Fungal Genetics Reports

Volume 43 Article 14

Heterokaryon formation in Ascobolus immersus is not affected by mating type

Bernard C. Lamb
Imperial College of Science, Technology and Medicine

Wei Mun Chan
Imperial College of Science, Technology and Medicine

Recommended Citation

Lamb, B. C., and W.M. Chan (1996) "Heterokaryon formation in Ascobolus immersus is not affected by mating type," *Fungal Genetics Reports*: Vol. 43, Article 14. https://doi.org/10.4148/1941-4765.1310

Heterokaryon formation in Ascobolus immersus is not affected by mating type

Abstract

The ability of *Ascobolus immersus* to form vegetative heterokaryons between strains of the same mating type and between strains of opposite mating type was tested using auxotrophs and morphological markers. Heterokaryon formation, confirmed by hyphal-tip culture, was not affected by mating type; in several other filamentous fungi, vegetative heterokaryons only form between strains of the same mating type.

Heterokaryon formation in *Ascobolus immersus*is not affected by mating type

Bernard C. Lamb and Wei Mun Chan -
Department of Biology, Imperial College of Science, Technology and Medicine, London SW7 2BB, UK

The ability of *Ascobolus immersus* to form vegetative heterokaryons between strains of the same mating type and between strains of opposite mating type was tested using auxotrophs and morphological markers. Heterokaryon formation, confirmed by hyphal-tip culture, was not affected by mating type; in several other filamentous fungi, vegetative heterokaryons only form between strains of the same mating type.

In filamentous fungi, vegetative fusion in heterokaryon formation is often controlled by mating type and by a number of vegetative incompatibility loci. For example, in *Neurospora crassa*, hyphae of opposite mating type, *A* and *a*, will not form a viable heterokaryon as incompatibility reactions at the point of fusion lead to death, although only opposite mating types can cross sexually. Fusion of hyphae of the same mating type is controlled by a number of *het* loci, for which alleles must be identical in the two fusing hyphae. Mating- type-associated vegetative incompatibility also occurs in *Aspergillus heterothallicus* and *Ascobolus stercorarius* (references in Glass and Kuldau, 1992 Annu. Rev. Phytopathol. **30**: 201- 224).

We have used auxotrophic (Lamb and Helmi, 1991 Fungal Genetics Newsl.**38**: 75-77) and morphological mutants to test whether mating type affects heterokaryon formation in the Pasadena strains of *Ascobolus immersus*. These mutants were obtained in strains derived ultimately from two original cultures from ascospores of opposite mating type, P5(+) and K5(-)(Emerson and Yu-Sun,1967 Genetics **55**: 475-485). Inocula were obtained by placing small (about 2 mm length cube) blocks of complete medium on plates of minimal medium (media as in Yu-Sun,1964 Am. J. Bot. **51**: 231-237), and taking off small blocks of this minimal medium when hyphae had grown over them from the complete medium blocks. Inocula of different mutants were placed side by side in the centre of 9 cm Petri dishes of minimal medium at 17.5 C, with (+) and (+) mating type combinations, or (-)and (-),or (+) and (-)combinations. Platings of two different types of mutation, e.g. methionine- requiring and leucine- requiring, were made, and the amount of growth produced was compared with that of each auxotroph on its own on minimal medium.

The auxotrophic mutations used were: methionine, leucine, valine, and alanine requirements; morphological mutants *slow growth* and *thin* (with sparsely branched hyphae) were also tried. All strains were haploid, and both mating types were available for each strain. The various auxotrophs grown on their own on minimal medium gave from 2 to 16 mm radial growth after 10 days. When any combination of different auxotrophs, or of different morphological mutants, was tried in any of the three mating type combinations, (+) with (+), (-) with (-),(+) with (-),on minimal medium, the whole Petri dish was covered in wild- type hyphal growth in under 10 days, showing complementation between the mutants at different loci. The only exception was methionine (+) and leucine (-), for unknown reasons. Complementation between the two

morphological mutants showed as colonies with wild- typegrowth rate and normal morphology, including hyphal branching patterns. The combinations tested and their results are shown in Tables 1 and 2.

To distinguish between heterokaryon formation and joint growth of two homokaryons from mutual cross- feeding, single hyphal tips were isolated under a dissecting microscope from potential heterokaryons and were plated on minimal medium to test for further growth. In all cases, the tips continued to grow. It has therefore been shown that heterokaryon formation in *Ascobolus immersus* is independent of mating type, unlike the cases mentioned above from *N. crassa*, *Aspergillus heterothallicus* and *Ascobolus stercorarius*. There also appear to be no, or very few, non- mating-type vegetative incompatibility genes differing between these *Ascobolus immersus* strains.

Acknowledgement. We are most grateful to Muhammad Saleem for providing the mutants used, and to the Frederick Gregory Fund for a bursary for W. M. C.

Table 1. Heterokaryon- formation test results in relation to mating type, for auxotrophs.

		Strain 1							
		methionine -		leucine -		valine -		alanine -	
	Mating type	+	-	+	-	+	-	+	-
methionine-	+	m*	m	wt*	wt	wt	wt	wt	wt
	-	m	m	wt	wt	wt	wt	wt	wt
leucine-	+	wt	wt	m	m	wt	wt	wt	wt
	-	m	wt	m	m	wt	wt	wt	wt
valine-	+	wt	wt	wt	wt	m	m	wt	wt
	-	wt	wt	wt	wt	m	m	wt	wt
alanine-	+	wt	wt	wt	wt	wt	wt	m	m
	-	wt	wt	wt	wt	wt	wt	m	m

* m, mutant phenotype, less than 16 mm radial growth after 10 days; wt, wild- type growth, Petri dish completely covered in under 10 days.

Table 2. Heterokaryon- formation test results in relation to mating type, for morphological mutants.

		Strain 1			
		thin-		slow-	
	Mating type	+	-	+	-
thin-	+	thin*	thin	wt	wt
	-	thin	thin	wt	wt
slow-	+	wt	wt	slow	slow
	-	wt	wt	slow	slow

* Thin, thin growth, sparsely branched hyphae; slow, normal branching but slow growth; wt, wild- type, normal growth-rate and hyphal branching.

Last modified 7/25/96 KMC