African Journal of Biotechnology Vol. 7 (3), pp. 276-281, 5 February, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Somatic incompatibility in *Agaricus bitorquis* (Quel.) Sacc.

Perihan Guler

Kırıkkale University, Faculty of Science and Literature, Department of Biology, 71450 Yahsihan-Kırıkkale, Turkey, E-mail: perihangler@yahoo.com.

Accepted 28 November, 2007

The somatic incompatibility in *Agaricus bitorquis* was studied using ten wild strains. Heterokaryons from each isolate were all paired with the same unrelated heterokaryon and also paired together in all combinations. Two different types of somatic incompatible interaction were observed lightly or heavily pigmented lines developing between the two isolates. In addition to, some elements and photographs of hyphae were determined by means of scanning electron microscopy.

Key words: *Agaricus bitorquis*, somatic incompatibility, elements.

INTRODUCTION

Somatic or vegetative incompatibility is widespread in macrofungi. The variability of sexual incompatibility genes has been intensively studied in fungi, particularly in higher basidiomycetes (Noel et al., 1991). Fungi possess features that render many ideals for genetic research. Most fungi can be grown in pure culture under controlled conditions. This means that environmental variability, which could conceal or be confused with genetic differences, can be minimized. Many have predominantly haploid life cycles with most genes expressed in the haploid phase. Mutant alleles of such genes are readily detected (Carlile and Watkinson, 1996).

Somatic incompatibility regulates allorecognition and allorejection following somatic contacts in many groups of organisms. Allorecognition many trigger two reactions that have different implications should be distinguished. One is prevention of successful somatic anastomoses, the accompanying cytoplasmic and nuclear exchange. The term somatic incompatibility as applied to Basidiomycetes. The other macroscopic interaction may be called mycelial incompatibility. Mycelial incompatibility has been the most criterions evaluating somatic incompatibility in Basidiomycetes (Worral, 1997). The control of somatic incompatibility also has been studied recently in several Basidiomycetes (Marcais et al., 2000). Interactions between 42 higher fungi were studied *in vitro* (Napierala and Werner, 2000). The mating type alleles were determined by mating tests for a sample of 17 wild isolates of *Pleurotus ostreatus* (Theochari et al., 2000). Variability of the sexual incompatibility genes of *Agrocybe aegerita* was investigated in the homokaryotic progeny of 13 wild dikaryotic strains originating from 5 distinct European regions (Noel et al., 1991). Somatic incompatibility, the interaction that usually develops between the mycelia of two unrelated dikaryons whenever they come into contact on agar medium, has been successfully used to study the structure of the population of many Basidiomycetes (Marcais et al., 2000)

In many Ascomycetes, Basidiomycetes and Deuteromycetes, fusion between vegetative hyphae (hypal anastomosis) of the same colony is common during colony development. If the hyphae that fuse carry genetically different nuclei, the colony that develops may be a heterokaryon. Bringing together different mutants that have been derived from the same parental strain can readily produce heterokaryons. An encounter between hyphae of different strain of the same species may result in vegetative incompatibility, also known as somatic incompatibility (Carlile and Watkinson, 1996).

In this paper, we studied the somatic incompatibility in the different strains of *A. bitorquis* (Quel.) Saccardo. *A. bitorquis* like most cultivated edible fungi is a Basidiomycete. *A. bitorquis* is widespread in nature. It appears to have a great variety of genetically determined morphological and physiological characteristics which it can be managed in a breeding program. The life cycle of *A. bitorquis* allows a great advantage in breeding potential over the homothallic *A. bisporus.* Its heterothallic nature

Table 1. Sources of the *Agaricus bitorquis* isolates used in somatic incompatibility tests.

Isolate	Year of isolation	Geographical origin
A	1998	Aşağı Hacıosmanoğlu village
B	1999	Aşağı Hacıosmanoğlu village
C	1999	Aşağı Hacıosmanoğlu village
D	1998	Aşağı Hacıosmanoğlu village
E	2000	Aşağı Hacıosmanoğlu village
F	2000	Aşağı Tüfekçioğlu village
G	1999	Aşağı Tüfekçioğlu village
H	1998	Aşağı Tüfekçioğlu village
J	1998	Aşağı Tüfekçioğlu village
K	1999	Aşağı Tüfekçioğlu village

Table 2. Pairings between heterokaryons of *Agaricus bitorquis* strains.

Heterokaryons	A	B	C	D	E	F	G	H	J	K
A	AA	AB	AC	AD	AE	AF	AG	AH	AJ	AK
B		BB	BC	BD	BE	BF	BG	BH	BJ	BK
C			CC	CD	CE	CF	CG	CH	CJ	CK
D				DD	DE	DF	DG	DH	DJ	DK
E					EE	EF	EG	EH	EJ	EK
F						FF	FG	FH	FJ	FK
G							GG	GH	GJ	GK
H								HH	HJ	HK
J									JJ	JK
K										KK

and visibly distinct sexual interaction in compatible mating greatly facilitate the making of controlled crosses to combine genetic traits (Raper, 1978). *A. bitorquis* is a tetraspored heterothallic bipolar Homobasidiomycete mushroom of the order Agaricales. Only the dikaryotic mycelium can differentiate fruit bodies (Valjalo and Labarere, 1989).

MATERIAL AND METHODS

In this study, ten wild dikaryotic strains of *A. bitorquis* were used as monosporous isolates from spore prints of naturally occurring fruiting bodies. The source and geographic origin of each strains are indicated Table 1. Dikaryotic strains were collected in Middle Anatolia in Turkey. The letters from A to K designated all wild-type strains.

Homokaryotic and dikaryotic mycelia were cultured vegetatively on solid 2% malt extract medium (MEA) at 30ºC in the dark. Transfers to fresh slants were made monthly. Differentation of isolation and germination of basidiospores were carried out previously described (Raper, 1978). Strains were maintained on MEA slants at 4ºC.

Somatic incompatibility was detected by pairing two different homokaryotic mycelia were inoculated on MEA. Inoculum blocks (8 mm diameter) were cut from stock plates and placed 1.5 cm apart in the centre of petri dishes (90 mm diameter) and incubated at 30ºC in darkness. According to the strains, two mycelia formed zone of contact in about 20 days. Two plates were done for each pairing. The width of the interaction was measured in terms of relative of aerial hyphae. Each somatic incompatibility reactions were self-pairing and pairings between completed heterokaryons as control (Hansen et al., 1993). The somatic incompatibility of each groups were compared. The results of heterokaryons were produced by pairing AxA, BxB, AxB, AxC, AxD for example. All paired heterokaryons are shown in Table 2.

Four milimeter squares of the compatibility and incompatibility zone on agar media were fixed in 3.0% glutheraldehyde solution for 1.5 h. at room temperature. All materials were post fixed in 1.0% osmium tetroxide 1.5 h. dehydrated, embedded and they were coated under vacuum with a thin layer of gold and examined by means of scanning electron microscope (JOEL, Model JMS 5600). In addition to; the concentrations of some elements of hyphae were examined by SEM.

RESULTS AND DISCUSSION

Although an increasing number of studies dealing with the somatic incompatibility in fungi have been reported (Rayne et al., 1994; Carlile and Watkinson, 1996; Marcais et al., 1998), no somatic incompatibility data is available in *A. bitorquis*.

The control of somatic incompatibilty was examined in ten wild-type strains. Almost all pairings between geogra-

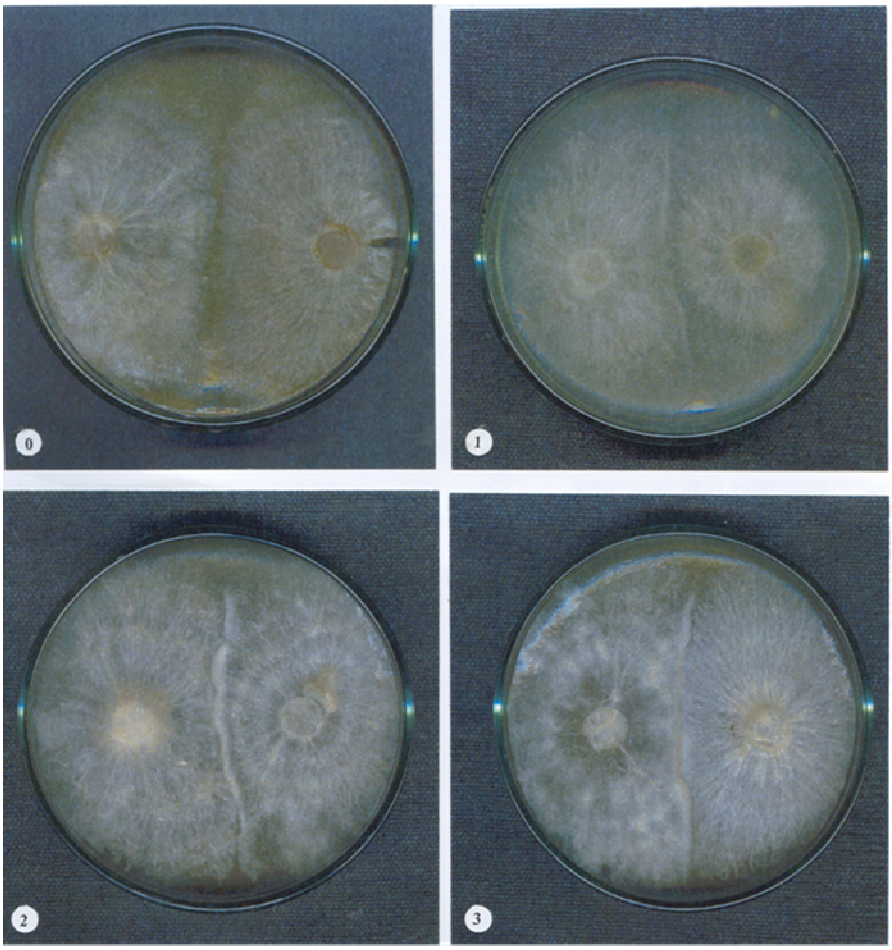

Figure 1. Somatic incompatibility between two *Agaricus bitorquis* isolates on MEA in 20 days. 0 = no visible reaction; 1 = slight incompatibility; 2 = moderate incompatibility; and 3 = strong incompatibility.

phically diverse isolates of *A. bitorquis* showed incompatible reactions. Incompatible reactions began as a gap between two isolates at 2 - 3 weeks and thick, white line formed between two isolates after 4 weeks. The line formed pigmentation at 5 or more weeks. In some pairings the pigmentation was light while in the others clear pigmented line was present.

The results showed that colony type was white, aerial, fluffy almost slower growing. The width of the interaction was rated on a scale of 0 - 3. (0 = no visible reaction; 1= slight reaction; 2 = moderate reaction; and 3 = strong reaction) (Figure 1). The resulting of somatic incompatibility reactions between heterokaryons was given (Table 3).

Gap reactions are sometimes visible when heterokaryons differ at two loci. The reaction ratings of heterokaryon 1x heterokaryon 2 = 0 and 1x3 = 0 but 2x3 = 1. If 1 and 2 differed at somatic incompatibility locus a, 1 and 3 differed at locus b, then 2 and 3 differed at both a and b and their interaction produced a visible gap reaction (Hansen et al., 1993). No reactions were recorded between all sibling heterokaryons AA, BB, CC, DD for example. The line between heterokaryon was colour (white pigmentation), with ratings, 1 or 2. Intensive pigmentation and fluffy line between heterokaryon was named as 3 and strong incompatibility were obtained AxH, AxK, ExJ, GxJ.

Marcais et al. (2000) explained that no strong incompatibility was present in pairings between sibling dikaryons. Our data relate to visual and morphological changes between heterokaryotic mycelia of different strains on agar. During comparisons, a gap among two strains and pigment formation were determined as main features. The vegetative compatibility of *C. fusipes* isolates was examined by Marcais et al. (1998). They examined the vegetative compatibility of *C. fusipes* isolates and explained that the morphology of the interaction observed between those unrelated dikaryons was very similar to the one observed among the sib related and the sibling dikaryons. Pigment formation between mycelia is very important at incompatibility in fungi. Such incompatibility is often visible to the naked eye as barrage, the formation

Table 3. Somatic incompatibility reactions between *Agaricus bitorquis* heterokaryons. 0 = no visible reaction; 1 = slight incompatibility; 2 = moderate incompatibility; and 3 = strong incompatibility.

Heterokaryons	A	B	C	D	E	F	G	H	J	K
A	0	2	0	0	1	0	0	3	2	2
B		0	1	1	0	0	1	2	2	1
C			0	1	1	1	1	1	1	0
D				0	1	1	0	2	0	1
E					0	0	1	1	3	0
F						0	0	1	0	0
G							0	1	3	0
H								0	2	1
J									0	2
K										0

Table 4. Scanning electron microscopy analysis about to elements of *Agaricus bitorquis* hyphae.

Elements	Line	Intensity (c/s)	Concentration (wt%)
C	Ka	0.00	0.000
O	Ka	4.56	0.000
Mg	Ka	0.23	0.267
Al	Ka	1.03	1.080
P	Ka	0.21	0.162
K	Ka	7.88	4.994
Ca	Ka	36.68	25.114
Fe	Ka	2.30	2.868
Cu	Ka	22.70	52.460
Zn	La	0.21	0.745
Se	Ka	1.35	11.529
Mo	La	0.31	0.601
Cd	La	0.10	0.180

of a demarcation zone of sparse mycelium sometimes with black pigmentation. Post fusion incompatibility is widespread in filamentous fungi, for example *Ophiostoma novo-ulmi*. The points of inoculation of the two strains are visible. A barrage of fuzzy white aerial mycelium, narrow between the inoculums sites and wider further out, has been produced where the strains have met (Carlile and Watkinson, 1996). Pigmented barrages described with wood-rot species (Rayner et al., 1994). Pigment accumulation in the medium and sometimes in hyphal walls often occurs (Wilson, 1991). *Neurospora crassa*, *Aspergillus nidulans* and *Saccharomyces cerevisiae* are among the genetically best understood organisms and their study has enabled fundamental biological advances to be made (Carlile and Watkinson, 1996).

In our study, sibling heterokaryons, AA, BB, CC for example, do not produce visible line formation. In this study, pigmentation is white at first and later change to intensive color. They showed compatible reactions (Table 3). In other group; especially those located in different geographical regions (approximately 5 km) were observed incompatibility reactions. The results indicated that 0.69 pairs of the groups were somatically incompatible and 0.31 of pairs between strains were compatible. Volk and Leonard (1989) indicated that cultural studies demonstrated a genetic basic for different types of interactions between mycelia from sister and non sister spores. They observed that mycelial reaction between mycelia from non-sister spores and non mycelial reaction shows no aerial ridge of hyphae formed at the line of junction. The mating test of the cultivated mushroom *A. bitorquis* identified 13 different alleles at the incompatibility by Martinez et al. (1995). In this paper, compatible and incompatible reactions were examined by using SEM. SEM photographs related to hyphae are given in Figure 2.

In common with much of the information on the composition of the mushroom, that for minerals gives little detail about the samples analyzed. One of the most comprehensive mineral analyses of *A. bisporus* to date is

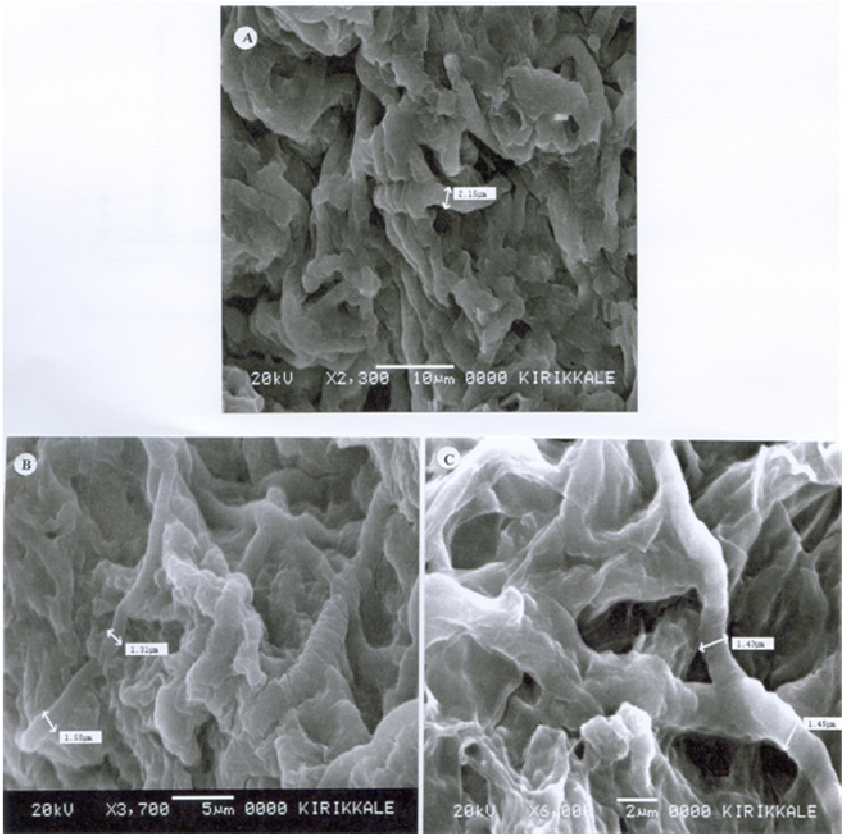

Figure 2. Scanning electron microscopy analysis in the compatible and incompatible hyphae. A- compatible hyphae, B,C- incompatible hypha.

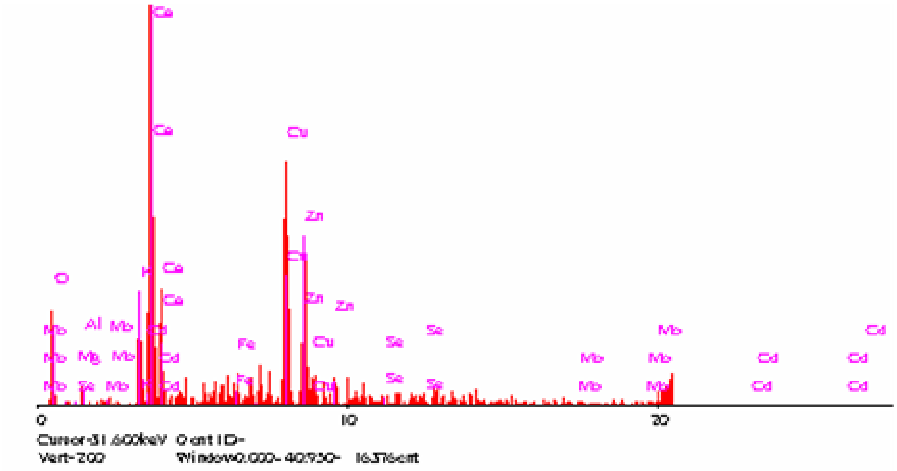

Figure 3. Some elements in *Agaricus bitorquis* hyphae.

that of Varo et al. (1980). Their values give some idea of the relative proportion of various minerals. For example, K, P, Cu and Fe were 6.2 g, 0.75 g, 9.4 mg and 7.8 mg, respectively. Mushroom contains considerable amounts of potassium, phosphorus, copper and iron but do not contain appreciable quantities of calcium. A significant proportion of phosphorus is predominant in the gills of the sporophore. Copper is accumulated by members of the *Agaricus* and is most abundant in the outer layers and in the cap and gills. The mushroom also supplies significant quantities of other elements associated with manganese, molybdenum and especially zinc (Manning, 1985). In our

study, some elements at the hyphae were analyzed by SEM. Elements quantities of *A. bitorquis* hyphae were given in Figure 3 and Table 4.

The functions of various elements were investigated by Griffin (1981) and these elements play similar roles in the nutrition of *Agaricus*. For example, iron-containing compounds would promote primordium formation in cultures grown on 2% malt extract agar. Calcium is essential for fruit body formation and also forms a sheath of calcium oxalate crystals which surround mushroom hyphae (Wood and Fermor, 1985).

ACKNOWLEDGMENTS

The authors are thankful to Ass. Prof. Kültigin Çavuşoğlu, Giresun University and Ass. Prof. Talip Kırındı Kırıkkale University for scan-ning electron microscopy examinations. The financial support by T.R. Prime Ministry State Planning Organiza-tion (DPT), via a grant number DPT 2002 K120430-03, is acknowledged gratefully.

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