Genet. Res., Camb. (1971), **18**, *pp.* 153–166
With 1 *text-figure*
Printed in Great Britain

Phosphatases of *Coprinus lagopus*: the conditions for their production and the genetics of the alkaline phosphatase

By JANE NORTH* and D. LEWIS
Department of Botany and Microbiology, University College London

(*Received* 12 *May* 1971)

SUMMARY

1. *Coprinus lagopus* produces two non-specific phosphatases: a constitutive acid phosphatase, and an alkaline phosphatase which is repressed during growth on media with a high inorganic phosphate concentration.

2. The alkaline phosphatase is also repressed when *Coprinus* is grown on an organic phosphate source; but if the acid phosphatase is selectively inhibited by fluoride the alkaline phosphatase is de-repressed and growth is comparable to that observed on an inorganic phosphate source.

3. Alkaline phosphatase is not repressed in aerial mycelium or sporophores even when grown on high phosphate medium.

4. Mutants altered in their capacity to synthesize alkaline phosphatase were selected from two compatible wild-type strains, H 2 and H 5.

5. Mutants producing a higher level of alkaline phosphatase than wild-type ('regulator' mutants) fall into four (or possibly five) complementation groups. Assuming five separate genes, two pairs are linked; the remaining one is independent and on another chromosome.

6. Mutants deficient in alkaline phosphatase synthesis fall into at least three groups. They were tested for linkage to 'regulator' loci but so far there is no evidence of this.

1. INTRODUCTION

Repressible non-specific phosphatases have been detected in a variety of bacteria. The genetical control has been investigated in some of them, e.g. *Escherichia coli* and *Bacillus subtilis* (Garen & Echols, 1962*a*, *b*; Le Hegarat & Anagnostopoulos, 1969). In both these species the enzyme is localized outside the cell membrane, suggesting a nutritional role (Kusnarev & Smirnova, 1966; Takeda & Tsugita, 1967).

Non-specific phosphatases have also been found in fungi. *Neurospora crassa* produces two constitutive alkaline phosphatases and a constitutive acid phosphatase (Kuo & Blumenthal, 1961; Davis & Lees, 1969). In addition, it produces two repressible enzymes: an alkaline phosphatase (Nyc, Kadner & Crocken, 1966) and an acid phosphatase which is secreted into the culture medium (Nyc, 1967). The constitutive alkaline phosphatase activity is released from the mycelium by osmotic shock (Wiley, 1970). *Aspergillus nidulans* produces a constitutive alkaline phosphatase, two repressible acid phosphatases, and a repressible alkaline phosphatase (Dorn & Rivera, 1966). Mutations in many genes can affect phosphatase synthesis, some mutations affecting more than one enzyme (Dorn, 1965).

* Present address: Biological Laboratory, University of Kent, Canterbury, Kent.

Coprinus lagopus is a basidiomycete fungus suitable for biochemical and genetical analysis (e.g. Lewis, 1961, 1963; Casselton, 1965; Casselton, Fawole & Casselton, 1969). In addition, the *Coprinus* fruiting body is large enough for biochemical analysis. The object of this investigation was to look for non-specific phosphatases in *Coprinus*, and to analyse the physiological and genetical control of their production.

2. MATERIALS

The wild-type strains of *Coprinus lagopus* used for selection of mutants were a compatible pair, H 2 (A_6B_5) and H 5 (A_5B_6), which were isolated in Bayfordbury, Herts.

Growth and biochemical experiments were carried out on H 2, H 5 and their dikaryon, H 2 × H 5.

The life-cycle of *C. lagopus* was described by Lewis (1961). Marker strains used in mapping experiments are listed in Table 1.

Table 1. *Marker strains of* Coprinus lagopus *used for mapping experiments*

Strain	Mating type	Markers
SR 54	A_6B_6	*ad*-8 *me*-5 *Chol*-1
C 692	A_3B_1	*ad*-3
PR 2301	A_2B_3	*me*-1
WMR 66A	A_6B_6	*nic*-4 *paba*-2
P 809	A_3B_3	*ad*-6
CC 201	A_5B_5	*ac*-2
CC 1	A_5B_5	*ac*-1
ARR 33	A_5B_6	*nic*-4 *paba*-2 gp^s-4
MAE 161	A_6B_5	*paba*-1

3. METHODS

(i) *Culture media*

Minimal, complete and fruiting media were described by Lewis (1961). Basidiospores were germinated on complete medium supplemented with 0·01 % furfuraldehyde. Supplemented media for growth of auxotrophs were described by Casselton (1965). Phosphate-free medium was adapated from minimal medium by the replacement of phosphate by 0·05 M HEPES (*N*-2-hydroxyethyl piperazine *N'*-2-ethane sulphonate: Sigma). In addition, liquid phosphate-free medium contained 0·178 g/l. K_2SO_4. Media with defined phosphate content were made by the addition of a solution of equimolar KH_2PO_4 and Na_2HPO_4. Sodium *beta*-glycerophosphate* (Sigma) and sodium phytate (B.D.H.) were added as sterile filtered solutions after media had been autoclaved. Acetate medium was adapted from minimal by the omission of glucose and asparagine, the addition of 10 g/l. anhydrous sodium acetate and increasing the ammonium tartrate content to 5 g/l. (L. A. Casselton, personal communication).

* Abbreviation for sodium *beta*-glycerophosphate = BGP.

(ii) *Culture techniques*

Methods for growth tests on solid media and fruiting were described by Lewis (1961) and Casselton (1965). All growth tests were incubated for 72 h unless otherwise stated. Liquid cultures were inoculated with spore suspensions (oidia or chlamydospores) prepared by standard methods. Flasks were incubated on a shaker at 37 °C.

Dry weights were used to measure growth in liquid culture.

(iii) *Preparation of cell-free extracts*

Mycelium grown in liquid culture was filtered through fine nylon net, washed and homogenized for 3 min with a 'Vertis 45' blender in 0·1 M *tris*, pH 7·6. The homogenate was centrifuged at 18000 rev/min for 20 min and the supernatant used for enzyme assays. All operations were carried out at 4 °C.

(iv) *Assays for phosphatase activity*

(*a*) Preliminary tests on whole mycelium were carried out by the method of Neumann & van Vredendaal (1967) for alkaline phosphatase and a modification of the method of Giri *et al.* (1952) using 0·6 M acetate buffer, pH 4·8, for acid phosphatase.

(*b*) Test for small colonies (used for selection of mutants and scoring of complementation tests and basidiospore colonies) was adapted from Dorn (1965), using for each Petri dish 1 mg *alpha*-naphthyl phosphate (Koch–Light) and 10 mg Fast Violet salt B (George Gurr) in 4 ml. *tris* pH 9·0.

(*c*) Activity in cell-free extracts was measured using *p*-nitrophenyl phosphate (Koch–Light) as substrate. Assay tubes contained 0·5 ml extract, 3 ml buffer and 0·5 ml substrate (4 mg/ml). Tubes were incubated at 37 °C and the reaction was stopped by the addition of 0·5 ml 2 M sodium hydroxide solution. *p*-Nitrophenol was measured by OD at 400 nm. The buffers used in the assay are described in Table 2. All buffers were used at a concentration of 0·2 M.

Table 2. *Buffers of phosphatase assays*

Buffer	pH range
Acetate	4·1–6·05
Imidozole	5·8–7·5
Ammediol*	7·5–9·85
Ammonium	9·6–10·5

* Ammediol (Koch–Light) is 2-amino 2-methyl-1,3-propane diol.

(v) *Phosphate assay*

Phosphate was assayed by the method of Delsal & Manhuri (1958).

(vi) *Protein assay*

Protein was assayed by the method of Lowry *et al.* (1951), with crystalline bovine serum albumen (B.D.H.) as standard.

(vii) *Ultraviolet irradiation*

Ultraviolet irradiation of spores was described by Lewis (1961).

4. RESULTS

(i) *Growth and alkaline phosphatase production on solid media of varying phosphate concentrations*

A preliminary investigation (Lewis, unpublished) suggested that *Coprinus* produces a repressible alkaline phosphatase. To confirm this, growth tests on H 2 and H 5 were set up on media of increasing phosphate concentrations. In addition to radial growth measurements, production of aerial mycelium was scored. Alkaline phosphatase production was tested on standard mycelial blocks. The results shown in Table 3 indicate that *Coprinus* produces a repressible alkaline phosphatase with the repression threshold at about 5×10^{-4} M phosphate. This result applies *only* to the horizontal mycelium. The aerial mycelium, which is only produced on the high phosphate media, is derepressed for alkaline phosphatase production. The sporophore of H 2 × H 5 is also de-repressed.

Table 3. *Growth in 72 h and alkaline phosphatase production of H 2 and H 5 on media with increasing concentrations of phosphate*

	H 2			H 5		
$[P_i]$ (M)	Radial growth (mm)	Aerial mycelium	Enzyme detected*	Radial growth (mm)	Aerial mycelium	Enzyme detected
0	11·0	−	+	22·0	−	+
5×10^{-7}	13·0	−	+	23·0	−	+
5×10^{-6}	14·0	−	+	23·0	−	+
5×10^{-5}	19·0	−	+	28·0	+	+
5×10^{-4}	21·0	+	−	28·0	+	−
5×10^{-3}	22·0	+	−	30·0	+	−

P_i = inorganic phosphate.
Alkaline phosphatase present: +; absent: −.
Aerial mycelium present: +; absent: −.
* These results refer to horizontal mycelium only.

(ii) *Enzyme production in liquid culture*

Cell-free extracts were made from mycelia of H 2, H 5 and H 2 × H 5 grown in liquid minimal and in phosphate-free medium. The extracts were assayed for phosphatase activity over the pH range 4·0–10·4. A typical result is shown in Fig. 1. There are two peaks of activity in the alkaline pH range which are markedly increased in the extracts from mycelia grown in phosphate-free medium. Further tests on the repression of both peaks during growth on solid medium confirmed that the minimum $[P_i]$ required to repress is the same for both peaks. In addition, there is a constitutive acid phosphatase.

Levels of de-repression varied between strains. As with *E. coli* alkaline phos-

phatase (Jones & Gallant, 1964) there is a low basal level of activity under repressing conditions. The enzyme levels in the three strains are shown in Table 4, both as specific activities and, to eliminate variations due to other protein changes during phosphate starvation, as ratios of one peak to another.

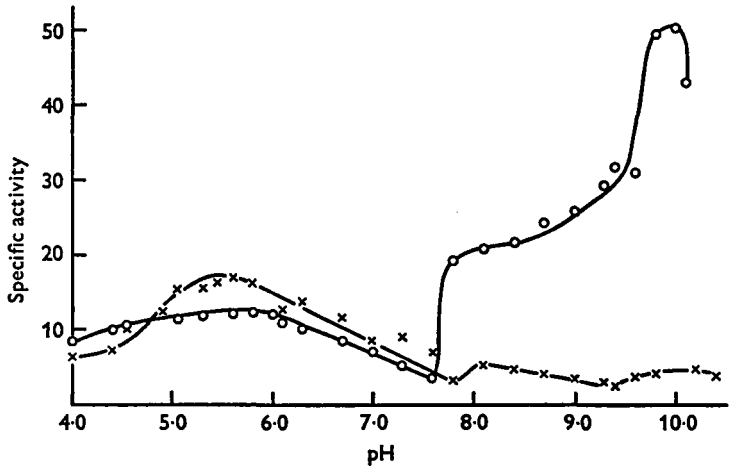


Fig. 1. Phosphate spectrum of strain H5. Assay conditions are described under methods. ×, Activity of extract of mycelium grown in liquid minimal medium. ○, Activity of extract of mycelium grown in liquid phosphate-free medium. Specific activity = μmoles *p*-nitrophenol produced/mg protein/h.

Table 4. *Phosphatase levels in extracts of repressed and de-repressed cultures*

		Specific activities*			Ratio (pH peaks)		
pH ...	Phosphate	5·4–5·6	8·0–8·4	9·8–10·2	1:2	1:3	2:3
Peak ...	level	1	2	3			
H2	High	6·90	1·25	0·85	5·5	8·0	1·5
	Low	13·20	11·45	29·80	1·0	0·45	4·0
	De-repression	× 2	× 9	× 36			
H5	High	2·60	0·80	0·60	3·0	4·5	1·5
	Low	1·80	2·20	7·80	1·0	0·25	3·0
	De-repression	× 1	× 3	× 13			
			Dikaryon				
H2 × H5	High	4·70	0·85	0·60	5·5	8·0	1·5
	Low	5·35	5·75	15·20	1·0	0·35	4·0
	De-repression	× 1	× 7	× 26			

* Specific activity is expressed as μmoles *p*-nitrophenol produced/mg protein/h.

(iii) *Inhibition of alkaline phosphatase by inorganic phosphate*

In *E. coli*, alkaline phosphatase is both inhibited and repressed by inorganic phosphate (Torriani, 1960). Extracts of *Coprinus* containing alkaline phosphatase were assayed in the presence of increasing concentrations of phosphate. The results,

which are shown in Table 5, indicate that the cut-off in detection of alkaline phosphatase shown in Table 2, at a point where the enzyme is only about 30% inhibited, is a result of repression rather than inhibition.

(iv) *Growth and enzyme production on organic phosphates*

Both constitutive and phosphataseless mutants of alkaline phosphatase in *E. coli* have been selected by virtue of the fact that wild-type bacteria can grow on *beta*-glycerophosphate (BGP) as sole carbon and phosphate source using its alkaline phosphatase (Levinthal, 1959; Torriani & Rothman, 1961). In order to find out if a similar technique was possible in *Coprinus*, growth tests were made on phosphate-free media supplemented with various organic phosphates. Enzyme production was tested on whole mycelium. The results are shown in Table 6. It is clear that both organic and inorganic phosphates repress alkaline phosphatase production. There are three possible explanations for the repression by organic phosphates:

(*a*) The alkaline phosphatase is de-repressed and hydrolyses this substrate rapidly, releasing phosphate which represses the enzyme again: this effect has been reported by Torriani (1960) in *E. coli*.

Table 5. *Inhibition of alkaline phosphatase by inorganic phosphate*

	% initial activity	
$[P_i]$ (M)	pH 8·3	pH 10·0
0	100	100
10^{-5}	91	100
10^{-4}	86	85
10^{-3}	64	76
10^{-2}	16	12

Table 6. *Repression of alkaline phosphatase on different phosphate sources*

	Concentration of phosphate (M) required to repress alkaline phosphatase		
Phosphate source	H2	H5	H2 × H5
Orthophosphate	10^{-4}	10^{-4}	10^{-4}
Pyrophosphate	10^{-4}	10^{-3}	10^{-3}
beta-Glycerophosphate	10^{-4}	5×10^{-4}	5×10^{-4}
Phytate	10^{-4}	10^{-4}	10^{-4}

(*b*) There is repression of the enzyme by organic phosphates as such, even though these are *in vitro* substrates for the enzyme: yeast acid phosphatase is repressed by glucose-1-phosphate (Heredia, Yen & Sols, 1963).

(*c*) Organic phosphates are substrates for the acid phosphatase in *Coprinus*; their hydrolysis releases phosphate which keeps the alkaline phosphatase repressed.

The third hypothesis is easy to test, since it predicts that if the acid phosphatase is inhibited in the presence of organic phosphate, the alkaline phosphatase should be de-repressed. Dorn (1965) has shown that sodium fluoride specifically inhibits the acid phosphatase of *Aspergillus nidulans*.

The effect of sodium fluoride on the growth and phosphatase activities of *Coprinus* are shown in Table 7. These results confirm that sodium fluoride at the conce ntration used has some specificity as an inhibitor of acid phosphatase while not totally inhibiting growth. Similar results were obtained with H 2.

The effect of 0·01 M fluoride on growth and phosphatase production on media with organic and inorganic phosphate sources is shown in Table 8. It is clear t hat in the presence of BGP and fluoride the alkaline phosphatase is de-repressed, as predicted by hypothesis (*c*) above. This indicates that BGP is normally a substrate for the acid phosphatase, although it can also act as a substrate for the alkaline phosphatase (growth is greater on phosphate-free medium with BGP and fluoride than on phosphate-free medium with fluoride alone).

Table 7. *The effect of 0·01 M fluoride on growth and phosphatase activity of H 5**

[F⁻]	Radial growth (72 hr)†	Enzyme activities†	
		pH 5·5	pH 10·0
0	100	100	100
0·01 M	30	7	81

* H 5 grown on solid minimal (growth) or liquid minimal (enzyme assay).
† Growth and activity as a percentage of those without fluoride.

Table 8. *The effect of 0·01 M fluoride on growth on inorganic and organic phosphate sources*

Phosphate source	Growth*		Enzymes detected			
			H 2		H 5	
	H 2 +F	H 5 +F	−F	+F	−F	+F
None	0	0	Both	N.D.	Both	N.D.
$1·4 \times 10^{-2}$ M P_i	22	29	AcP	0	AcP	0
5×10^{-4} M BGP	145	89	AcP	AkP	Both	AkP

AcP = acid phosphatase; AlkP = alkaline phosphatase; 0 = no enzyme detected; N.D. = not determined; P_i = inorganic phosphate, BGP = *beta* glycerophosphate.
* Growth as a percentage of growth without fluoride.

(v) *The use of organic and inorganic phosphate sources*

Growth of H 2, H 5 and H 2 × H 5 was measured on media supplemented with various phosphate sources. The results, shown in Table 9, indicate that inorganic phosphate and pyrophosphate both act equally well as phosphate sources for *Coprinus*. The effect of the organic phosphates is not so clear-cut; phytate, although it represses alkaline phosphatase (Table 5) does not stimulate growth at all. Glycerophosphate stimulates growth in two of the strains and inhibits H 2 (this difference is more apparent on higher BGP concentrations). Analysis of the basidiospores from the cross H 2 × H 5 showed that the growth difference is due to mutation at a single locus. The mapping of this locus will be described later.

A closer examination of the formula for the phosphate-free medium showed that it is deficient in potassium ions. In order to check that the results obtained were not artifacts of this deficiency, growth and enzyme tests were repeated on glycerophosphate medium supplemented with $1{\cdot}2 \times 10^{-3}$ g-ions/l. of potassium (as K_2SO_4). This gives a Na^+/K^+ ratio the same as in minimal medium. There were no differences in enzyme production between media with and without potassium. The growth results are shown in Table 10. It is evident that if *Coprinus* is supplied with potassium ions both H 2 and H 5 can use BGP as well as they can use inorganic phosphate.

Table 9. *Growth of* Coprinus *on different phosphate sources*

Phosphate source	Growth as % of growth without phosphate		
	H 2	H 5	H 2 × H 5
10^{-4} M P_i	125	120	110
10^{-4} M PP_i	145	140	140
5×10^{-4} M BGP	60	110	130
10^{-4} M phytate	100	100	100

PP_i = inorganic pyrophosphate.

Table 10. *Effect of potassium ions on the growth of H 2 and H 5 on P_i and BGP*

Phosphate source	Growth as % growth without phosphate or potassium			
	H 2		H 5	
	−K	+K	−K	+K
None	100	100	100	100
0·02 M BGP	50	250	140	180
0·014 M P_i (minimal)	N.D.	300	N.D.	200

−K = without potassium; +K = sodium/potassium ratio = 3·0; N.D. = not determined

There is a stimulation of the growth of H 2 on BGP in the presence of fluoride (Table 8). This suggests that the inhibition by BGP may be connected with the activity of the acid phosphatase. However, as noted in the introduction, phosphatases in *E. coli*, *B. subtilis* and *N. crassa* are found outside the cell membrane. We have found that the acid phosphatase in *Coprinus* can be detected without cell breakage; Matile (1969) has located acid phosphatase in lysozyme like bodies in *Coprinus*, suggesting that it is located outside the cell. If this is so, the inhibition of growth by BGP may be connected with the absorption by the cell of the products of hydrolysis by the acid phosphatase. Goodman & Rothstein (1957) showed that the uptake of phosphate by *S. cerevisiae* is stimulated by potassium ions.

(vi) *Selection of mutants altered in their ability to synthesize alkaline phosphatase*

Suspensions of oidia from H 2 and H 5 were irradiated with ultraviolet light and plated at suitable levels on minimal and phosphate-free media. The oidia had a viability before irradiation of $1{\cdot}5–0{\cdot}5 \times 10^{-1}$ and after irradiation of $1{\cdot}0–0{\cdot}6 \times 10^{-2}$. After 3 days incubation colonies were scored for alkaline phosphatase production. Viability was scored directly from the minimal plates.

Two types of mutant were selected: (*a*) from both media; colonies producing more alkaline phosphatase than wild-type ('regulator' (*reg*) mutants, AR series); (*b*) from phosphate-free medium; colonies with reduced or no alkaline phosphatase activity (phosphataseless (*pho*) mutants, AP series). Mutants were isolated to minimal medium and tested. Those which persisted were purified by re-isolation from a single oidial colony. The results of the selection are shown in Table 11.

Table 11. *Selection of mutants with altered alkaline phosphatase production*

	Total mutants isolated		Frequency of mutations	
Strain	*reg*	*pho*	*reg*	*pho*
H 2	22	15	$5{\cdot}6 \times 10^{-3}$	$8{\cdot}9 \times 10^{-3}$
H 5	11	3	$3{\cdot}0 \times 10^{-2}$	$9{\cdot}3 \times 10^{-3}$

* Frequency $= \dfrac{\text{no. of mutants isolated}}{\text{total viable oidia plated}}$.

Table 12. *Complementation groups of* reg *and* pho *mutants*

			No. of mutants		
Series	Group	Dominance	From H 2	From H 5	Example used
AR	*reg*-1	Recessive	3	4	AR 45
	reg-2*	Semi-dominant	2	2	AR 72
	reg-3	Dominant	1	0	AR 202
	reg-4*	Semi-dominant	11	0	AR 162
	reg-5	Recessive	3	5	AR 132
AP	*pho*-1†	Recessive	4	2	AP 43
	pho-2	Recessive	4	1	AP 54
	pho-3*	Recessive	3	0	AP 23

* These groups may not be homogenous.

† One case of interallelic complementation was found in this group.

(vii) *Complementation of* reg *and* pho *mutants*

In order to carry out mapping experiments mutants were divided into complementation groups. To test for dominance, each mutant was mated to its compatible wild-type strain and the dikaryons tested on phosphate-free medium (for *pho* mutants) and on $2{\cdot}5 \times 10^{-4}$ M phosphate, which is just above repression threshold

for the two wild-type monokaryons (for *reg* mutants). Complementation tests were carried out on the same media using dikaryons between all compatible pairs of mutants within the AP and AR series. The two sets of mutants were divided into groups by their dominance and complementation patterns. The groups are set out in Table 12. The dominance of mutations in certain of the *reg* groups may give an indication of the product of those genes. This is the subject of a further investigation.

(viii) *Mapping the* reg *loci*

So far, seven linkage groups have been identified in *Coprinus lagopus* (Moore, 1967). A selected mutant from each *reg* group (see Table 12) were tested for linkage with all the centromere markers available. The phenotype of all the *reg* mutants used was apparent on phosphate-free medium; all basidiospores were tested on this medium, supplemented where appropriate.

Table 13. *Linkage data for* reg-*3 and* reg-*4 loci*

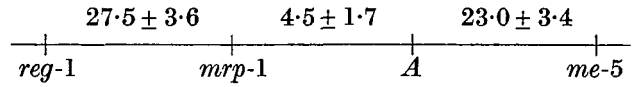
Interval	Total progeny	Recombinants	Recombination (%)	S.E.
ad-3–*ac*-2	443*	129	29·0	2·2
ad-3–*reg*-3	152	37	24·3	3·5
ad-3–*reg*-4	291	66	22·6	2·5
ad-2–*reg*-3	152	56	37·0	6·2
ac-2–*reg*-4	291	92	31·5	2·7

* This result is calculated from the data of both crosses.

(*a*) *The* reg-*1 locus*

The *reg*-1 strain used, AR 45, carried both regulator and morphological mutations. A cross with H 2 indicated that the two mutations were linked, and linked to the *A* mating-type locus. A cross was made between a *reg*-1 strain and a strain carrying *me*-5, a marker on the *A* chromosome.

The results of the cross indicate the order:



Moore (1967) puts the centromere of this linkage group 14 units from *A* distal to *me*-5. The *reg*-1 locus is therefore on the centromere side of *A*.

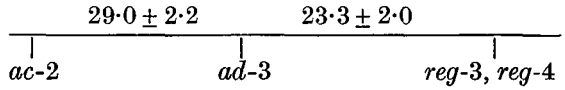
(*b*) *The* reg-*2 locus*

The *reg*-2 strain used, AR 72, was also a double mutant. The morphological mutation, *mrp*-2, was not linked to the *reg*-2 locus. The *mrp*-2 mutation conferred low viability on the strains which carried it; all strains with this marker had died within 12 months of their isolation. Recombinants from the cross AR 72 × H 5 carrying the *reg*-2 marker were used in further experiments.

The cross AR 72 × H 5 indicated that the *reg*-2 locus was independent of the *A* and *B* mating-type loci. Crosses with marker strains (see Table 1) showed no linkage of *reg*-2 to *ad*-3 (group G), *me*-1 (group III), *nic*-4 (group IV), *paba*-2 (group VI) or *ad*-6 (group V).

(c) *The* reg-*3 and* reg-*4 loci*

AR 202 (*reg*-3) and AR 162 (*reg*-4) were crossed to the marker strains shown in Table 1. Both loci showed weak linkage to the *ad*-3 locus on group G. Recombinants carrying each *reg* locus with *ad*-3 were crossed with strain CC 201 which carries the *ac*-2* mutation, which maps 29 units from *ad*-3 (L. A. Casselton, personal communication). The results, shown in Table 13, indicate the order *ac*-2–*ad*-3–*reg* 3 and *reg*-4. The data show that the *reg* loci are very closely linked, if not at the same site; therefore the map distances shown below were calculated as if only one locus were involved.



A cross set up between a *reg*-4 and a *reg*-3 strain failed to fruit. Further experiments are being carried out on this system.

A cross between a *reg*-5 and a *reg*-2 strain showed that the two loci are 5 units apart. As the *reg*-2 locus shows no linkage to the markers available, no further attempt was made to map the *reg*-5 locus.

(ix) *Mapping of the* pho *loci*

Mutants lacking alkaline phosphatase could be of two types: negative (super-repressed) control mutants, which should map close to the *reg* loci: and mutations in a structural gene for alkaline phosphatase. In bacterial systems structural genes often map near a control gene (e.g. Torriani & Rothman, 1961) but in fungal systems this is not generally so (e.g. Dorn, 1965).

Examples of the three groups of *pho* mutants were tested for linkage with markers to which the *reg*-1, *reg*-3 and *reg*-4 loci show linkage. One mutant from each class was crossed with strain C 692; progeny were tested for linkage of *pho* markers to *A* and *ad*-3. The *pho*-1 and *pho*-3 loci show no linkage to the *ad*-3 and *A* loci and are therefore not linked closely to any of the three mapped *reg* loci. However, the *pho*-2 locus shows weak linkage to the *A* locus ($40 \pm 4{\cdot}4$ map units). As the *reg*-1 locus is $32 \pm 3{\cdot}8$ units from *A* the *pho*-2 locus was mapped with respect to the centromere of group 1. The cross AP 54 × MAE 161 (A_6B_5 *paba*-1) confirmed the linkage of *pho*-2 to *A*, but *paba*-1 is too close to *A* to give the gene order. A cross was made between APR 1 (A_3B_1 *pho*-2) and CC 1 (A_6B_6 *ac*-1). The *ac*-1 locus maps 15 units from *A* on the centromere side (L. A. Casselton, personal communication). The results of the cross indicate that the *pho*-2 locus is independent of the *ac*-2 locus, and therefore of the *reg*-1 locus.

There is therefore no evidence so far of linkage of *pho* and *reg* loci.

(x) *Mapping of the sensitivity shown by H 2 to BGP*

As shown in Table 8, the growth of H 2 is inhibited by the addition of BGP to the medium. This is a recessive character, as shown by the normal growth of H 2 × H 5 on BGP.

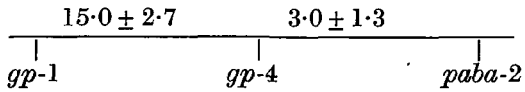
* Acetate (*ac*) mutants cannot use acetate as a sole carbon source.

https://doi.org/10.1017/S0016672300012556 Published online by Cambridge University Press

A cross between H 2 and H 5 indicated that the inhibition segregated as a single allele difference unlinked to *A* or *B*. The sensitive allele was designated *gp*^{s}-1, the resistant one *gp*^{r}-1. During attempts to map the *reg*-3 locus, strain AR 202 (derived from H 2) was crossed to strain ARR 33 which carries a similar mutation, *gp*^{s}-4, distinguishable from *gp*^{s}-1 by greater inhibition and by complementation in dikaryons.

The results of the cross were scored for auxotrophic, regulator and BGP phenotype. It was not possible to distinguish between *gp*^{s}-4 *gp*^{s}-1 and *gp*^{s}-4 spores; their number was estimated from the total showing the very sensitive phenotype and the reciprocal recombinant class. Double mutants were identified within these classes by complementation tests.

The results give the order



Paba-2 is 9·5 units from the centromere of group VI (Moore, 1967).

5. DISCUSSION

(i) *The role of the acid and alakaline phosphatases in the cell*

On the basis of the results described above, and by analogy with other fungal systems, possible functions for the acid and alkaline phosphatases, and a mechanism for repression of the latter, will be suggested here.

The experiments described in sections (iv) and (v) above indicate that organic phosphates in the medium are substrates for the constitutive acid phosphatase. Unless supplies of phosphate (direct from the medium or via the acid phosphatase) are restricted, the alkaline phosphatase is repressed in horizontal mycelium. However, the enzyme can be detected in aerial mycelium and sporophores on high phosphate media.

Dorn & Rivera (1966) suggested that the repressible phosphatases in *Aspergillus* are involved in the recycling of phosphate within the mycelium under starvation conditions. Kritskii & Kulaev (1965) have proposed that phosphate is transported within the mycelium of *Agaricus bisporous* as a specific phosphate ester. In particular, both fruiting and spore production in *Agaricus* are accompanied by the rapid accumulation of phosphate in sporophore tissue (Kulaev, Kritskill & Belozerskii, 1960; Kritskii & Kulaev, 1965).

In *Coprinus*, sporulation and fruiting are specific functions of the aerial mycelium. We suggest that the alkaline phosphatase is involved in the recycling of phosphate under starvation conditions, and in the transport of phosphate within the mycelium, perhaps by hydrolysing a transport ester in aerial mycelium and sporophores. In fungi, aerial mycelium is dependent on the horizontal mycelium for nutrients, causing a gradient between the two parts of the colony. Since fungi esterify phosphate as they take it up (e.g. Kulaev & Okarov, 1967; Borst-Pauwels, 1962; Borst-Pauwels, Loef & Havinga, 1962) it is likely that the co-repressor of alakaline phosphatase in *Coprinus* is also an ester which is synthesized only when the phosphate level in the cell is above a certain value. If this value is above that normal in aerial

mycelium, alkaline phosphatase should be de-repressed there. Our results confirm this. Differences in enzyme composition in different parts of a mycelium have also been noted by Gooday (1968) in *Mucor haemalis* and Marzluf (1970) in *Neurospora crassa*.

(ii) *The genetics of alkaline phosphatase synthesis*

In *Coprinus* alkaline phosphatase synthesis is under the control of 5 'regulator' and at least 3 'structural' loci. In this respect it is broadly similar to the *Aspergillus* system, where there are 3 and 7 loci respectively. The fungal systems appear to differ fundamentally from the two bacterial systems analysed (see Introduction), each of which have one structural gene and two regulator genes, one of which is closely linked to the structural gene. Dorn (1965) suggested that the *Aspergillus* alkaline phosphatase is a heteropolymer; however, there is little evidence for this. There is some evidence that the *Coprinus* enzyme has subunits, as one case of interallelic complementation was observed (Table 12): but this does not explain the presence of three structural loci.

Semidominant constitutive mutants have been reported by Cove (1969) for nitrate reductase, and by Hynes & Pateman (1970) for acetamidase, both in *Aspergillus nidulans*. The exact properties of the dominant 'regulator' mutations in the *Coprinus* alkaline phosphatase system and the relationship between the 'regulator' and structural loci are currently being investigated.

One of us (J.N.) wishes to thank the Science Research Council for a postgraduate Studentship.

REFERENCES

Anagnostopoulos, C. (1960). Alkaline phosphatase formation in *Bacillus subtilis*. *Federation Proceedings* **19**, 48.

Borst-Pauwels, G. W. F. H. (1962). The uptake of radioactive phosphate by yeast. I. The uptake of inorganic phosphate by yeast as compared to that by higher plants. *Biochimica et biophysica acta* **65**, 403.

Borst-Pauwels, G. W. F. H., Loef, W. H. & Havinga, E. (1962). The uptake of radioactive phosphate by yeast. II. The primary phosphorylation products. *Biochimica et biophysica acta* **65**, 407.

Casselton, L. A. (1965). The production and behaviour of diploids of *Coprinus lagopus*. *Genetical Research* **6**, 190.

Casselton, P. J., Fawole, M. O. & Casselton, L. A. (1969). Isocitrate lyase in *Coprinus lagopus* (*sensu* Buller). *Canadian Journal of Biochemistry* **15**, 637.

Cove, D. J. (1969). Evidence for a near-limiting intracellular concentration of a regulator substance. *Nature* **224**, 272.

Davis, F. W. J. & Lees, H. (1969). Alkaline phosphatases of *Neurospora crassa*: I. *Canadian Journal of Biochemistry* **15**, 455.

Delsal, J. L. & Manhuri, H. (1958). Etude des dosages colorimétriques du phosphore. *Bulletin de la Société de chimie biologique* **40**, 1623.

Dorn, G. (1965). Genetic analysis of the phosphatases in *Aspergillus nidulans*. *Genetical Research* **6**, 13.

Dorn, G. & Rivera, W. (1966). Kinetics of fungal growth and phosphatase production in *Aspergillus nidulans*. *Journal of Bacteriology* **92**, 1618.

Garen, A. & Echols, H. (1962*a*). Genetic control of the induction of alkaline phosphatase. synthesis in *Escherichia coli*. *Proceedings of the National Academy of Sciences of the U.S.A.* **48**, 1398.

Garen, A. & Echols, H. (1962*b*). Properties of two regulator genes for alkaline phosphatase. *Journal of Bacteriology* **83**, 297.

GIRI, K. V., PRASAD, A. L. N., GOWRI DEVI, S. & SRI RAM, J. (1952). A technique for the identification and separation of enzymes by paper chromatography. *Biochemical Journal* **51**, 123.

GOODAY, G. W. (1968). The localisation of some enzymes in the mycelium of *Mucor haemalis*. *Archiv für Microbiologie* **63**, 11.

GOODMAN, J. & ROTHSTEIN, A. (1957). The active transport of phosphate into the yeast cell. *Journal of General Physiology* **40**, 915.

HEREDIA, C. F., YEN, F. & SOLS, A. (1963). The role and formation of the acid phosphatase in yeast. *Biochemical and Biophysical Research Communications* **10**, 14.

HYNES, M. J. & PATEMAN, J. A. J. (1970). The genetic analysis of the regulation of amidase synthesis in *Aspergillus nidulans*. I. Mutants able to utilize acrylamide. *Molecular and General Genetics* **108**, 97.

JONES, T. C. & GALLANT, J. A. (1964). The genetic control of basal levels of alkaline phosphatase. *Genetics* **50**, 260.

KRITSKII, M. S. & KULAEV, I. S. (1965). Translocation of phosphates in the sporophores of *Agaricus bisporous*. *Biochemistry, USSR* **30**, 688.

KULAEV, I. S., KRITSKII, M. S. & BELOZERSKII, A. N. (1960). The metabolism of polyphosphates and some other phosphorus compounds in the development of fruiting-bodies of *Agaricus bisporous*. *Biochemistry, USSR* **25**, 563.

KULAEV, I. S. & OKAROV, L. A. (1967). Primary stages in the entry of inorganic phosphate from the medium into the mycelium of *Penicillium chrysogenum*. *Biochemistry, USSR* **32**, 571.

KUO, M. H. & BLUMENTAL, H. J. (1961). Purification and properties of an acid phosphomonoesterase from *Neurospora crassa*. *Biochimica et Biophysica Acta* **52**, 13.

KUSNAREV, V. M. & SMIRNOVA, T. A. (1966). Electron microscopy of alkalaine phosphatase in *Escherichia coli*. *Canadian Journal of Microbiology* **12**, 605.

LE HEGARAT, J.-C. & ANAGNOSTOPOULOS, C. (1969). Localisation chromosomique d'un gene gouvernant la synthèse d'une phosphatase alcaline chez *Bacillus subtilis*. *Comptes rendus des séances de l'Acadamie des Sciences* (série D) **269**, 2048.

LEVINTHAL, C. F. (1959). Genetic and chemical studies with alkaline phosphatase of *Escherichia coli*. *Brookhaven Symposia* **12**, 76.

LEWIS, D. (1961). Genetical analysis of methionine supressors in *Coprinus*. *Genetical Research* **2**, 141.

LEWIS, D. (1963). Structural gene for the methionine activating enzyme and its mutation as a cause of resistance to ethionine. *Nature* **200**, 151.

LOWRY, O. H., ROSEBROUGH, N., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* **193**, 265.

MARZLUF, G. A. (1970). Genetic and biochemical studies of distinct sulfate permease species in different development stages of *Neurospora crassa*. *Archives of Biochemistry and Biophysics* **138**, 254.

MATILE, P. (1969). Vacuoles as lysozomes of plant cells. *Biochemical Journal* **111**, 26P.

MIKI, T., MINAMI, Z. & IKEDA, Y. (1965). The genetics of alkaline phosphatase production in *Bacillus subtilis*. *Genetics* **52**, 1093.

MOORE, D. (1967). Four new linkage groups in *Coprinus lagopus*. *Genetical Research* **9**, 351.

NEUMANN, H. & VAN VREDENDAAL, M. (1967). An improved alkaline phosphatase determination with *p*-nitrophenyl phosphate. *Clinica chimica acta* **17**, 183.

NYC, J. F. (1967). A repressible acid phosphate in *Neurospora crassa*. *Biochemical and Biophysical Research Communications* **27**, 183.

NYC, J. F., KADNER, R. J. & CROCKEN, B. J. (1966). A repressible alkaline phosphatase in *Neurospora crassa*. *Journal of Biological Chemistry* **241**, 1468.

TAKEDA, K. & TSUGITA, A. (1967). Phosphoesterases of *Bacillus subtilis*. II. Crystallisation and properties of the alkaline phosphatase. *Journal of Biochemistry Tokyo* **61**, 231.

TORRIANI, A. (1960). Influence of inorganic phosphate on the formation of phosphatases by *Escherichia coli*. *Biochimica et biophysica acta* **38**, 460.

TORRIANI, A. & ROTHMAN, F. (1961). Mutants of *Escherichia coli* constitutive for alkaline phosphatase. *Journal of Bacteriology* **81**, 835.

WILEY, W. R. (1970). Tryptophan transport in *Neurospora crassa*: a tryptophan binding protein released by cold osmotic shock. *Journal of Bacteriology* **103**, 656.