R-Glucanase Activity and Susceptibility of Hyphal Walls to Degradation in Mutants of *Schizophyllum* with Disrupted Nuclear Migration

By J. G. H. WESSELS

Department of Botany, University of Groningen, Haren, The Netherlands

AND Y. KOLTIN

Genetics Unit, Faculty of Life Sciences, Tel-Aviv University, Tel-Aviv, Israel

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SUMMARY

R-glucanase activities, and susceptibility of native-wall preparations to degradation by R-glucanase, were determined in strains of *Schizophyllum commune* with three different mutations that disrupt nuclear migration. These mutations, *M11*, *M15*, and *M16* appeared to affect the susceptibility of the hyphal wall to R-glucanase but *M11* and *M15* also had an effect on the activity of R-glucanase. Both effects may be related to the stability of complex septa in these mutants and account for their specific mating behaviour.

INTRODUCTION

The formation of the dikaryon of tetrapolar basidiomycetes, such as *Schizophyllum commune*, is regulated by two incompatibility factors, *A* and *B*, and occurs normally if the two interacting monokaryons carry different incompatibility factors ($A \neq B \neq$). One of the key events in the transition from the uninucleate to the binucleate state is the migration of invading nuclei following nuclear exchange between the mates. Nuclear migration is only a transient process in the formation of the dikaryon, but it occurs continuously in a common-*A* heterokaryon ($A = B \neq$) and in a homokaryon carrying a mutation in the *B* incompatibility factor (Raper, 1966).

The problem of nuclear migration became more clearly defined with the discovery of a complex septal apparatus which prevents the passage of nuclei from cell to cell (Girbardt, 1962). A solution to this problem was suggested in electron micrographs of common-*A* heterokaryons and homokaryons with a mutation in the *B* factor which revealed that septal disintegration occurs simultaneously with the initiation of nuclear migration (Giesy & Day, 1965; Jersild, Mishkin & Niederpruem, 1967; Koltin & Flexer, 1969).

In *Schizophyllum commune*, both the common-*A* heterokaryon and the homokaryon with a mutated *B* factor not only have disintegrated septa but they also grow with very little aerial mycelium and the hyphae are irregularly shaped (Raper, 1966). Biochemical studies (Wessels & Niederpruem, 1967; Wessels, 1969*a*) indicated that the appearance of these morphological characteristics is correlated with a decrease in the alkali-insoluble glucan (R-glucan) in the hyphal wall and a parallel increase in the specific activity of R-glucanase in the mycelium. The presence of an unlinked mutation (*M11*) in a *B*-factor mutant, which restores the wild-type morphology of the hyphae and a normal distribution of nuclei (Raper & Raper, 1966), again causes the deposition of a normal amount of R-glucan in the wall and

leads to a low R-glucanase activity similar to that of the wild-type strain (Wessels, 1969*a*). There is, therefore, a positive correlation between aberrant hyphal morphology, septal dissolution, low R-glucan content of the wall, and high R-glucanase activity. In addition, the fact that the combination of R-glucanase and chitinase can completely dissolve the septa but not the longitudinal walls in hyphal-wall preparations (Janszen & Wessels, 1970), supports the hypothesis that R-glucanase is involved in septal dissolution.

Several mutations (modifier mutations), at different sites and unlinked to the incompatibility factors, may restore normal hyphal morphology and complete septa to a strain carrying a *B*-factor mutation (Koltin, 1967, 1970). However, strains carrying these modifier mutations are unilateral in a mating: they can donate nuclei but are unable to accept them. To explain this situation, two suggestions have been considered (Koltin, 1970): (i) the modified strains lack certain enzymes necessary for septal disintegration; (ii) the septa in the modified strains have become resistant to hydrolysing enzymes.

To distinguish between these alternatives, the level of R-glucanase activity, the ratio of S-glucan to R-glucan, and the susceptibility of the hyphal wall to R-glucanase were determined in strains possessing a modifier mutation (*M11*, *M15*, or *M16*) in addition to a mutation in the *B* factor. Since restoration of the wild-type morphology is reflected in the morphology of both the hyphal wall and the septum, and since both the longitudinal wall and the septum contain R-glucan, a study of changes in hyphal-wall susceptibility to R-glucanase may indicate changes in the septum.

METHODS

The strains of *Schizophyllum commune* used were 699, C10-46, and B10-1 described by Wessels (1969*a*), and various strains carrying modifier mutations, isolated and characterized by Koltin (1967). The genotypes are detailed in Table 1. All strains were grown for 5 days in liquid-sand cultures. Culture conditions, estimation of S-glucan and R-glucan, and the R-glucanase assay were as described by Wessels (1969*a*).

RESULTS

S-glucan to R-glucan ratios in the hyphal walls. S-glucan to R-glucan ratios for some of the strains were determined (Table 1). The presence of a *B*-factor mutation which causes abnormal hyphal morphology and disintegration of septa raises this ratio to over twice that of the wild-type strain. The additional presence of one of the modifier mutations reduces this ratio towards the wild-type ratio. This is correlated with complete restoration of wild-type hyphal morphology and septal structure.

R-glucanase activities. R-glucanase activities were measured in dialysed extracts of a number of strains (Table 1). A mutation in the *B* factor greatly increases the specific activity of R-glucanase. In most of the strains carrying both a mutated *B* factor and a modifier mutation the R-glucanase activity is reduced towards the wild-type level. A partial reduction of R-glucanase occurs in only one of the strains carrying an *M11* mutation (B10-1-M5-7-9) and in the strain with the *M16* mutation. The *M11* mutation in an otherwise wild-type strain (1871) has little effect on R-glucanase activity.

The R-glucanase activities after concentration and subsequent dilution of the extracts were determined for some strains. An increase in the activity of R-glucanase is noticeable after concentration of extracts from the *M11*-modified strains.

Susceptibility of hyphal-wall preparations to R-glucanase. Table 1 shows that a low S-glucan to R-glucan ratio is maintained in the strains carrying *M11* and *M16*, although high R-

Table 1. *S*-glucan/*R*-glucan ratios and specific *R*-glucanase activities in homokaryons of *Schizophyllum commune*

Nuclear migration	Strain	Genotype	S-glucan/ <i>R</i> -glucan	R-glucanase activity*	
				A	B
Inducible	699 (wild-type)	<i>B</i> α 3- β 2	0.61	21	20
Constitutive	C10-46	<i>B</i> α 3- β 2(<i>r</i>)†	1.30	319	404
	1868-F11	<i>B</i> α 3- β 2(<i>r</i>)	—	312	—
Disruptive	1871	<i>B</i> α 3- β 1 <i>M</i> 11‡	—	12	—
	B10-1	<i>B</i> α 1- β 2(<i>r</i>) <i>M</i> 11	0.77	30	182
	B10-1-3	<i>B</i> α 3- β 2(<i>r</i>) <i>M</i> 11	—	19	98
	B10-1-M5-7-9	<i>B</i> α 3- β 2(<i>r</i>) <i>M</i> 11	—	238	314
	1869	<i>B</i> α 3- β 2(<i>r</i>) <i>M</i> 15	—	36	—
	1888	<i>B</i> α 3- β 2(<i>r</i>) <i>M</i> 15	—	29	27
	1883	<i>B</i> α 3- β 2(<i>r</i>) <i>M</i> 15	0.91	35	49
	1868	<i>B</i> α 3- β 2(<i>r</i>) <i>M</i> 16	0.67	202	218

* Expressed as μ g of solubilized glucose equivalents per mg protein after 3 h of incubation. A = extracts only dialysed against 0.05 M-McIlvain buffer (pH 5.6). B = extracts subsequently concentrated tenfold by dialysis against 30% (w/v) polyethylene glycol in 0.05 M-McIlvain buffer (pH 5.6) and again diluted to the original volume.

† β 2(*r*) is a mutation in the β locus of the *B* factor which leads to constitutive migration of nuclei.

‡ *M*11, *M*15 and *M*16 are mutations at different loci which disrupt the migration of nuclei.

glucanase activities can be detected. A decreased susceptibility of the wall to degradation by *R*-glucanase could explain why the *S*-glucan to *R*-glucan ratio in these strains does not rise as it does in strain C10-46 which has only the mutation in the *B* factor.

Hyphal walls were isolated and incubated with two enzyme preparations containing *R*-glucanase, and the amount of soluble carbohydrate released was used as an indication of the degradation of *R*-glucan (Table 2). The release of soluble carbohydrate from briefly washed walls of three strains carrying both a *B*-factor mutation and a modifier mutation is low when compared with the wild-type strain. Continuation of the washing procedure increases the susceptibility of the walls of all strains but largely eliminates the differences between them. This indicates that components which confer protection against enzymatic wall degradation can be removed by the buffer used in the cleaning procedure. Extraction of the walls with alkali, which removes the *S*-glucan and essentially only leaves the *R*-glucan-chitin complex, gives a further increase in the release of soluble carbohydrate by the enzyme preparations. The values obtained after alkaline extraction probably reflect the susceptibility of the *R*-glucan proper, unprotected by other components. It appears that only the *R*-glucan from the wall of strain B10-1 differs significantly in its susceptibility to *R*-glucanase.

Comparison of the release of soluble carbohydrate from briefly washed walls with that from KOH-extracted walls (Table 1, values in parentheses) indicates that the native-wall preparations are better protected against degradation by the *R*-glucanase preparation obtained from the culture fluid of strain K8 than against the enzyme in the mycelial extract of strain C10-46.

DISCUSSION

The apparent specific activities of *R*-glucanase observed in strains carrying both a modifier mutation and a *B*-factor mutation were clearly lower than those found in strains with only a mutation in the *B* factor. With the exception of two of the modified strains, the *R*-glucanase activities were close to that of the wild-type strain, as was earlier reported for one

Table 2. *Release of soluble carbohydrate from native and extracted hyphal walls of homokaryons of Schizophyllum commune by two different R-glucanase preparations*

Native hyphal walls were prepared according to Wessels (1966) except that the walls were broken and washed in 0.05 M-KH₂PO₄-NaOH buffer (pH 7.0). Walls were extracted with 1 N-KOH at 60 °C for 20 min to remove S-glucan. All incubation mixtures (1 ml) contained 0.05 M-McIlvain buffer (pH 5.6) and isolated walls standardized on 1.5 mg R-glucan. The enzyme preparations were an acetone precipitate (0.5 mg/ml) from the culture fluid of a 12-day-old culture of *S. commune* dikaryon κ8 (Wessels, 1969*b*) or a mycelial extract from a 5-day-old culture of the *B*-mutant strain C10-46.

Enzyme source	Walls from strain	Nuclear migration	Release of soluble carbohydrate.* Treatment of walls		
			Washed 5 times	Washed 15 times	Extracted with KOH
Dikaryon κ8	699 (wild-type)	Inducible	102 (27)	264 (69)	384 (100)
	B10-1 (<i>Bmut M11</i>)	Disruptive	22 (8)	218 (77)	285 (100)
	1883 (<i>Bmut M15</i>)	Disruptive	66 (15)	215 (49)	442 (100)
	1868 (<i>Bmut M16</i>)	Disruptive	53 (13)	245 (62)	399 (100)
<i>B</i> -mutant C10-46	699 (wild-type)	Inducible	38 (59)	41 (63)	65 (100)
	B10-1 (<i>Bmut M11</i>)	Disruptive	6 (30)	—	21 (100)
	1883 (<i>Bmut M15</i>)	Disruptive	18 (22)	46 (58)	79 (100)
	1868 (<i>Bmut M16</i>)	Disruptive	14 (20)	35 (48)	72 (100)

* Expressed as μ g solubilized glucose equivalents from the hyphal-wall preparations after 3 h incubation with enzymes, and in parentheses are the values as percentages of the values obtained with the KOH-extracted product of that particular wall preparation.

M11-modified strain (Wessels, 1969*a*). However, such strains carrying an *M11* mutation in addition to a *B*-factor mutation contained an inactive form of R-glucanase rather than were repressed for synthesis of the enzyme (compare results in Table 1 before and after concentration of extracts). Although stimulation of R-glucanase activity could not be found after concentration of extracts from the *M15*-modified strains, it would appear premature to consider the low R-glucanase activities in these strains as being due to repressed enzyme synthesis.

The effect of the *M11* and *M15* mutations on R-glucanase activities in *B*-factor mutants could explain the restoration of a low S-glucan to R-glucan ratio and normal hyphal morphology and also the absence of septal disintegration. However, the restoration of these wild-type characteristics in the *M16*-modified *B*-factor mutant in the presence of a high R-glucanase activity calls for another explanation in at least this strain.

The results suggest that not only the *M16* mutation but also the *M11* and *M15* mutations confer on the hyphal walls an increased resistance to enzymatic breakdown. The occurrence of differences between strains in the susceptibility of hyphal walls to enzymatic degradation has earlier been demonstrated in *Schizophyllum commune* in connexion with the breakdown of R-glucan during carbon starvation (Wessels, 1966; Niederpruem & Wessels, 1969). These differences in susceptibility could be eliminated by hot-water extraction of the walls. In the modified strains studied here it appears that the protecting components can even be removed by extensive cold washing of the walls.

Assuming that a change in both the activity of R-glucanase and the susceptibility of the walls to R-glucanase relate to the same mutation, two of the modifier mutations, *M11* and *M15*, apparently have a pleiotropic effect. However, it is conceivable that the two phenomena are biochemically related. The protecting substances in the wall preparations might actually

be inhibitors of R-glucanase. Because these substances can be washed from the walls, their presence in the mycelial extracts is probable and this could account for the inhibition of R-glucanase in these extracts. Alternatively, these inhibitors were originally present in the cytoplasm and became secondarily attached to the walls during their isolation.

Although the nature of the protecting substances remains unresolved, their presence due to the modifier mutations could explain the unilateral mating behaviour of strains carrying such mutations. If they are part of the wall structure, the septa in the modified strains may also be less susceptible to dissolution by R-glucanase and other enzymes. If the protecting substances are actually cytoplasmic inhibitors of R-glucanase, their presence would also prevent septal dissolution in the modified strain.

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