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MYCOSTASIS IN NUTRIENT AMENDED AND RE-INOCULATED SOIL MINERALS

BY

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Microbial nutrient-sink theory was tested by using soil mineral substrates. Unamended soil mineral supported germination of nutrient-independent *H. oryzae* and *C. lunata*, but failed to support the germination of nutrient-dependent spores of *P. chrysogenum*. Amendment of Fe_2O_3 and SiO_2 with nutrients supported good germination of *P. chrysogenum*. Inoculation of nutrient-amended soil minerals with soil microorganisms rendered them fungistatic within 7 days of inoculation. No fungistatic substances(s) could be extracted from inoculated fungistatic soil minerals. Loss of glucose from inoculated silica as a function of time was correlated with decrease in germination of nutrient-dependent spores of *P. chrysogenum*. Germination of nutrient independent spores of *H. oryzae* remained unaffected till the available nutrients were exhausted. No fungistatic substance could be extracted from re-inoculated fungistatic silica substrate.

INTRODUCTION

Nutrient-sink imposed by soil microorganisms is considered to be the cause of 'biotic' fungistasis of natural soils (Ko and Lockwood, 1967 ; Steiner and Lockwood, 1969 ; Filonow and Lockwood, 1983 ; Bhattacharya and Somaddar, 1986). Steiner and Lockwood (1970) provided further evidence in support of microbial nutrient-sink theory using sterilized and re-inoculated soils

It is now well established that fungistasis in sterilized soil is restored by re-inoculation with soil microbes which cause depletion of the available nutrients released due to sterilization (Park, 1956 ; Old and Wilcoxson, 1962 ; Lockwood and Lingappa, 1964 ; Steiner and Lockwood, 1969). Since the exact nutrient status of soils is difficult to ascertain, soil minerals lacking any nutrient were used in this study as model substrate systems for testing nutrient-sink theory.

Mycostasis on soil mineral substrates with or without nutrients and after inoculation with soil microbes was investigated. The relationship between nutrient depletion and restoration of mycostasis on soil minerals was also examined.

MATERIALS AND METHODS

Soil minerals used were : aluminium oxide (Al_2O_3), ferric oxide (Fe_2O_2), silicon dioxide (SiO_2), montmorillonite and kaolin. Montm rillonite and kaolin were washed 5 times with O.2N HCl followed by several rinsings with distilled water to remove chloride. The pH of the slurry was adjusted to 6.5 with O.lN NaOH and centrifuged. The residue was air dried and stored. Other minerals used were of 'Analar' grade. Moisture of each mineral was adjusted to 60% watar holding capacity before use.

Soil microorganisms were isolated by dilution plate technique following Pramer and Schmidt (1967). The soil fungi were isolated using rose bengal agar containing 30 μg/ml streptomycin. Fungal species differing in colony morpholgy were transferred to PDA slopes. Soil bacteria and Actinomycetes were isolated using soil-extract agar (Bhattacharya 1975). Soil dilution plates were incubated at 37 C and the colonies differing in morphology were transferred to nutrient agar slants.

Organisms used for assay of fungistasis were nutrient dependent *Penicillium chrysogenum* (PC), and nutrientindependent *Curvularia lunata* (CL) and *Helmint-hosporium* oryzae (HO). The maintenance of the fungi and preparation of conidial suspensions were done following the method of Setua and Samaddar (1980). Gram positive *Bacillus subtilis* and gram negative *Escherichia coli* were used for antibiotic assay. The organisms were maintained on nutrient agar with weekly subculturing.

Aqueous extracts of minerals were prepared by shaking one part mineral with two volumes of distilled water on a rotary shaker for 1h, centrifuged at 3000 X g for 10 min, concentrated ten fold in a vacuum evaporator at 37°C and filter sterilized. Chloroform: ethanol (2 : 1, v/v) extracts of minerals were prepared as described previously (Bhattacharya and Samaddar, 1986).

The double cellophane (DC) mathod of Weltzien (1963) was used for assaying the fungitoxic property of soil minerals. Fungistatic activity of aqueous extracts of soil minerals was tested by slide germination bioassay technique (APS Committee, 1943). Antibiotic property of the extracts of soil min rals was assayed by agar-cup method (Kruger, 1961). The glucose content of the soil mineral extracts was determined by the anthrone method of Morris (1948).

Nutrient utilization and fungistaic activity in inoculated soil minerals was determined by the method of Steiner and Lockwood (1970) with minor modific tions. Two hundred grams of silica were sterilized (15 p.s i. for 15 min). The sterilized silica was amended with 8 ml of sterile 5% glucose solution and 4 ml of sterile Czapek's salt solution per flask. The concentration of glucose was 2 mg g of dry silica. Moisture was adjusted to 60% of the dry weight of silica with sterile distilled water. The sterilized and amended silica was inoculated with each of *Fusarium* sp., *Streptomyces*, *B. subtilis* and *Serratia* sp. The flasks were incubated at 28°C for

7 days. Portions of silica were removed at intervals and assayed for : (i) germination of conidia of PC and HO, (ii) amount of residual glucose, and (iii) extractable fungistatic or antibiotic substances. The culture filtrates of the three bacteria used were not inhibitory to the germination of spores of PC and HO.

RESULTS

To test the role of nutrient deprivation by microorganisms in the causation of soil fungistasis, model soils were prepared using soil minerals. Germination of test conidia on minerals with or without supplemental nutrients was determined.

Conidia of nutrient-dependent *P. chrysogenum* failed to germinate on any of the unamended soil minerals (Table 1). Aqueous extracts of the soil minerals were not inhibitory to the germination of spores of PC suspended in 0.1% glucose solution. When the soil minerals were amended with 0.5% glucose and 0.25% casein hydrolysate separately or in combination significant stimulation of germination (26-100%) was observed on iron oxide and silica but not on other minerals. Nutrient-independent conidia of *H. oryzae* and *C. lunata* germinated readily on unamended soil minerals. Amendment of soil minerals caused further stimulaton of the germination of these test organisms (Table 1).

In another experiment, soil minerals were enriched with 0.5% glucose and 0·25% casein hydrolysate and inoculated with soil microorganism isolated previously. Two each of fungal, actinomycete, and bacterial isolates were selected, scrapped off with spatula, added to the minerals and mixed thoroughly. Unit weight (100 g) of each of the minerals received scrappings equivalent to the culture tube of each of the organisms. After one week of incubation at 28°C, mineral plates were prepared and assyed to determine the fungistatic aetivity of the minerals. Water extracts of the minerals were tested for fungitoxic and antibiotic activity. Results (Table 1) indicated that soil minerals supplemented with nutrients and then inoculated with soil microbes became inhibitory to the germination of both nutrient-dependent and independent spores after 7 days of incubation. Aqueous extracts of soil minerals at 0 h or after 7 days of incubation with soil microorganisms were not inhibitory to the germination of conidia of HO or PC. Apparently, the fungistasis of inoculated soil minerals was due to nutrient competition.

The relationship between nutrient utilization and fungistatic activity of inoculated soil mineral was tested. Fungistasis was assayed by the DC method. Fungitoxicity or antibiotic activity of the extracts were determined. Results (Fig, 1) indicated that as the glucose level decreased in the inoculated silica system there was a corresponding decrease in the germination of nutrient-dependent spores of *P. chrysogenum* on the surface of silica. After a days of inoculation anthrone positive glucose was completely utilized and germination of conidia of PC was reduced to 10-12%. On the 5th day, germination of PC was nil. The germination

Table 1. *Germination*[a] *of fungal conidia on unamendad and nutrient-amended soil minerals.*

Treatment of soil minerals	Mean (%) germination of conidia of fungi in														
	Al_2O_3			Fe_2O_3			SiO_2			Montmorillonite			Kaolin		
	SC	CL	HO	PC	CL	HO	PC	CL	HO	PC	CL	HO	PC	CL	HO
Unamended (control)	0	69	78	0	90	77	2	73	59	0	33	50	0	72	72
+ 0.5% glucose	0	90	86	100	90	86	50	80	76	0	84	60	0	80	87
+ 0.5% glucose + 0.25% casein hydrolysate	0	100	90	100	100	95	100	100	99	0	85	70	0	100	100
+ 0.5% glucose + 0.25% casein hydrolysate + soil organisms[b]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fungistatic activity[c] of aqueous extracts		Nil			Nil			Nil			Nil			Nil	
Antibiotic activity[d] of aqueous and chloroform ethanol extracts		Nil			Nil			Nil			Nil			Nil	

a Germination of *P. chrysogenum* (PC), *C. lunata* (CL) and *H. oryzae* (HO) in distilled water was 2,90,81% respectively.

b. Soil organisms used were 2 species of fungi one *Streptomyces* Sp. and one each of *Bacillus* sp. and *Serratia* sp.

c Water extracts of soil minerals were made at 0 hr and after 7 days of incubation and the fungitoxicity was assayed by spore germination bioassey.

d. Antibiotic activity of the extracts assayed by agar-cup method.

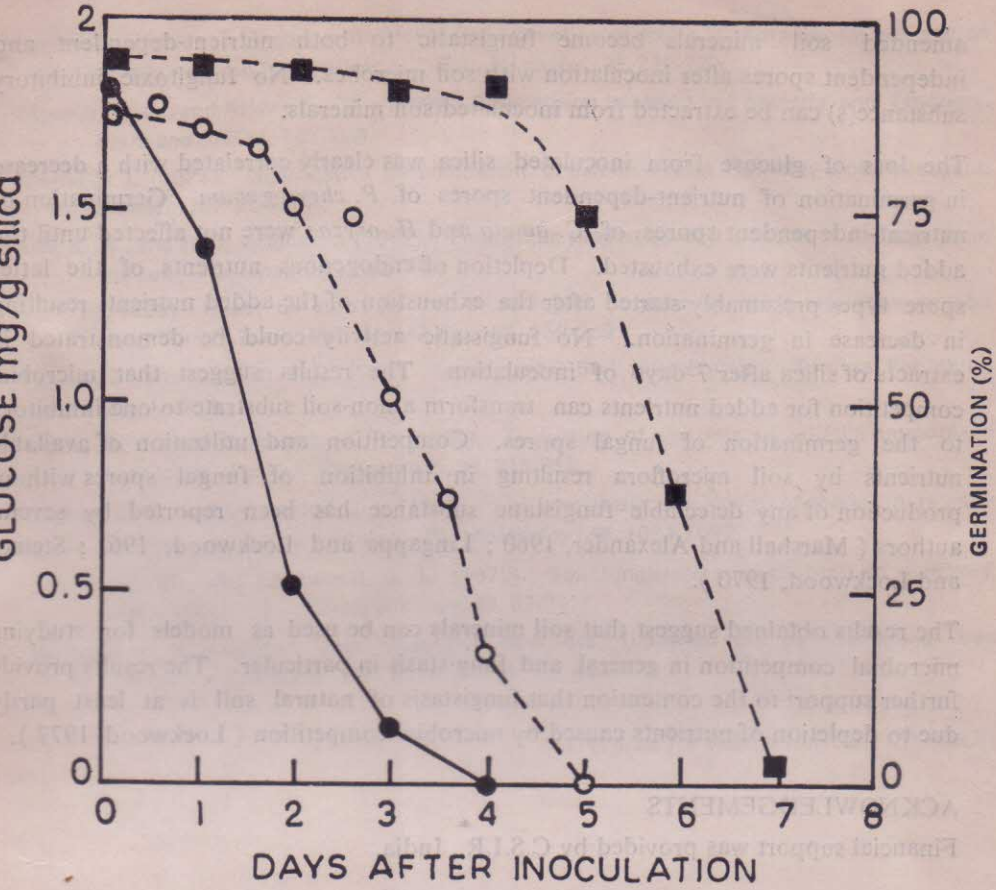

Fig. 1. Relation between amount of glucose ●—● remaining in silica incubated with soil microbes and the decrease in germination of spores of *P. chrysogenum* (o - - o) and *H. cryzal* (■ - - ■).

of nutrient-independent *H. oryzae* remained uneffected upto five days aftei inoculation. After the exhaustion of glucose, however, the germination of HO also declined rapidly and within two subsequent days was reducəd to zero. Aqueous and chloroform : ethanol extracts of inoculated silica after 7 days were not inhibitory to the germination of conidia of PC or HO.

DISCUSSION

The results of this study indicate that soil minerals amended with glucose and mineral salts and inoculated with soil microorganisms provide a suitable model system for studying relationship between nutrient depletion and fungistasis. It is evident from this study that nutrients can annul fungistasis of nutrient-dependent spores in soil mineral systems where microbes are absent. It is of interest that amended soil minerals become fungistatic to both nutrient-dependent and independent spores after inoculation with soil microbes. No fungitoxic inhibitory substance(s) can be extracted from inoculated soil minerals.

The loss of glucose from inoculated silica was clearly correlated with a decrease in germination of nutrient-dependent spores of *P. chrysogenum* Germination of nutrient-independent spores of *C. lunata* and *H. oryzae* were not affected until the added nutrients were exhausted. Depletion of endogenous nutrients of the latter spore types presumably started after tha exhaustion of the added nutrients resulting in decrease in germination. No fungistatic activity could be demonstrated in extracts of silica after 7 days of inoculation. The results suggest that microbial competition for added nutrients can transform a non-soil substrate to one inhibitory to the germination of fungal spores. Competition and utilization of available nutrients by soil microflora resulting in inhibition of fungal spores without production of any detectable fungistatic substance has been reported by several authors (Marshall and Alexander, 1960 ; Lingappa and Lockwood, 1961 ; Steiner and Lockwood, 1970).

The results obtained suggest that soil minerals can be used as models for studying microbial competition in general, and fungistasis in particular. The results provide further support to the contention that fungistasis of natural soil is at least partly due to depletion of nutrients caused by microbial competition (Lockwood, 1977).

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