Studies on the Growth and Sporulation of Some Species of *Penicillium*

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SUMMARY

The effect of certain carbohydrates, nitrogen compounds and accessory growth factors on the growth and sporulation of several strains of *Penicillium vermiculatum* and *P. wortmanni*, as well as of many commonly occurring non-perithecial species, was studied. An initial low concentration of soluble sugar was conducive to perithecial formation in the two sexual species, and perithecial growth was best with polysaccharides as carbon source. Peptone was the most favourable nitrogen source; asparagine exerted an inhibitory effect in many cases. Of six B-group vitamins tested only thiamine had any marked effect. Similar effects of the nutritional factors were seen with the non-perithecial species generally.

INTRODUCTION

In 1948-49 seven isolations of *Penicillium vermiculatum* and six of *P. wortmanni* were made in this laboratory from rotting jute materials, and were found to display considerable variability in sporulation and other characteristics (Basu, 1951). By 1953, three strains of the first-named were no longer producing perithecia. Physiological studies relating to perithecial formation in *Penicillium* are rare; the present work has been concerned with the effect of nutritional and other factors on the growth and fruiting of *P. vermiculatum* and *P. wortmanni*. A similar but less detailed study has been made also of growth and conidial formation in a number of non-perithecial *Penicillium* species. In the identification of species the classification of Raper & Thom (1949) was followed. Strains of *P. vermiculatum* were given the integral code number 77; those numbered 77.29, 77.62 and 77.81 regularly produced perithecia, and 77.80B did so on malt agar, but only occasionally on potato glucose agar. The six strains of *P. wortmanni* fell into two rather distinct morphological groups (numbered 126 and 130; Basu, 1951); all fruited normally. Of the non-perithecial species, twenty-nine isolates comprising twenty-two species belonging to the main divisions of the genus were studied; they are enumerated in Table 2. Tests for heterothallism, which was stated by Derx (1926) to be responsible for certain features of fruiting in some strains of *P. luteum*, showed that the species used in this investigation were truly homothallic.

METHODS

All the fungi used here have been subcultured since isolation alternately on malt agar and potato glucose agar at intervals of 6 months. The basal medium used contained: NaNO_3 , 2 g.; K_2HPO_4 , 0.75 g.; KH_2PO_4 , 0.25 g.; KCl , 0.5 g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g.; water to 1 l.; adjusted to pH 7.0. To the medium was added glucose (unless another carbon source was being investigated) usually to 3% (w/v). Cultures were ordinarily made in liquid media, of which 10 ml. portions were taken in 50 ml. Pyrex conical flasks and closed with aluminium caps. For solid media (agar 15 g./l.) 20 ml. quantities were taken in 10 cm. Petri dishes. Sterilization was at 115° for 15 min., and incubation at 27° . As a measure of growth mycelial dry weight was estimated; for this purpose the harvested mycelia, after washing with distilled water, and being freed of surplus water with blotting paper, were dried at 105° for 4 hr. and weighed. When cellulose (Whatman filter paper no. 80) was used as carbon source, growth was estimated from the loss in weight of the paper.

RESULTS

Carbon sources

Eight different carbohydrates were tested as carbon source, at 3% (w/v) in the liquid medium, excepting cellulose which was about 1% (w/v). The figures in Table 1 show that these carbohydrates differed widely in their capacity to promote growth and to stimulate fruiting of various strains of *Penicillium vermiculatum* and *P. wortmanni*. Maltose was generally the most effective and xylose the least for vegetative growth; but mycelial weight after an arbitrary time does not necessarily give a true measure of the maximum growth attainable. Some strains of *P. vermiculatum* and *P. wortmanni* only attacked cellulose feebly and did not produce perithecia; but two other strains of *P. vermiculatum* which attacked cellulose vigorously also fruited well thereon. Starch and dextrin allowed good vegetative growth of all strains, but encouraged fruiting of *P. wortmanni* only. The contrast between cellulose and starch as substrates for production of strong perithecial growth in these two species is noteworthy.

In similar tests with the non-perithecial species there was a considerable variation in the capacity of the carbohydrates to stimulate sporulation (Table 2). When the medium contained 8% (w/v) carbohydrate sporulation was often greatest on the polysaccharides and least on glucose; maltose was often superior to other simple sugars. Eight species did not sporulate on cellulose media; all of these were very weakly cellulolytic. Eight strongly cellulolytic species all sporulated very vigorously; but instances were noted in which quite vigorous conidial formation was associated with rather weak cellulolytic capacity, e.g. *Penicillium roseo-purpureum* 148 and *P. notatum* 138. There is a fair degree of agreement between the present observations and those of Marsh, Bollenbacher, Butler & Raper (1949) who made a survey of cellulolysis by *Penicillium* species under quite different experimental conditions.

Experiments in which glucose only was supplied in a wide range of concentrations confirmed that for *Penicillium vermiculatum* and *P. wortmanni*, as for many other fungi, fruiting was favoured by a low concentration of soluble carbohydrate in the medium (Table 3). Of the four strains of *P. vermiculatum* tested none formed

Table 1. *Effect of various carbohydrates on growth and fruiting of Penicillium vermiculatum and P. wortmanni*

Strain no.	Day of appearance of perithecia								Intensity of fruiting at 14 days							Mycelial dry weight (mg.) at 14 days							Loss of wt (%)			
	Day of appearance of perithecia								Intensity of fruiting at 14 days							Mycelial dry weight (mg.) at 14 days										
	Xylose	Glucose	Fructose	Sucrose	Maltose	Dextrin	Starch	Cellulose	Xylose	Glucose	Fructose	Sucrose	Maltose	Dextrin	Starch	Cellulose	Xylose	Glucose	Fructose	Sucrose	Maltose	Dextrin		Starch	Cellulose	
77.29	> 14	> 14	> 14	> 14	7	> 14	> 14	7	<i>P. vermiculatum</i>	7	0	0	0	0	0	3	31	36	53	38	85	83	79	14.2		
77.62	> 14	> 14	> 14	> 14	7	> 14	> 14	7		7	0	0	0	0	0	4	36	37	54	46	87	83	73	10.0		
77.80B	> 14	> 14	> 14	> 14	> 14	> 14	> 14	9		0	0	0	0	0	0	2	35	36	67	41	84	87	80	8.1		
77.81	> 14	> 14	> 14	> 14	7	12	6	14		0	0	0	0	2*	1*	1*	0	27	14	10	24	54	50	47	0.6	
126.24	12	12	8	10	8	10	10	> 14	<i>P. wortmanni</i>	> 14	3	2	2	2	3	0	35	46	45	56	94	65	44	2.3		
126.31	12	11	7	6	8	6	9	> 14		> 14	2	2	2	2	3	4	0	47	51	51	60	99	75	58	2.1	
126.78	8	8	7	7	8	11	7	> 14		> 14	2	2	3	3	4	4	0	32	36	44	36	89	66	58	2.0	
130.30	> 14	> 14	> 14	6	5	7	5	> 14		> 14	0	0	0	2	2	3	4	0	40	39	55	32	74	63	53	0.6
130.63	> 14	> 14	> 14	6	5	7	5	> 14		> 14	0	0	0	2	2	3	4	0	37	38	52	28	79	59	53	0.8
130.64	> 14	> 14	> 14	6	5	7	5	> 14		> 14	0	0	0	2	2	3	4	0	44	37	61	29	80	75	78	0.9

Table 2. *Effect of various carbohydrates on the growth and sporulation of various non perithecial Penicillium species*

All carbohydrates 3 % concentration (except cellulose, 1 %). Increasing intensity of sporulation denoted by numbers 0 to 4.

Fungus and ref. no.	Intensity of sporulation at 14 days							Mycelial weight at 14 days (mg.)							Loss (%)
	Xylose	Glucose	Fructose	Sucrose	Maltose	Dextrin	Cellulose	Xylose	Glucose	Fructose	Sucrose	Maltose	Dextrin	Starch	
<i>Penicillium adametzi</i> 128	1	1	1	1	1	1	0	44	27	36	21	72	71	73	0.8
<i>P. canescens</i> series 136	2	1	2	2	3	4	3	41	37	43	45	42	50	43	20.5
<i>P. citrinum</i> 37	2	2	2	2	2	3	1	42	64	48	60	53	53	66	5.8
<i>P. citrinum</i> 37-1	2	1	2	1	3	4	2	72	82	74	64	89	72	71	4.5
<i>P. citrinum</i> 37-2	2	2	3	2	3	4	3	56	71	62	50	84	81	74	4.9
<i>P. cyaneum</i> type 124	1	0	1	0	1	1	2	75	97	79	100	76	79	84	0.5
<i>P. fellutanum</i> 7	2	2	2	2	2	2	1	42	51	51	41	84	86	85	0.7
<i>P. fellutanum</i> 125	0	1	1	1	1	1	0	8	27	20	25	20	22	9	3.4
<i>P. fellutanum</i> series 135	2	2	2	3	2	4	0	8	50	24	30	55	28	94	1.1
<i>P. funiculosus</i> series 150	2	2	2	2	3	4	3	22	32	39	35	22	43	48	36.2
<i>P. implicatum</i> 53	2	2	2	2	2	3	4	47	60	66	78	58	73	88	0.6
<i>P. implicatum</i> series 101	0	0	1	0	0	1	0	70	98	84	71	110	81	60	3.8
<i>P. luteum</i> 28	0	0	0	0	1	2	1	71	75	85	85	98	101	99	0.5
<i>P. notatum</i> 138	2	1	1	1	2	3	3	74	70	71	73	79	79	74	5.7
<i>P. ochro-chloron</i> 153-1	0	0	1	1	1	3	3	103	120	101	67	106	92	100	37.9
<i>P. oxalicum</i> 96	2	2	3	2	3	3	3	66	54	74	58	73	78	105	21.6
<i>P. purpurogenum</i> 134	2	2	2	3	2	4	3	35	42	58	52	69	80	51	0
<i>P. roseo-purpureum</i> 148	1	1	1	1	2	3	3	78	53	81	50	99	72	69	6.0
<i>P. rubrum</i> 127	0	0	1	0	0	2	2	59	59	74	54	67	95	52	11.8
<i>P. simplicissimum</i> 4	0	0	0	0	2	3	4	110	120	101	102	111	97	94	0.9
<i>P. steckii</i> 137	1	1	1	1	1	2	3	63	48	81	65	88	68	85	13.3
<i>P. tardum</i> 132	1	1	2	1	1	2	1	23	52	86	50	41	56	54	0
<i>P. variable</i> 34	2	2	3	1	2	2	1	54	59	55	21	28	91	15	1.7
<i>P. variable</i> 121	2	0	2	1	2	2	3	89	61	82	49	76	96	62	32.0
<i>P. variable</i> 121-1	0	1	0	0	2	1	2	64	32	43	47	52	94	58	38.0
<i>P. variable</i> 121-2	2	0	2	1	3	2	3	73	69	86	53	71	91	68	22.4
<i>P. verruculosum</i> 158	1	1	1	1	2	1	1	26	22	28	36	44	35	52	12.9
<i>Penicillium</i> sp. 40	0	0	0	0	0	0	0	27	25	36	20	50	52	72	0
<i>Penicillium</i> sp. 98	1	0	0	0	1	2	3	53	48	58	37	52	50	68	50.0

perithecia within 14 days on the 1.5% (w/v) glucose medium. The three 126 strains of *P. wortmanni* were relatively less sensitive to the sugar concentration, but although perithecial frequency was still high at glucose concentrations > 0.5% (w/v), the perithecia themselves were still immature on the 14th day. In contrast, the three 130 strains of *P. wortmanni* were the most sensitive of all to sugar concentration. Similar experiments with maltose or sucrose gave the same type of result, although on maltose *P. wortmanni* 126 strains gave optimum fruiting at a rather higher sugar concentration, namely 2% (w/v).

Table 3. *Effect of glucose concentration on fruiting of Penicillium vermiculatum and P. wortmanni*

Intensity of fruiting judged visually and rated from 0 to 4 (maximum). * = immature perithecia only.

	Day of appearance of perithecia					Intensity of fruiting at 14 days				
	Glucose % (w/v)					Glucose % (w/v)				
	0.1	0.5	1.0	1.5	2.0	0.1	0.5	1.0	1.5	2.0
<i>P. vermiculatum</i>										
77.29	6	9	> 14	> 14	> 14	3	2*	0	0	0
77.62	7	11	11	> 14	> 14	3	2*	2*	0	0
77.80B	> 14	> 14	> 14	> 14	> 14	0	0	0	0	0
77.81	7	8	> 14	> 14	> 14	2	1	0	0	0
<i>P. wortmanni</i>										
126.24	6	7	7	7	7	2	2	2*	2*	2*
126.31	6	6	7	6	6	2	3	3*	3*	3*
126.78	7	7	7	7	7	2	3	3*	3*	3*
130.30	6	10	> 14	> 14	> 14	2	1*	0	0	0
130.63	6	10	> 14	> 14	> 14	2	1*	0	0	0
130.64	6	10	> 14	> 14	> 14	2	1*	0	0	0

In a further experiment the amount of sugar actually present in the medium at the onset of fruiting (i.e. when immature perithecia were visible) was determined, with liquid media containing four different initial glucose concentrations. Although exhaustion of sugar did not alone necessarily induce fruiting it seemed that, irrespective of the initial concentration, relatively little glucose ever remained when perithecia appeared. Particularly with the *P. wortmanni* 126 strains it was noted that with increasing initial sugar concentrations there was an increasing time-lag between the first appearance of immature perithecia and the final production of ascospores (Table 4).

On agar media, fruiting was always much more rapid and vigorous than in liquid media of the same glucose concentration. Strain 77.80B of *Penicillium vermiculatum* formed perithecia in glucose concentrations up to 0.5%; the other three strains of this species produced ascospores at all glucose concentrations tested, as did also the three 130 strains of *P. wortmanni*. The three 126 strains of *P. wortmanni*, which had given only immature perithecia in liquid media, produced ascospores.

Table 4. *The amount of glucose remaining in the medium at onset of fruiting, or after 30 days*

Strain no.	Initial concentration of glucose											
	0.1 % (10 mg./flask)			0.5 % (50 mg./flask)			1.0 % (100 mg./flask)			2.0 % (200 mg./flask)		
	Day of appearance of perithecia	Day of appearance of ascospores	Total glucose remaining (mg.)	Day of appearance of perithecia	Day of appearance of ascospores	Total glucose remaining (mg.)	Day of appearance of perithecia	Day of appearance of ascospores	Total glucose remaining (mg.)	Day of appearance of perithecia	Day of appearance of ascospores	Total glucose remaining (mg.)
<i>P. vermiculatum</i>												
77.29	7	11	0.16	10	80	0.90	> 30	> 30	0.94	> 30	> 30	2.26
77.62	7	11	0.24	11	18	0.13	22	> 30	1.06	> 30	> 30	2.00
77.80B	> 30	> 30	0.19	> 30	> 30	0.98	> 30	> 30	1.10	> 30	> 30	1.20
77.81	7	11	0.50	8	14	0.48	12	24	1.35	25	> 30	1.55
<i>P. wortmanni</i>												
126.24	7	10	0.59	7	12	1.70	7	16	2.20	7	24	1.35
126.31	7	10	0.50	7	12	0.70	7	16	0.93	7	22	6.10
126.78	7	10	0.40	7	12	0.60	7	17	0.89	7	22	3.00
130.30	6	10	0.21	10	18	0.14	> 30	> 30	0.32	> 30	> 30	1.11
130.63	6	10	0.40	10	18	0.15	> 30	> 30	0.48	> 30	> 30	0.78
130.64	6	10	0.16	> 30	> 30	0.48	> 30	> 30	0.63	> 30	> 30	1.06

Table 5. *Effect of different nitrogen sources on fruiting of Penicillium vermiculatum and P. wortmanni*

Intensity of fruiting judged visually and rated 0 to 4 (maximum). * = immature perithecia.

Strain no.	Day of appearance of perithecia						Intensity of fruiting at 14 days					
	Glucose 0.1 % (w/v)			Glucose 1.0 % (u/v)			Glucose 0.1 % (w/v)			Glucose 1.0 % (w/v)		
	Asparagine	Casein hydrolysate	Peptone	Ammonium sulphate	Sodium nitrate		Asparagine	Casein hydrolysate	Peptone	Ammonium sulphate	Sodium nitrate	Asparagine
77.29	> 14	> 14	> 14	> 14	> 14	<i>P. vermiculatum</i>	> 14	> 14	> 14	> 14	> 14	0
77.62	> 14	> 14	> 14	> 14	> 14		> 14	> 14	> 14	> 14	> 14	0
77.80B	> 14	> 14	> 14	> 14	> 14		> 14	> 14	> 14	> 14	> 14	0
77.81	> 14	> 14	> 14	> 14	> 14		> 14	> 14	> 14	> 14	> 14	0
126.24	5	5	11	5	8	<i>P. wortmanni</i>	11	0	1	2	2	2*
126.31	6	5	11	5	8		6	0	1	2	3	2
126.78	6	5	10	6	6		6	1	1	1*	3	2
130.30	6	5	8	5	6		7	0	1	0	3	1
130.63	6	4	8	4	6		7	0	1	0	3	2
130.64	6	4	8	4	6		9	0	1*	0	3	1*

Table 6. *Effect of different nitrogen sources on the growth and sporulation of various non-perithecial Penicillium species*

Fungus and ref. no.	Glucose 3% (w/v). Increasing intensity of sporulation denoted by numbers 0 to 4.					
	Intensity of sporulation at 14 days			Mycelial weight at 14 days (mg.)		
	Sodium nitrate	Ammonium sulphate	Peptone	Casein hydrolysate	Asparagine	Casein hydrolysate Asparagine
<i>Penicillium adametzi</i> 128	1	0	0	0	2	83 95
<i>P. canescens</i> series 136	1	1	1	0	1	83 22
<i>P. citrinum</i> 87	2	2	2	3	2	98 90
<i>P. citrinum</i> 87.1	2	2	3	3	2	72 91
<i>P. citrinum</i> 87.2	2	2	3	3	2	64 78
<i>P. cyaneum</i> type 124	0	0	0	0	0	117 133
<i>P. felutanum</i> 7	3	3	3	3	3	74 62
<i>P. felutanum</i> 125	1	0	1	1	1	80 31
<i>P. felutanum</i> series 135	2	0	2	1	1	78 63
<i>P. funiculosum</i> series 150	3	1	2	2	2	71 72
<i>P. implicatum</i> 53	2	2	3	2	2	59 70
<i>P. implicatum</i> series 101	0	0	0	0	0	100 167
<i>P. luteum</i> 28	0	0	2	1	1	90 73
<i>P. notatum</i> 138	2	1	3	2	2	54 82
<i>P. ochro-chloron</i> 153.1	0	0	2	0	1	119 136
<i>P. ovalicum</i> 96	2	1	3	1	2	71 51
<i>P. purpurogenum</i> 134	2	0	2	1	3	60 65
<i>P. roseo-purpureum</i> 148	1	0	3	1	2	46 57
<i>P. rubrum</i> 127	0	0	3	1	1	101 94
<i>P. simplicissimum</i> 4	0	0	2	0	0	133 122
<i>P. steckii</i> 137	1	0	0	1	1	91 75
<i>P. tardum</i> 132	2	2	2	2	2	75 51
<i>P. variable</i> 84	2	1	1	2	2	105 53
<i>P. variable</i> 121	0	0	0	0	0	71 58
<i>P. variable</i> 121.1	1	0	1	1	0	52 38
<i>P. variable</i> 121.2	0	0	0	0	0	73 66
<i>P. verruculosum</i> 158	2	1	3	3	2	56 40
<i>Penicillium</i> sp. 40	0	0	1	0	0	143 108
<i>Penicillium</i> sp. 98	0	0	4	0	4	40 14

Nitrogen sources

Five nitrogen sources (Na nitrate, ammonium sulphate, peptone, casein acid hydrolysate, asparagine), were tested, all at concentrations equivalent to 0.033 % (w/v) N, in conjunction with glucose (0.1 %, 1.0 %, w/v). At both concentrations of sugar, peptone (Oxo Ltd.) or casein acid hydrolysate ('vitamin-free', University of Bombay) gave the maximum mycelial growth, but this may have been due in part to the fact that these substances increased the amount of available carbon in the medium. It is evident from Tables 5 and 6 that as regards fruiting the picture is complicated. With 0.1 % (w/v) glucose the inorganic sources of nitrogen most often permitted perithecial formation while asparagine (Difco; twice recryst.) and the casein hydrolysate did so but rarely. With 1 % (w/v) glucose organic nitrogen compounds were usually more favourable, the best being peptone. Because these variations in fruiting response emphasized the importance of the concentrations of carbon and nitrogen sources, the effect of different glucose concentrations in a peptone medium was examined (Table 7).

Table 7. *Effect of glucose-peptone balance on fruiting of Penicillium vermiculatum and P. wortmanni*

Intensity of fruiting judged visually and rated 0 to 4 (maximum. * = immature perithecia. Peptone concentration throughout equivalent to 0.033 % N.

Strain no.	Day of appearance of perithecia					Intensity of fruiting at 14 days				
	Glucose % (w/v)					Glucose % (w/v)				
	0.1	0.3	0.5	0.7	1.0	0.1	0.3	0.5	0.7	1.0
<i>P. vermiculatum</i>										
77.29	> 14	8	7	8	10	0	2*	4	4	4*
77.62	> 14	8	6	7	10	0	2*	4	4	4*
77.80B	8	6	8	9	9	1*	3	3	3	1
77.81	7	6	7	7	7	2	3	3	3	3
<i>P. wortmanni</i>										
126.24	11	10	9	9	8	1	2	3	3	3
126.31	11	9	9	9	8	1	2	3	3	3
126.78	7	6	6	6	7	1	2	3	3	3
130.30	7	6	6	6	7	2	3	3	4	4
130.63	7	6	6	6	7	2	3	3	4	4
130.64	7	6	6	6	7	1	1	2	3	3

With 0.2 % (w/v) peptone (equiv. 0.033 % N), the optimum glucose concentration for fruiting was between 0.3 and 0.7 % (w/v) in most instances for *Penicillium vermiculatum* and *P. wortmanni*. Although a low concentration of sugar was found generally favourable for fruiting of these fungi, *P. vermiculatum* 77.29 and 77.62 did not form perithecia on 0.1 % (w/v) glucose in the presence of peptone; at the same concentration of glucose, 77.80B formed more conidia than (immature) perithecia, but with 1.0 % (w/v) glucose few conidia appeared. The three 130 strains of *P. wortmanni* also formed conidia at the lower glucose concentration. One of the strains of *P. vermiculatum* (77.10) which had become non-perithecial in subculture

produced many conidia on these highly nutritive media, with a maximum on media containing about 20 % (w/v) glucose; no perithecia appeared on media with up to 40 % (w/v) glucose.

Responses to growth factors

Thiamine, riboflavin, nicotinic acid, pyridoxine and Ca pantothenate were added to the 1.0 % (w/v) glucose (nitrate as N source) medium to 0.1 μ g. and 0.01 μ g./ml., and biotin to 0.01 μ g. and 0.001 μ g./ml. (Sterilization at 109° for 5 min. only.) In no case was vegetative growth of *Penicillium vermiculatum* or of *P. wortmanni* appreciably affected, and the only significant effect on sporulation was enhancement of perithecial growth of *P. wortmanni* 130.30 and 130.64 by thiamine, and of 130.64 by nicotinic acid. Experiments in which the concentration of thiamine ranged from 0.001 to 1.0 μ g./ml. and the initial pH was between 4.4 and 5.8 showed that the effect of thiamine was much more positive and general at about initial pH 5.4. Mycelial growth was not particularly affected, but fruiting was often accelerated and increased; the three 126 strains of *P. wortmanni* showed acceleration only (Table 8).

Table 8. *Effect of thiamine at different pH values on fruiting of Penicillium vermiculatum and P. wortmanni*

Intensity of fruiting judged visually and rated 0 to 4 (maximum). * = immature perithecia. Glucose concentration 1.0 % (w/v) and thiamine HCl 0.1 μ g./ml., throughout.

Strain no.		Day of appearance of perithecia				Intensity of fruiting at 14 days			
		pH value				pH value			
		4.4	4.9	5.4	5.8	4.4	4.9	5.4	5.8
<i>P. vermiculatum</i>									
77.29	Without thiamine	8	8	10	> 14	1*	2*	1*	0
	With thiamine	8	6	8	> 14	1*	3*	2*	0
77.62	Without thiamine	8	8	10	> 14	1*	2*	1*	0
	With thiamine	8	7	6	> 14	1*	3*	2*	0
77.81	Without thiamine	10	9	11	11	2*	2*	2*	2*
	With thiamine	8	8	7	10	2*	2	3	2*
<i>P. wortmannii</i>									
126.24	Without thiamine	4	4	6	6	3	3	3	3
	With thiamine	4	4	4	6	3	3	3	3
126.31	Without thiamine	4	4	5	6	3	3	3	3
	With thiamine	4	4	5	6	3	3	3	3
126.78	Without thiamine	4	5	6	6	3	3	3	3
	With thiamine	4	4	4	6	3	3	3	3
130.30	Without thiamine	10	7	9	> 14	1*	1*	1*	0
	With thiamine	10	5	9	11	2*	3	1*	1*
130.63	Without thiamine	11	7	10	> 14	1*	1*	1*	0
	With thiamine	10	5	9	10	2*	3	1*	1*
130.64	Without thiamine	10	6	10	> 14	1*	1*	1*	0
	With thiamine	10	5	9	10	1*	3*	1*	1*

Wide differences in response to the growth factors, in media at pH 7, were seen with the non-perithecial species, as shown in Table 9; in some cases the stimulation of growth was considerable. Effects on sporulation were less marked, but panto-

thenate caused moderate development of conidia in one species (*Penicillium rubrum* 127) which did not sporulate at all on the control medium. Stimulation of vegetative growth and of sporulation did not always coincide. The effect of thiamine was enhanced at initial pH values around 5.0. Mycelial growth was increased in *P. citrinum* 37, *P. fellutanum* 7, *P. implicatum* 101, *P. luteum* 28, *P. roseo-purpureum* 148, and *P. verruculosum* 158; enhanced sporulation was seen in *P. adametzi* 128, *P. canescens* series 136, *P. fellutanum* 7, *P. steckii* 137, *P. variabile* 121.1, *P. verruculosum* 158 and *Penicillium* sp. 98. Even without thiamine, *P. luteum* 28, *P. rubrum* 127, *P. simplicissimum* 4 and *P. sp.* 98, which gave no conidia in 3% (w/v) glucose at pH 7, showed mild sporulation at pH 5.0.

Table 9. *Effect of vitamins on growth and sporulation of various non-perithecial Penicillium species*

Species and ref. no.	Increased mycelial growth (%) in presence of	Increased sporulation in presence of
<i>P. adametzi</i> 128	Ca pantothenate (85 %)	—
<i>P. canescens</i> series 136	Nicotinic acid (20 %) biotin (29 %)	—
<i>P. citrinum</i> 37.1	—	Nicotinic acid, riboflavin
<i>P. cyaneum</i> type 124	Biotin (11 %)	—
<i>P. fellutanum</i> series 135	Thiamine (87 %), riboflavin (16 %), pyridoxine (83 %)	Thiamine, biotin
<i>P. funiculosum</i> , series 150	—	Thiamine, nicotinic acid, riboflavin, pyridoxine, Ca pantothenate, biotin
<i>P. notatum</i> 138	—	Thiamine, riboflavin, Ca pantothenate, biotin
<i>P. oxalicum</i> 96	Thiamine (25 %), Ca pantothenate (22 %)	Thiamine, nicotinic acid, riboflavin, pyridoxine, Ca pantothenate
<i>P. purpurogenum</i> 134	Nicotinic acid (86 %), biotin (66 %)	—
<i>P. roseo-purpureum</i> 148	—	Riboflavin, Ca pantothenate
<i>P. rubrum</i> 127	—	Nicotinic acid, Ca pantothenate
<i>P. simplicissimum</i> 4	—	Thiamine, nicotinic acid, riboflavin
<i>P. steckii</i> 137	Pyridoxine (13 %), biotin (34 %)	—
<i>P. tardum</i> 132	Riboflavin (11 %), pyridoxine (25 %), Ca pantothenate (14 %), biotin (12 %)	—
<i>P. variabile</i> 121	Pyridoxine (14 %), Ca pantothenate (12 %), biotin (20 %)	Thiamine, nicotinic acid, pyridoxine
<i>P. variabile</i> 121.2	Thiamine (14 %), riboflavin (16 %), pyridoxine (20 %), biotin (20 %)	—
<i>Penicillium</i> sp. 40	Thiamine (27 %), pyridoxine (87 %), Ca pantothenate (20 %), biotin (20 %)	—

DISCUSSION

Klebs's principle that sexual and asexual sporulation in fungi is generally favoured by exhaustion of nutrients is supported by the results obtained here with various carbohydrates as carbon sources. The polysaccharides markedly stimulated sporulation, possibly because their insolubility did not allow a high concentration of free sugar to accumulate in the medium. However, it is not clear why the strains of *Penicillium vermiculatum* which fruited best on cellulose and gave maximum growth on starch and dextrin did not fruit on starch or dextrin, since these also gave a low concentration of 'sugar' in the medium. The polysaccharides are liable to be contaminated with micronutrients; fungal growth factors have for instance been reported in soluble starch (Cochrane, 1958). The presence of such contaminants may therefore have affected sporulation. It may be significant that thiamine, found by Schopfer (1934) in some samples of maltose prepared from starch, proved to be the most potent of the six growth factors tested here. Fergus (1952), however, found that starch and maltose were utilized but poorly for growth by *P. digitatum*.

Organic nitrogen sources such as peptone are also likely to be contaminated with micronutrients, and another complicating factor is the fact that they may also serve as carbon source. That the two perithecial species gave better and more rapid fruiting with 0.1 % (w/v) glucose when the nitrogen was supplied in the inorganic form may be due to the fact that the organic nitrogen compounds by supplementing glucose as a source of carbon maintained a higher sugar concentration and thus delayed fruiting. In general ammonium-N is believed to be more easily utilized by fungi than nitrate-N. But the results reported here seem to indicate that the reverse is true for growth for the majority of *Penicillium* spp. tested. In no case was the production of conidia better with ammonium sulphate than with sodium nitrate.

Reports of growth factor stimulation of *Penicillium* spp. are rare, but from the striking effects on growth and fruiting shown by natural media such as malt agar and potato-glucose agar it may perhaps be assumed that other still undefined micronutrients are important. Organisms such as *P. implicatum* 101 and *P. variable* 121, which sporulated well on defined media when first isolated but which formed conidia sparsely or not at all throughout the present experiments, seem to have developed a requirement for some undefined micronutrients, since they were still able to sporulate on malt agar. The loss of sexual fertility observed in three strains of *P. vermiculatum* can hardly be due to nutritional deficiency since even the use of natural media did not result in production of perithecia.

The seven strains of *Penicillium vermiculatum* can be divided into three groups: 77.29, 77.62 and 77.81 fruited regularly and more or less consistently under ordinary conditions; 77.C, 77.10 and 77.32 did not fruit under any conditions in these experiments; 77.80B produced perithecia only under certain circumstances and then not consistently. This strain may represent a stage in the process of loss of sexual fertility. As regards the six strains of *P. wortmanni* the subgrouping previously proposed by Basu (1951), mainly on morphological and cultural characteristics, appears to be valid also on nutritional grounds; it seems that these should be recognized as different varieties, if not species.

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