basidiomyceten *Schizophyllum commune.* Diss. Bochum

Stahl, U.; Esser, K. (1976): Genetics of fruit body production in higher basidiomycetes. I. Monokaryotic fruiting and its correlation with dikaryotic fruiting in *Polyporus ciliatus.* Mol. Gen. Genet. **148,** 183–197

Received June 3, 1981
Communicated by H. F. Linskens

Dr. F. Meinhardt
Dr. F. Saleh
Prof. Dr. Dr. K. Esser
Lehrstuhl für Allgemeine Botanik
Ruhr-Universität
Postfach 10 21 48
D-4630 Bochum (Federal Republic of Germany)