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The Influence of certain Micro-organisms on the Formation of Perithecia by *Chaetomium globosum*

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SUMMARY: The localized formation of perithecia in colonies of *Chaetomium globosum* growing in association with certain other micro-organisms (e.g. *Aspergillus fumigatus*) is associated with the presence of organic phosphates, especially phosphoglyceric acid, in the medium. These phosphates are normal metabolic products of the associated micro-organism, and only when they diffuse into the medium and locally reach a critical concentration does the fruiting response follow.

The stimulatory effect of one fungus on the sporulation of another was reported by Heald & Pool (1908), and has since been noted frequently. The subject was reviewed by Asthana & Hawker (1936) who carried out one of the most detailed studies of the subject. Working mainly with *Melanospora destruens* Shear they found that the intensity of sporulation of this fungus in mixed cultures was governed by a number of interdependent factors, one of which was the rate of production of a chemical stimulant of sporulation by the associated organism. Buston & King (1951), who had observed stimulation of production of perithecia of *Chaetomium globosum* Kunze following contamination by a strain of *Aspergillus fumigatus* Fresen, obtained active extracts from the mycelium of the latter organism and from the staled medium, but were unable to isolate or characterize the substance responsible. Hawker (1948) demonstrated the stimulant effect of glucose-1-phosphate and of fructose-1, 6-diphosphate on the fruiting of *Melanospora destruens*, but Buston & King (1951) showed that these compounds, although possibly present, did not entirely account for the activity of the *Aspergillus* extract. Buston, Jabbar & Etheridge (1953) applied a chromatographic method to the investigation of a 'fruiting factor' present in an aqueous extract of jute (Buston & Basu, 1948), and concluded that its activity could be accounted for by the presence of glucose-6-phosphate, fructose-1, 6-diphosphate and calcium ions in a particularly favourable balance of concentrations. The same chromatographic technique has now been applied to extracts of *A. fumigatus* and other micro-organisms which stimulate fruiting of *Chaetomium globosum* in mixed culture.

RESULTS

The strain of *Chaetomium globosum* was that previously used in this laboratory (Basu no. 79); the strain of *Aspergillus fumigatus* was isolated by Buston & King from a chance infection. The cultural methods used were those previously described (Buston & King, 1951). Extracts of the mycelium of *A. fumigatus*, grown in liquid media, were made with cold 0.1 N-NaOH in the presence of

a little ether, and after neutralization with HCl were evaporated almost to dryness *in vacuo* at as low a temperature as possible. The residue was taken up in 95 % (w/w) ethanol in water, undissolved NaCl removed, and the solution again evaporated *in vacuo*, after which the residue was dissolved in a little water. From the staled liquid media, material was obtained in a similar manner, after removal of the greater part of the inorganic salts by precipitation with ethanol. The final products still contained small amounts of inorganic salts.

Preliminary tests having shown that these extracts accelerated the formation of perithecia of *Chaetomium globosum*, suitable portions were examined for the presence of organic phosphates by paper chromatography as described by Buston *et al.* (1953). The mycelial extract was found to contain glucose-1-phosphate, fructose-1, 6-diphosphate and larger amounts of phosphoglyceric acid; the staled medium contained phosphoglyceric acid and a trace of fructose-1, 6-diphosphate. Phosphoglyceric acid has not been studied hitherto in connexion with perithecial formation, and experiments showed that like the hexose phosphates it has a stimulant effect within certain limits of concentration (Table 1). It seemed reasonable, therefore, to conclude that the observed

Table 1. *Effect of phosphoglyceric acid (Ca salt) on sporulation of Chaetomium globosum*

Phosphoglyceric acid (Ca salt) added (mg./100 ml. medium)	Perithecial frequency* (5th day)
20	0.6
30	1.2
40	2.2
50	2.8
60	1.4

* Asthana & Hawker (1936). Average number of perithecia/field; counts made on three fields in each of ten sectors.

stimulation of fruiting was due to the presence of suitable amounts of these organic phosphates, and especially of phosphoglyceric acid, in the medium. However, it must be borne in mind that in previous experiments, although known amounts of the various organic phosphates were added to the original medium, nothing is known of any change in concentration that may occur subsequently. Thus neither the *effective* concentration at the moment when fruiting is initiated, nor the exact stage of development of *C. globosum* at which the chemical stimulus can evoke the fruiting response, is known. For this reason estimations of the amount of organic phosphate in the medium at the moment when perithecia are first visible may be of little significance; nevertheless, it seemed of interest to attempt such estimations, and for this purpose portions of the agar medium were removed from positions adjacent to the fruiting zones as soon as perithecia were seen, and the phosphates were separated on a chromatogram. Total organic phosphate was estimated in

eluates from the chromatogram according to the method of Berenblum & Chain (1938). In two such experiments the local concentration of organic phosphate was estimated as equivalent to *c.* 30 mg. phosphoglyceric acid/100 ml. of medium.

The stimulation of fruiting of *Chaetomium globosum* in the presence of other micro-organisms was studied by Basu (1947), who tested the influence of fifteen different species grown side by side with *C. globosum* on agar plates. Under the conditions which he used, Basu found no effect on the sporulation of *C. globosum*; perithecia appeared at the same time as in the controls, and developed from the centre of the colony rather than at the edges nearest the second organism. Among the species tested by Basu was a strain of *Aspergillus fumigatus*, and in view of the results now obtained with this organism some further tests were made both with our strain and with that used by Basu. It was found that the discrepancies between our results and those of Basu were due in part to the different distances between the colonies of the two organisms. Maximum fruiting resulted when the centres of the colonies were about 20 mm. apart; at 50 mm. the effect was barely perceptible (Table 2). Dr Basu has kindly repeated his experiments with *A. fumigatus*, and he now reports that 'the response was more marked at 20 mm. than at 40 mm'.

Table 2. *The effect of distance between colonies of Chaetomium globosum and Aspergillus fumigatus on the formation of perithecia by C. globosum*

C. globosum was inoculated 1 day before *A. fumigatus*

Distance between centres of colonies (mm.)	Perithecial frequency (8th day)	
	In 90° sector nearest colony of <i>A. fumigatus</i>	Average over whole colony
20	12	3.9
30	10	2.9
40	9 (small)	2.3
50	3 (small)	2.3

The fruiting of *Chaetomium globosum* is also affected by the relative age of the two colonies, and the greatest response is found when this organism is inoculated one day earlier than the *Aspergillus fumigatus*, at a distance of 20 mm. When the *Chaetomium globosum* is inoculated 4 days earlier, growth of *Aspergillus fumigatus* is completely inhibited.

The influence of some of the other organisms tested by Basu was also re-examined; in two instances a definite positive response was obtained when the distance between the colonies was suitable. In one other case a feeble response was observed, but the rest of the organisms gave negative results at all the distances tested. The distribution of organic phosphates in the mycelia of these organisms, and in their staled media, was also examined, and the results are summarized in Table 3. In all instances mycelial extracts contained organic phosphates, phosphoglyceric acid predominating, but organic phosphate was detected in the media only in those instances where a positive fruiting response had been observed.

Table 3. *Phosphorylated compounds in mycelia of certain micro-organisms, and in staled media*

Organism	Strain	Phosphates in		Relative degree of fruiting response by <i>C. globosum</i>
		Mycelial extract	Staled medium	
<i>Aspergillus fumigatus</i>	B. & K.	FP PGA G-1-P	FP (f.) PGA	+++
<i>A. fumigatus</i>	Basu	n.i.	PGA	++
<i>A. flavus</i>	Kew	FP PGA XP	FP PGA XP	++
<i>A. terreus</i>	Kew	FP PGA	FP PGA	++
<i>A. ustus</i>	I.C.	PGA (f.) XP	n.d.	—
<i>Penicillium citrinum</i>	Kew	PGA (f.) XP	n.d.	—
<i>P. islandicum</i>	I.C.	FP PGA (tr.)	n.d.	—
<i>P. notatum</i>	Kew	FP PGA G-6-P	FP PGA	+
<i>Trichoderma viride</i>	I.C.	PGA (tr.)	n.d.	—

Sources of strains: B. & K. = Buston & King, 1951; Basu = Basu, 1947; Kew = Commonwealth Mycological Institute; I.C. = Department of Mycology, Imperial College.

Phosphates: FP = fructose-1, 6-diphosphate; PGA = phosphoglyceric acid; G-1-P = glucose-1-phosphate; G-6-P = glucose-6-phosphate; XP = unidentified organic phosphate; (f.) = faint; (tr.) = trace. n.d. = no phosphate detected; n.i. = not investigated.

Relative degree of fruiting response is scored + + +, + +, + in decreasing order of magnitude; — = no response.

DISCUSSION

The effect of phosphoglyceric acid in stimulating production of perithecia has not been recorded hitherto, but in view of the close relationship between this substance and the hexose phosphates it may be presumed that the mechanism of its action is the same. The occurrence of glucose-1-phosphate in the mycelium of *Aspergillus fumigatus* was somewhat unexpected. Buston & King (1951) noted the relative resistance to mild acid hydrolysis of extracts of *A. fumigatus* as compared with active jute extracts; this is in agreement with the comparative stability of phosphoglyceric acid, which does not occur in jute extract.

Although all the mycelia examined were found to contain organic phosphates, there were considerable differences in the amounts present; this probably merely reflects a difference in the rate and not the pathway of metabolism. What is more significant in regard to the present problem is the variation in the amount of phosphate which passes into the surrounding medium. This might be due to differences in the permeability of the mycelial membrane as well as to differences in the concentration of phosphate within the tissues, and

since production of perithecia seems to require a rather narrow range of concentrations of the various organic phosphates (alone or in combination) it is reasonable to conclude that the rate of diffusion of these substances from the mycelium and through the medium will play a major part in deciding whether any stimulation of fruiting shall occur. The observed differences in response with different inter-colony distances can also be explained as a diffusion-concentration phenomenon. In all cases studied in which the presence of a second organism caused accelerated or increased production of perithecia by *Chaetomium globosum*, the effect was associated with the presence in the medium of phosphoglyceric acid and other organic phosphates.

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ADDENDUM

Our colleague Mr M. W. McDonough has shown that *Aspergillus fumigatus* excretes organic phosphates into the medium in the earliest stages of its growth, and the fruiting response of a neighbouring colony of *Chaetomium globosum* may follow later even if the colony of *Aspergillus fumigatus* has been removed in the meantime. In his experiments that portion of the agar containing the latter colony was cut out on the second or third day after inoculation, and the gap in the medium immediately refilled with fresh agar (in order to avoid stimulation of fruiting of *Chaetomium globosum* being caused by