Genet. Res., Camb. (1973), **22**, *pp.* 181–185
Printed in Great Britain

Variation in the basal level of alkaline phosphatase in *Coprinus lagopus* wild-type strains

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(*Received* 24 *April* 1973)

SUMMARY

Two wild-type strains of *Coprinus lagopus* isolated from a single basidiocarp differ by a factor of two in their basal level of alkaline phosphatase. The gene responsible for this difference is allelic to *reg-2* and unlinked to the *pho* loci; the allele conferring a lower basal level is dominant both in diploids and dikaryons.

1. INTRODUCTION

The production of alkaline phosphatase (AP) in *C. lagopus* is under the control of at least four regulator loci. Previous results indicate that although the two wild-type strains H2 and H5 from which mutants were selected were isolated from a single basidiocarp, they differ in their basal level of enzyme (North & Lewis, 1971).

It is possible in *Coprinus* to compare gene action between and within nuclei because regular dikaryons and stable diploids are available. The experiments described here confirm the difference between H2 and H5 and show that the action of the gene concerned is not confined to the nucleus.

2. MATERIALS

Wild-type strains H2 and H5 and the life-cycle of *C. lagopus* were described by North & Lewis (1971). Diploids were selected by the method of Casselton (1965) and tested by the dikaryon test (Casselton, personal communication). Origins and genotypes of the strains used are shown in Table 1.

3. METHODS

Culture media and techniques were as used by North & Lewis (1971). Mycelium was grown in liquid culture for 3–4 days (high phosphate medium) or 7–8 days (low phosphate medium), harvested by filtration, washed, blotted dry, weighed and stored at −25 °C until used. Cell-free extracts were made and assays carried out as previously (North & Lewis, 1971).

https://doi.org/10.1017/S0016672300012969 Published online by Cambridge University Press

4. RESULTS

We have shown that the specific activity of acid phosphatase in *Coprinus* is not as much affected by the phosphate concentration of the medium as alkaline phosphatase (North & Lewis, 1971). Table 2 gives the specific activities of haploids, dikaryons and diploids grown on high and low phosphate medium. The activities

Table 1. *Strains of* Coprinus *used in this investigation*

Nuclear type	Strain	Mating type A	Mating type B	Genotype	Origin
Haploid	H2	6	5	+	Wild
	H5	5	6	*reg-2*	Wild
	ARR14	6	5	*reg-2*	AR72 (mutant exH2) × H5
	ARR15	5	5	*reg-2*	AR72 (mutant exH2) × H5
	ARR30	2	3	*reg-2 me-1*	ARR14 × PR2301
	ARR31	6	5	*reg-2 nic-4 paba-2 ad-me*	ARR15 × multiple marked strain WMR66
	ARR49	6	6	*reg-2 adhi-6*	ARR15 × mutant P809 × H9
	AP51	5	6	*pho-1*	Mutant ex H5
	AP23	6	5	*pho-3*	Mutant ex H2
	PR2301	2	3	*me-1*	68 (wild) × mutant ex wild H9
	SR16	2	6	*me-5*	Mutant ex wild H9
Dikaryon	H2 × SR16			(+ +) × (+ *me-5*)	Made from haploids described above
	H2 × H5			(+ +) × (*reg-2* +)	
	ARR14 × SR16			(*reg-2* +) × (+ *me-5*)	
	ARR14 × ARR30			(*reg-2* +) × (*reg-2 me-1*)	
	ARR14 × H5			(*reg-2* +) × (*reg-2* +)	
	ARR30 × H5			(*reg-2 me-1*) × (*reg-2* +)	
Diploid	ARR2301.16			(+ + *me-1*) / (+ *me-5* +)	Made from: PR2301 and SR16
	ARR30.16			(*reg-2* + *me-1*) / (+ *me-5* +)	ARR30 and SR16
	ARR31.49			(*reg-2 nic-4 paba-2 ad-me* +) / (*reg-2* + + + *adhi-6*)	ARR31 and ARR49

do not vary between strains of the same nuclear type and are independent of the phosphate concentration. There is obviously some difference between nuclear types, particularly the haploids and the dikaryons. For this reason in later experiments comparisons were only made within nuclear types.

Enzyme activities in fungi are variable, and are often expressed as the mean of several replicate experiments (Cove, 1969). To measure the alkaline phosphatase of *Coprinus* strains several cultures of each were grown and extracted as described under methods. Cook & Sorger (1969), working on *Neuropora crassa*, suggest that the activity of a repressible enzyme can be expressed relative to that of another

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Table 2. *Specific activity of* Coprinus *acid phosphatase*

Nuclear type	Strain		Specific activity: High phosphate	Specific activity: Low phosphate
Haploids	H2	$n = 3$	24·87 ± 4·04	22·33 ± 4·32
	H5	$n = 3$	24·45 ± 4·94	24·53 ± 2·62
	ARR14	$n = 3$	19·53 ± 1·42	19·47 ± 1·36
Dikaryons	H2 × H5	$n = 3$	13·42 ± 2·82	10·75 ± 1·76
	ARR14 × H5	$n = 3$	10·00 ± 0·49	10·62 ± 1·10
Diploids	ARR2301.16	$n = 4$	18·01 ± 0·12	14·58 ± 1·81
	ARR31.49	$n = 3$	16·50 ± 0·29	13·58 ± 1·70

Specific activity = optical density at 400 nm/mg protein/h.
n = number of experiments.

Table 3. *C/L ratios of haploids grown on high phosphate medium*

Strain	Partial genotype	C/L
H2	+	28·35 ± 2·54, $n = 5$
H5	*reg-2*	11·42 ± 1·16, $n = 6$ }
ARR14	*reg-2*	15·84 ± 1·85, $n = 7$ }

The values within the parentheses are not significantly different. The two groups of values differ at the 1 % level (t-test).

Table 4. *C/L ratios of diploids and dikaryons*

Strain	Partial genotype	C/L	Group
Dikaryons			
SR16 × H2	(+) × (+)	22·58 ± 3·53, $n = 6$	A
SR16 × ARR14	(+) × (*reg-2*)	22·50 ± 2·22, $n = 10$	A
H2 × H5	(+) × (*reg-2*)	20·48 ± 0·85, $n = 9$	A
ARR14 × H5	(*reg-2*) × (*reg-2*)	11·70 ± 0·84, $n = 8$	B
ARR30 × H5	(*reg-2*) × (*reg-2*)	9·06 ± 0·81, $n = 6$	B
ARR14 × ARR30	(*reg-2*) × (*reg-2*)	11·64 ± 1·58, $n = 5$	B
Diploids			
ARR2301.16	+/+	15·24 ± 1·15, $n = 8$	C
ARR30.16	*reg-2*/+	15·94 ± 1·87, $n = 5$	C
ARR31.49	*reg-2*/*reg-2*	7·11 ± 0·88, $n = 7$	D

Values within parentheses are not significantly different. Group A differs from B, and group C from D at the 1 % significance level.

enzyme known to have constant specific activity under the experimental conditions. We have shown (Table 2) that within nuclear types acid phosphatase activity in *Coprinus* is unaffected by medium phosphate concentration or genetic background; in the experiments described below alkaline phosphatase is expressed as

$$\frac{\text{units acid phosphatase}}{\text{units alkaline phosphatase}} \quad \text{(the C/L ratio)},$$

where 1 unit gives an OD of 1·0 at 400 nm after 30 min. at 37 °C.

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Table 3 gives C/L ratios for the wild-types H2 and H5, and a recombinant ARR14. H5 and ARR14, designated *reg*, produce twice as much enzyme as H2. In order to test the dominance of the *reg* mutation, diploids and dikaryons heterozygous for the *reg* mutation were constructed and their C/L ratios compared to those of homozygous strains. The full genotypes of the strains used are shown in Table 1. The C/L ratios indicate (Table 4) that the allele conferring the lower basal level is dominant in both diploids and dikaryons.

Table 5. *Crosses of* reg-2 *strains with* pho *mutants*

Cross	Genotypes	Number of spores
ARR14 × AP51 (*pho-1*)	+ +	35
	reg-2 +	21
	reg-2 pho-1 }* + *pho-1*	61 Total = 117
ARR30 × AP23 (*pho-3*)	+ +	28
	reg-2 +	22
	reg-2 pho-3 }* + *pho-3*	34 Total = 84

* The two genotypes bracketed together are phenotypically identical. Spores were tested by the diazocoupling technique on high and low phosphate media.

A cross between H5 and ARR14 produced no wild-type spores out of 100 progeny tested. Dikaryons between H5 and *reg-2* mutants, AR12, AR52 and AR182 and between ARR14 and *reg-2* mutants AR65 and AR75 showed the *reg* phenotype when tested by the diazocoupling technique, indicating that H5 and ARR14 are *reg-2*. Previous results show no linkage between *pho-2* and *reg-2* (North & Lewis, 1971); results from crosses with *pho-1* and *pho-3*, shown in Table 5, indicate that *reg-2* recombines freely with these loci.

5. DISCUSSION

Coprinus lagopus is an outbreeding organism with a tetrapolar incompatibility system. Variability in outbreeding fungi is under genetic control and subject to selection (Blatherwick & Wills, 1971; Jinks & Connolly, 1972). In *C. lagopus* Moore & Stewart (1971) report that the expression of 2-deoxy-D-glucose resistance depends on the background of the strain; wild-types vary in their ability to grow on amino acid analogues (Lewis, 1963; S. Senathirajah & D. Lewis, in press) and *beta*-glycerophosphate (North & Lewis, 1971). In *Escherichia coli* AP synthesis is controlled by two regulator genes, *pho R* and *pho S* (Echols *et al*. 1961). The basal level of activity in repressed wild-type strains is controlled by separate genes which are thought to affect the concentration of aporepressor (Jones, 1969). The results we report above indicate that the basal level of AP in *Coprinus* wild-types is under similar control.

Regulator gene mutations in fungi often show a dosage effect, suggesting a limiting concentration of regulator molecules (Cove, 1969, 1970; Hynes & Pateman,

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1970*a*, *b*; Valone, Case & Giles, 1971). In most fungal systems it is not possible to test whether the regulator molecules are confined to the nucleus because of uneven nuclear distribution in heterokaryons (Clutterbuck & Roper, 1966). In *Coprinus* diploids and dikaryons are directly comparable (Casselton & Lewis, 1967; Day & Roberts, 1969); our results show that the product of the regulator gene *reg-2* is neither confined to the nucleus nor present in limiting concentration.

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