EFFECT OF DIFFUSIBLE INHIBITORS IN SOIL ON SPORE GERMINATION AND GERM TUBE GROWTH OF *PENCILLIUM EXPANSION*

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Abstract

Pencillium expansum spore germination was inhibited on agar discs placed on cellophane on the unsterilized soil surface. But germination was not inhibited on discs separated from the soil by glass slides. Thus there is evidence for the presence of diffusible non-volatile inhibitors in these soils. The germination of spores on agar discs placed on autoclaved sterilized soil occur and shows that micro-organisms may be one of the producers of non-volatile inhibitors.

Introduction

Pencillium expansum Link ex.S.F. Gray spores did not germinate in unsterilized soil (Zardari, 1984); for that one factor might be the diffusible inhibitory substances which inhibit spore germination. Lockwood(1959) suggested that diffusiable toxins could be present in soils as even when the soil and fungus were separated by agar or cellophane barriers, inhibition still occurred. He also found that some actinomycetes, particularly ***Streptomyces*** spp. produced diffusible fungitoxic substances and suggested that they might play a role in natural fungistasis. Stevenson (1956) observed that specific morphological changes were produced in ***Helminthosporium sativum*** by an antibiotic produced by an actinomycetes. Similar morphological changes were produced in soils inoculated with actinomycetes. Thus micro-organisms in the vicinity of spores in soil may produce antibiotic which inhibit spore germination (Lingappa and Lockwood, 1964).

Hora and Baker (1970) used soil emanation agar method to differentiate between inhibitors diffusing in solution and inhibitors capable of diffusing as volatile gases. This

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method or variations of it, have been used by a number of workers (Hora and Baker, 1972a, Ko & Hora, 1972, Romine & Baker 1973, Balis, 1976) and provide good evidence for the presence of volatile germination inhibitors in many types of soil. The volatile fungistatic compounds are probably mostly produced by the soil microflora. Thus Hora & Baker (1972 b) found that volatile inhibitors were produced when sterilized soil was recolonized by soil actinomycetes fungi or bacteria.

The present work was carried out to determine the effect of the presence of diffusible inhibitors in soil spore germination and germ tube growth of ***P.expansum***.

Materials and Methods

The method of Hora and Baker (1970) was followed to investigate the effect of diffusable inhibitors in soil on ***P. expansum***. Soil was collected and dried at room temperature before sieving through 2mm mesh sieve. Half of the bulk was sterilized by autoclaving. The moisture content and pH of both the sterilized fractions was 25% and 6.1 respectively.

'A' size geneco-biovessels were filled with 100g unsterilized soil. Boiled, sterilized cellophane was then placed on the top of the soil and agar discs (3% Oxoid purified agar + 1% peptone (Dix 1972)) 2-3mm thick and 12mm diameter were placed on top of the cellophane. Geneco-biovessels containing 100g sterilized soil served in one set of controls and another set of agar discs were placed on cellophane in contact with 1% Oxoid purified agar in petridishes.

To determine if the inhibitors were volatile some agar discs were placed on sterilized glass slides in the Geneco-biovessels instead of on cellophane. As a further control agar discs were placed on microscope slides in petridishes without soil.

All containers were incubated for 24h at 23°C and after 24h one agar disc from each Geneco-biovessel was removed and placed on a sterilized glass slide in a petridish whose internal atmosphere was maintained at a high humidity. These discs were called pre-activated discs. Other discs were left in the Geneco-biovessels in contact with soil. All agar discs were then inoculated with one drop of spore suspension (79.16×10^4 spores ml^{-1}) obtained from 7 day old cultures of ***P. expansum*** Link ex.S.F. Gray (CMI 39761). After 12h incubation at 23°C, the discs were removed, stained with cotton-blue and examined under microscope. At least 400 spores selected at random were counted to assess percent germination. The lengths of 50 germ tubes were also measured.

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Results

The results showing the effect of diffusible inhibitors on spore germination and germ tube growth are given in (Table 1 & 2). Only 9% of the spores germinated on agar discs placed on cellophane in contact with unsterilized soil. In contrast, 96-99% germinated on the agar discs on cellophane in contact with sterilized soil or in the 1% agar controls (Table 1). There was, however, no significant difference between the percentage germination on pre-activated agar discs (removed after 24h in contact with soil before inoculation) and that on agar discs which remained in contact with the soil. Similarly there was no significant differences between pre-activated discs and other discs in the control treatments.

Germ tube growth on agar discs in treatments containing sterilized soil or 1% agar was also greater than on discs in contact with unsterilized soil (Table 2). There was no marked difference between germ tube growth on pre-activated agar discs and on agar discs which were left in contact with unsterilized soil, but in contrast, there was a marked difference between growth on discs in the sterilized soil treatments. In these treatments the germ tube length was greater on discs which were left in contact with the soil than on the pre-activated discs. Germ tube growth on agar discs in contact with sterilized soil was also higher than on the discs in contact with 1% agar (Table 2).

The results of the volatility of the diffusible inhibitors (Table 3 & 4) show that the discs in all treatments supported a high percentage (97-99%) of spore germination and there were no significant differences between any of them (Table 3).

There were also no significant differences in germ tube growth between any of the treatments except in the case where the agar discs were left in contact with the sterilized soil where greater growth was found (Table 4).

Discussion

The results provide no evidence for volatile inhibitors affecting ***P. expansum*** spore germination and germ tube growth in soil. However non-volatile or relatively non-volatile inhibitory compounds capable of diffusing through agar were present, which could markedly reduce germination and germ tube growth. Dix (1972), using a similar method, which used filter paper instead of cellophane to separate the agar discs from the soil, found fungistatic effects on the speed of germination, the final percentage germination and the growth rate of germ tubes of ***P.expansum***.

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Table 1. Effect of diffusible inhibitors in the soil on *P. expansum* spore germination.

Treatment		Percentage spore germination after 12 hr.			
		Replicates			Mean ± S.E.
Unsterilized soil	Agar discs	1.	2.	3.	
	Preactivated* (24 hr)	9.22	25.97	8.67	14.620 ± 5.677
	Continuing activation**	9.76	9.03	5.88	8.233 ± 1.191
Sterilized soil (control)	Preactivated* (24 hr)	99.76	97.50	98.29	98.517 ± 0.622
	Continuing activation**	99.52	99.51	99.29	99.106 ± 0.300
1% Agar (control)		99.29	95.35	95.26	99.300 ± 0.955

* Agar discs removed after 24 hr in contact with the soil and placed on sterilized glass slides in petridishes befor inoculation with spores.
** Agar discs in contact with soil before and after inoculation with spores.

Table 2. Effect of diffusible inhibitors in the soil on germ tube growth of *P. expansum*.

Treatment		Germ tube length (μm) after 12 hr.				
			Replicates			Mean ± S.E.
Unsterilized soil	Agar discs		1.	2.	3.	
	Preactivated* (24 hr)	Mean	11.15	7.26	9.75	9.387 ± 1.138
		S.E.	1.07	0.60	0.91	
Sterilized soil (control)	Continuing activation**	Mean	13.19	9.68	4.32	9.063 ± 2.579
		S.E.	1.08	0.96	0.83	
	Preactivated* (24 hr)	Mean	47.32	40.23	30.59	39.380 ± 4.848
		S.E.	2.36	2.07	1.54	
	Continuing activation**	Mean	72.99	57.97	55.84	62.267 ± 5.397
		S.E.	3.67	2.79	2.67	
1% Agar (control)		Mean	30.83	28.52	30.13	29.823 ± 0.687
		S.E.	1.44	1.32	2.08	

*/** as in Table 1.
Values are means of measurements of 50 germ tubes. A mean of 10 measurements only.

Table 3. Volatility of diffusible inhibitors on spore germination of *P. expansum*.

Treatment		Percentage spore germination after 12 hr.			
		Replicates			Mean ± S.E.
Unsterilized soil	Agar discs	1.	2.	3.	
	Preactivated* (24 hr)	97.85	98.31	99.01	98.390 ± 0.337
	Continuing activation**	99.75	99.27	99.08	99.367 ± 0.199
Sterilized soil (control)	Preactivated* (24 hr)	99.50	99.57	98.84	98.637 ± 0.566
	Continuing activation**	97.96	97.14	98.58	97.893 ± 0.417
Without soil (control)		98.29	98.28	98.38	98.316 ± 0.032

*/** as in Table 1.

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Table 4. Volatility of the diffusible inhibitors on germ tube growth of *P. expansum*.

Treatment		Germ tube length (μm) after 12 hr.				
			Replicates			
			1.	2.	3.	Mean ± S.E.
Unsterilized soil	Agar discs Preactivated* (24 hr)	Mean	56.30	43.95	35.10	45.117 ± 6.148
		S.E.	3.07	2.97	1.08	
	Continuing activation**	Mean	65.40	56.88	56.94	59.740 ± 2.830
		S.E.	3.52	3.19	3.11	
Sterilized soil (control)	Preactivated* (24 hr)	Mean	55.99	51.03	49.26	52.093 ± 2.014
		S.E.	2.84	3.18	2.397	
	Continuing activation**	Mean	61.89	70.33	69.29	67.170 ± 2.657
		S.E.	2.80	2.98	3.66	
1% Agar (control)		Mean	56.02	53.87	49.58	53.157 ± 1.893
		S.E.	2.75	2.71	2.53	

*/** as in Table 1.
Values are means of measurements of 50 germ tubes.

The diffusible substances are probably produced by micro- organisms since the soil sterilized by autoclaving looses its activity. However, such treatments do not conclusively prove this because autoclaving may effect nutrient levels in the soil which may then reduce or remove the effect of toxic substances in the soil, thus allowing the spores to germinate and grow. According to Ko and Lockwood (1967) reduced germination on agar discs in contact with soil may also result from dilution of nutrients due to their diffusion from the disc into the soil. However, in these experiments, when agar discs placed on cellophane in contact with 1% purified agar to allow such diffusion to occur, the percentage germination remained high. Such results indicate that the effect cannot be attributed to depletion of nutrients from the discs. In any case these results indicate that germination inducers may diffuse into the discs from the soil since germ tube growth on discs placed on cellophane in contact with sterilized soil was greater than on discs on 1% agar.

Further support for the diffusion of growth stimulators from sterile soil is given by the observation that germ tube growth was lower on the pre-activated discs, which were incubated in petridishes after inoculation, than on discs left in contact with the sterilized soil after inoculation.

Thus it can be concluded that a major cause of the inhibition of germination and germ tube growth in natural soil appears to be the presence in the soil of relatively non-volatile diffusible inhibitors.

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