Mitochondrial Inheritance During Outcrossing In a Substrate Simultaneously Inoculated With Spores And Mycelium Of Agaricus bisporus

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Mitochondrial inheritance during outcrossing in substrate simultaneously inoculated with spores and mycelium of *Agaricus bisporus*

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Since two decennia, strains of *A. bisporus*, the button mushroom, have been isolated from several hundred of wild specimens but this biodiversity remains underexploited because it is is difficult to cross them. In the predominantly pseudohomothallic life cycle of *A. bisporus,* a low percentage of spores are homokaryotic (*n*), while most of them give rise to fertile heterokaryons (*n+n*) that cannot be easily crossed *in vitro*. In this context, the following method of hybridization *in vivo* was recently developed: compost trays are simultaneously inoculated with spores from one parent and with a homokaryotic mycelium from a second parent. It was shown that all the sporocarps produced on such trays were hybrids and that all or most of them resulted from Buller phenomenon, i.e. crosses between the inoculated homokaryon and heterokaryons issued from the inoculated spores. We propose here to analyse how mitochondria are inherited during this process. Firstly, we developed a new mitochondrial marker based on the presence/absence of certain introns of the COX1 gene, which allows us to distinguish the mitochondria of each parent. Secondly, analysing the genotypes of the hybrids sporocarps obtained with the new outcrossing method, we found that the mitochondrion of the parental homokaryon was systematically inherited in all the analyzed sporocarps. Thirdly, hybrid mycelia were also classically obtained *in vitro*, by confronting homokaryons issued from the two parents. In this case, genotype analysis revealed also a monoparental inheritance of the mitochondria, but, interestingly, the results were inverted: mitochondria were always inherited from the parent that never transmitted its mitochondrion in the 'in vivo' experiment. These data confirm that during the first experiment *in vivo*, (1) the rare homokaryotic spores did not participated to the crosses, and (2) during the outcrossing process, one nucleus migrates alone from a heterokaryotic spore or from the mycelium issued from it, towards the homokaryon.

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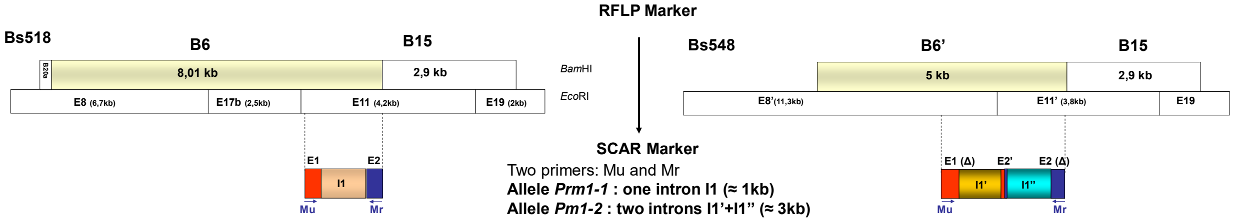
In *Agaricus bisporus* var. *bisporus* most of the basidiospores are heterokaryotic (predominant pseudohomothallic life cycle). From this, the outcrossing classical method (confrontation of homokaryons *in vitro)* is hampered by the difficulty to recover homokaryotic strains. However, it was recently shown that numerous hybrid sporocarps are easily produced *in vivo* by simultaneously inoculating a standard compost of culture with a homokaryon from one parent and the spores from a second parent (Callac *et al.* 2006). In this case, it was indirectly shown that these crosses occurred between the heterokarotic spores (or mycelia issued from them) and the inoculated homokaryon (Buller phenomenon).

Our aim was to determine from which parent the mitochondria are inherited during this *in vivo* process, in comparison to an *in vitro* confrontation of homokaryons. For this, a SCAR mitochondrial marker derived from a previously reported (Xu *et al.* 1998) RFLP marker was developed to distinguish the two parental haplotypes.

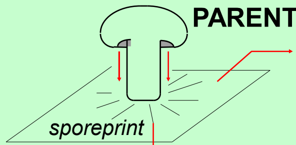
The PRM1 marker : a mitochondrial SCAR marker based on the polymorphism of the introns of the *Cox1* gene



Outcrossing between parents A and B, by using two different methods

A

B



PARENT A strain Bs 518 (brown cultivar C9) haplotype mt1 (genotype *Prm1-1*)

Agaricus bisporus is amphithallic: among the offspring, about 94% of the single spore isolates were heterokaryotic, while 6% were homokaryotic.

Spore suspension (about one billion) most of them are heterokaryotic (*n+n*)

12 homokaryotic single spore isolates (*n*)

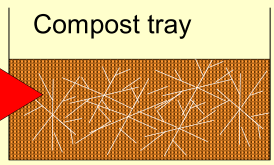
PARENT B strain Bs 548 (white hybrid cultivar S608) haplotype mt2, (genotype *Prm1-2*)

protoplastes

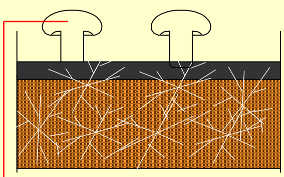
Homokaryon Bs 548-2

OUTCROSSING *IN VIVO*

- Spores and homokaryon are simultaneously inoculated in the compost of 4 culture trays



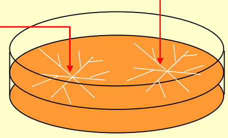
- Fructification of hybrid sporocarps
- Isolation of the AxB hybrid strains by tissue culture of the sporocarps



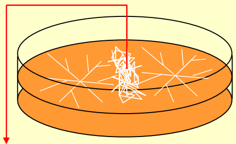
20 different hybrids (AxB) were isolated. Hybrid status was confirmed by using nuclear SCAR markers

OUTCROSSING *IN VITRO*

- Homokaryons are confronted in pairs in 12 compost agar plates



- Positive reaction (fluffy mycelium) between sexually compatible homokaryons
- Isolation of the AxB hybrid strains from the fluffy mycelium



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	Outcrossing *in vivo*	Outcrossing *in vitro*
genotype *PRM1*	The 20 hybrids possessed the *Prm1-2* allele	The 12 hybrids possessed the *Prm1-1* allele
CONCLUSION	**Mitochondria were inherited from the parent B**	**Mitochondria were inherited from the parent A**

DISCUSSION

Mitochondria inheritance in hybrid strains is uniparental and appears to depend on the used outcrossing method. When two homokaryons are mated *in vitro*, one of the two parental haplotypes is preferentially inherited. When crosses are performed *in vivo*, Buller phenomenon occurs and data show that one nucleus from the heterokaryotic spores (or the mycelium issued from them) migrates alone in the homokaryotic mycelium.

In a simple model where mycelia from spores (6% homokaryotic) would cross at random, 97% of the crosses would occur between homokaryons and heterokaryons. This would lead to hybrids without preferential haplotype inheritance (when homoA x heteroB and heteroA x homoB equally occurred). This agrees with the numerous different haplotypes found in wild populations.

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