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Microbiostasis by Nutrient Deficiency Shown in Natural and Synthetic Soils

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The germination pattern of six species of fungi on synthetic soil containing antibiotic-producing or non-antibiotic-producing micro-organisms was similar to that on natural soil. Sterilization by autoclaving destroyed the fungistatic effect of both natural and synthetic soils. Mixed micro-organisms were more effective than bacteria, actinomycetes or fungi alone in inducing fungistasis in synthetic soil. The percentage germination of *Exserohilum rostratum* and *Bipolaris maydis* on both natural and synthetic soils increased with increase in the proportion of silica sand added. Bacteriostasis, actinostasis and fungistasis occurred concurrently in the synthetic soil, which also induced lysis of mycelia of *Neurospora tetrasperma*. Preincubation on natural or synthetic soil rendered nutrient agarose blocks incapable of supporting germination of nutrient-dependent fungi without reducing their ability to support germination of nutrient-independent fungi. Individual groups of micro-organisms were not as effective as mixed micro-organisms in causing diffusion of nutrients from agarose blocks to synthetic soil.

INTRODUCTION

The inability of micro-organisms to multiply when added to natural soil was observed as early as 1909 for bacteria (Russell & Hutchinson, 1909) and 1940 for actinomycetes and fungi (Katznelson, 1940). Such antagonistic phenomena of soil against bacteria, actinomycetes and fungi have been termed bacteriostasis (Brown, 1973), actinostasis (Ko & Chow, 1977) and fungistasis (Dobbs & Hinson, 1953), respectively, and called microbiostasis collectively (Ko & Chow, 1976). Bacteriostasis, actinostasis and fungistasis exist concurrently in natural soils (Ho & Ko, 1982; Ko & Ho 1984). However, their antagonistic potential are differentially affected by environmental and edaphic factors (Ko & Ho, 1984; Ho & Ko, 1985). Lytic action on mycelia of most fungi is another unique antagonistic phenomenon of natural soil (Lockwood, 1960). Ko & Lockwood (1970) found that mycolysis occurred even when sterility of mycelia was maintained during incubation on soil by interposition of a membrane.

Ko & Lockwood (1967) suggested that sensitivity of nutrient-dependent spores to fungistasis is the result of the absence of available nutrients in the soil, and postulated the existence of a nutrient sink in soil created by microbial competition to account for fungistasis of nutrient-independent spores. Lysis of fungal mycelia in soil has been shown to be due to autolysis induced also by the action of a nutrient sink in natural soil (Ko & Lockwood, 1970). Based on the information currently available, nutrient deprivation has been considered to be the most likely overriding explanation for microbiostasis of soil (Ko & Chow, 1977; Ko, 1982; Lynch, 1982; Ko & Ho, 1984; Lockwood, 1984).

Inhibition of the spore germination and lysis of fungal mycelia under a leaching system have been used as supporting evidence for the importance of a nutrient sink in the creation of such phenomena in natural soil (Ko & Lockwood, 1967, 1970; Hsu & Lockwood, 1973). Although the

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leaching system provides an inhibitor-free condition for studying the antagonistic phenomena in soil, the assumption that microbial activity creates a nutrient sink in soil cannot be tested in such a system. Therefore, a synthetic soil was developed for assessing the role of micro-organisms in the creation of antagonistic phenomena in soil.

METHODS

Micro-organisms. Conidia of *Bipolaris maydis* (Nishikado) Shoemaker, *B. stenospila* (Drechsler) Shoemaker, *Exserohilum rostratum* (Drechsler) Leonard & Suggs emend. Leonard, *Mucor ramannianus* Moller, *Penicillium frequentans* Westling and *P. funiculosum* Thom were obtained by growing each fungus under continuous fluorescent light for 6–8 d at 24 °C on V-8 juice agar (Aragaki, 1964). Ascospores of *Neurospora tetrasperma* were obtained as described by Ko & Lockwood (1967) and used after heat activation at 58 °C for 20 min. *Penicillium funiculosum* was obtained from K. G. Rohrbach and the other species from M. Aragaki and J. L. Lockwood. Antibiotic-producing or non-antibiotic-producing bacteria, actinomycetes and fungi were isolated from soil as previously described (Ho & Ko, 1982).

Synthetic soil. The inorganic component of the synthetic soil consisted of 65% >0·2 mm silica sand, 20% <0·2 mm silica sand, 8% kaolinite and 7% montmorillonite. Silica sand was washed by shaking 100 g in 150 ml 1 M-HCl in a 250 ml flask on a shaker. After 24 h the HCl solution was replaced with fresh distilled water daily for 3 d. The washed silica sand was dried in an oven at 110 °C. The inorganic component was mixed with Canadian peat as the organic component at a ratio of 1 : 9 (w/w). Before use, 200 ml moist Canadian peat was autoclaved for 15 min and washed by shaking in 1500 ml distilled water in a 2000 ml flask for 1 week. The distilled water was changed every day. The washed Canadian peat was adjusted to pH 7 with 1 M-NaOH and dried in an oven at 50 °C for 3 d. Since extracts of natural soil contained about 4·2 μg carbohydrates ml^{-1} and 0·7 μg amino acids ml^{-1} (Ko & Lockwood, 1967), 10% (v/v) Hoagland's solution (Hoagland & Arnon, 1950) supplemented with 6 μg glucose ml^{-1} and 1 μg Casamino acids ml^{-1} was used as the soil solution for the synthetic soil. The ratio of soil solution and soil solid substance was 4:10 (v/w).

The numbers of micro-organisms in each component of synthetic soil were determined by the dilution plate method as described previously (Ho & Ko, 1982). No micro-organisms were detected in silica sand or kaolinite. There were an average of 5 bacteria and 1·7 fungi in 1 g dry Canadian peat, and 1390 bacteria and 1130 actinomycetes in 1 g montmorillonite. Before addition of micro-organisms, the numbers of bacteria, actinomycetes and fungi in 1 g synthetic soil were 88, 71 and 0, respectively. Suspensions of micro-organisms were obtained from 7-d-old fungi grown on V-8 agar, and 4-d-old bacteria and 14-d-old actinomycetes grown on nutrient agar. Suspensions of fungi, actinomycetes or bacteria, each consisting of five different isolates, were thoroughly mixed with non-inoculated synthetic soil at the ratio of 1 :10 (v/w). This soil was further mixed with non-inoculated synthetic soil at the same ratio. The resulting synthetic soil contained about 6×10^7 bacteria, 7×10^6 actinomycetes and 2×10^6 fungi g^{-1}. The inoculated synthetic soil was incubated at 24 °C for at least 1 month before use. The final populations of bacteria, actinomycetes and fungi were about 8×10^7, 2×10^6, and 5×10^5 (g dry soil)$^{-1}$, respectively. The pH of the synthetic soil was about 6·2.

Germination tests. One drop (approximately 0·05 ml) of spore suspension was spread on a sterile polycarbonate membrane (0·2 μm , 25 mm diam.; Nuclepore Co.) placed on a block (15 × 15 × 6 mm) of natural or synthetic soil in a small Petri plate (60 × 15 mm), and incubated at 24 °C. The incubation time for ascospores of *N. tetrasperma* and conidia of *B. maydis, B. stenospila* and *E. rostratum*, which are nutrient-independent, was 5 h, and that for conidia of *M. ramannianus, P. frequentans* and *P. funiculosum*, which are nutrient-dependent, was 16 h. After incubation, each polycarbonate membrane was transferred to a glass slide and spores on the membrane were stained with cotton blue (100 ml aniline blue in 100 ml 85% lactic acid). At least 200 spores were counted for each treatment. Two replicates were used and all experiments were done at least twice.

Assay of soil microbiostasis. If microbiostasis exists in a given soil, the number of micro-organisms should either decrease or remain more or less constant in it (Ko & Chow, 1977). If there was no increase in numbers of bacteria, actinomycetes and fungi in soil during a 3 d interval, the presence of bacteriostasis, actinostasis and fungistasis in soil, respectively, was indicated (Ho & Ko, 1982). Diluted soil suspensions prepared on days 0 and 3 were plated on PCNB-soil extract agar (Farley & Lockwood,1968), alkaline water agar (Ho & Ko, 1980*b*) and surfactant-PDA (Steiner & Watson, 1965) for determining populations of bacteria, actinomycetes and fungi, respectively. Five plates were used for each treatment and colony numbers were determined after 7 d for bacteria and fungi, and 14 d for actinomycetes at 24 °C.

Detection of a nutrient sink. Highly purified agarose (SeaKem HGT-P Agarose, Marine Colloids Division, FMC Co., Rockland, Maine, USA), which did not support germination of nutrient-independent fungi (Ho & Ko, 1980*a*), was used to study the ability of micro-organisms to create a nutrient sink in soil. Agarose blocks (0·8% SeaKem agarose, 100 μg glucose ml^{-1} and 100 μg peptone ml^{-1}) were placed on polycarbonate membranes and incubated on natural or synthetic soil. After incubation at 24 °C for 24 h, agarose blocks were transferred to sterile Petri plates and tested for spore germination as previously described.

To determine the rate of diffusion of nutrients from agarose blocks, 0·8% SeaKem agarose was supplemented with 800 μg glucose ml^{-1} and 250 μg asparagine ml^{-1}. Agarose blocks placed on sterile polycarbonate membranes were incubated on synthetic soil and removed at different times during incubation at 24 °C. Nutrients were extracted by shaking three blocks in 5 ml sterile distilled water for 3 min. The extract was filtered through a polycarbonate membrane (0·2 μm). Carbohydrates and amino acids in the extract were determined by the anthrone test (Morris, 1958) and ninhydrin test (Moore & Stein, 1954), respectively.

RESULTS

Antagonistic phenomena in synthetic soil

Nutrient-independent fungi (*N. tetrasperma*, *B. maydis*, *B. stenospila* and *E. rostratum*) germinated 91–99% but nutrient-dependent fungi (*P. frequentans* and *P. funiculosum*) germinated only 0–33% on the components of synthetic soil separated by a polycarbonate membrane (Table 1). The germination pattern was similar to that in distilled water, indicating the absence of inhibitory substances and nutrients in these components.

The germination pattern on synthetic soil containing antibiotic-producing or non-antibiotic-producing micro-organisms was similar to that on natural soil (Table 2). Ascospores of *N. tetrasperma*, which were not sensitive to fungistasis in natural soil, germinated freely on synthetic soils. Conidia of *B. maydis*, *B. stenospila*, *E. rostratum*, *P. frequentans* and *P. funiculosum*, which were sensitive to soil fungistasis, failed to germinate or germinated poorly on synthetic soils. Autoclaving destroyed the fungistatic effect of both natural and synthetic soils. Because similar results were obtained with synthetic soils inoculated with antibiotic-producing or non-antibiotic-producing micro-organisms, only synthetic soil with non-antibiotic-producing micro-organisms was used in the subsequent experiments.

The ability of different groups of micro-organisms to induce fungistasis in soil was determined by spore germination on synthetic soils inoculated with bacteria, actinomycetes, fungi or representative species of each group. Ascospores of *N. tetrasperma* germinated completely while conidia of *M. ramannianus* and *P. frequentans* failed to germinate on any of the synthetic soils tested (Table 3). When *B. maydis* was used as the test organism, mixed micro-organisms were more effective than bacteria, actinomycetes and fungi in inducing fungistasis in synthetic soils (Table 3); germination of conidia on synthetic soil with mixed micro-organisms was similar to that on natural soil.

Natural soil and synthetic soil containing mixed micro-organisms were mixed with various amounts of silica sand to dilute the micro-organisms, and germination of conidia of *E. rostratum* was tested on these soils. The percentage germination on both natural and synthetic soils increased with increasing proportion of silica sand (Fig. 1). Similar results were obtained when *B. maydis* was used as the test organism.

The numbers of bacteria, actinomycetes and fungi either decreased or remained more or less constant for 3 d on synthetic soil containing mixed micro-organisms (Table 4), indicating the concurrent existence of bacteriostasis, actinostasis and fungistasis. When synthetic soil was autoclaved and reinoculated with 1% natural soil, the populations of bacteria, actinomycetes and fungi all increased in 3 d, indicating the destruction of microbiostasis by autoclaving.

Lysis of fungal mycelia was tested by placing ascospores of *N. tetrasperma* on a polycarbonate membrane laid on synthetic soil. The fungus germinated within 5 h and lysis of mycelia was noticeable within 36 h (Fig. 2). More than 50% of the mycelia had lysed after 60 h and all mycelia had disappeared after 84 h. The mycelia of *N. tetrasperma* remained intact after 84 h on sterilized synthetic soil.

Nutrient sink in synthetic soil

To study the ability of micro-organisms to create a nutrient sink in soil it is necessary to incorporate known amounts of nutrients into a nutrient-free material to be placed on soil. A highly purified agarose (SeaKem HGT-P agarose), which supported germination of nutrient-independent fungi but not nutrient-dependent fungi (Ho & Ko, 1980), was selected for this study. Agarose (0·8% SeaKem agarose, 100 μg glucose ml^{-1} and 100 μg peptone ml^{-1}) blocks

Table 1. *Percentage germination of fungal spores on polycarbonate membranes placed on each component of synthetic soil*

The data are from one of two experiments with similar results. More than 200 spores were counted for each treatment in each experiment.

Test fungus*	Silica sand	Montmorillonite	Kaolinite	Canadian peat	Water
N. tetrasperma	96	94	96	91	93
B. maydis	98	95	99	96	97
B. stenospila	95	97	97	97	90
E. rostratum	96	98	96	92	89
P. frequentans	1	3	2	30	0
P. funiculosum	0	5	0	10	0

* Ascospores of *N. tetrasperma* and conidia of the other species were used.

Table 2. *Percentage germination of fungal spores on polycarbonate membranes placed on natural soil and synthetic soil*

The data are from one of two experiments with similar results. More than 200 spores were counted for each treatment in each experiment.

	Non-sterilized			Sterilized			
		Synthetic soil†			Synthetic soil†		
Test fungus*	Natural soil	NAP	AP	Natural soil	NAP	AP	Water
N. tetrasperma	93	94	96	97	92	92	93
B. maydis	4	9	5	95	99	98	97
B. stenospila	3	2	3	92	98	96	97
E. rostratum	9	6	9	96	97	95	96
P. frequentans	0	0	0	93	99	99	0
P. funiculosum	0	0	0	85	94	96	0

* Ascospores of *N. tetrasperma* and conidia of the other species were used.

† Synthetic soil was inoculated with five isolates each of non-antibiotic-producing (NAP) or antibiotic-producing (AP) bacteria, actinomycetes and fungi, and incubated at 24 °C for at least 1 month before use.

Table 3. *Percentage germination of fungal spores on polycarbonate membranes placed on synthetic soils containing different non-antibiotic-producing micro-organisms*

Synthetic soils were inoculated with (1) five isolates each of bacteria, actinomycetes and fungi, (2) five isolates of bacteria, (3) five isolates of actinomycetes and (4) five isolates of fungi, and incubated at 25 °C for at least one month before use. The final populations of micro-organisms in 1 g soil were: $6{\cdot}1 \times 10^7$ bacteria, $3{\cdot}6 \times 10^6$ actinomycetes and $1{\cdot}6 \times 10^6$ fungi; (2) $1{\cdot}0 \times 10^8$ bacteria; (3) $8{\cdot}1 \times 10^6$ actinomycetes; and (4) $4{\cdot}5 \times 10^6$ fungi. The data are from one of two experiments with similar results. More than 200 spores were counted for each treatment in each experiment. Conidia of *M. ramannianus* and *P. frequentans* did not germinate in any of the conditions tested.

	Micro-organisms in synthetic soil:					
Test fungus*	Mixed	Bacteria	Actinomycetes	Fungi	Natural soil	Water
N. tetrasperma	97	97	96	98	95	96
B. maydis	4	57	20	34	3	99

* Ascospores of *N. tetrasperma* and conidia of *B. maydis* were used.

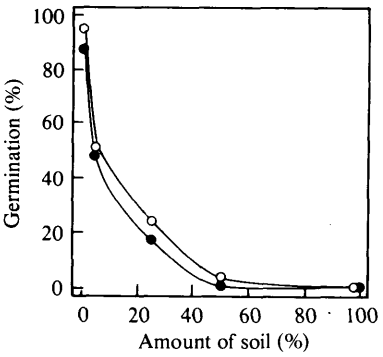

Fig. 1. Spore germination of *E. rostratum* on natural (●) or synthetic soil (○) soil mixed with various amounts of silica sand to dilute the micro-organisms. Synthetic soil contained five isolates each of non-antibiotic-producing bacteria, actinomycetes and fungi.

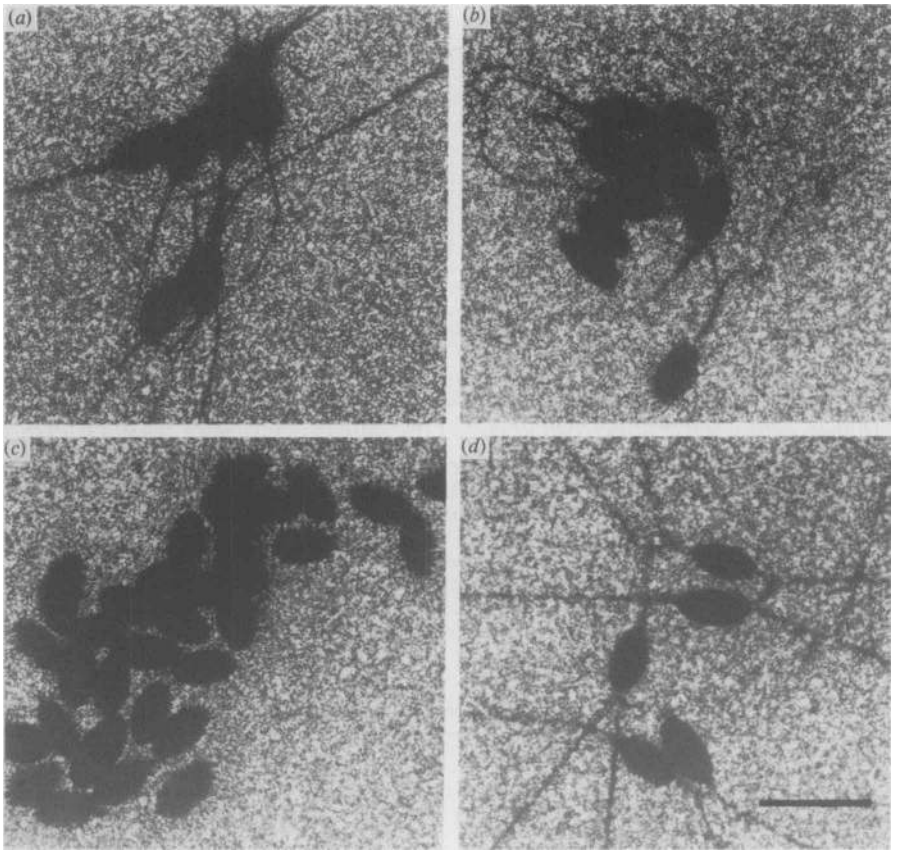

Fig. 2. Lysis of *N. tetrasperma* mycelia on polycarbonate membranes placed on synthetic soil containing non-antibiotic-producing micro-organisms (*a*) for 36 h, (*b*) for 60 h, and (*c*) for 84 h. (*d*) Mycelia incubated on sterilized synthetic soil for 84 h (control). Bar, 2 μm.

Table 4. *Microbiostasis in synthetic soil*

Synthetic soil was inoculated with five isolates each of non-antibiotic-producing bacteria, actinomycetes and fungi, and incubated at 24 °C for at least 3 months before the experiment. Six plates were used for each treatment, and experiments were repeated at least once. The results are given as no. (g dry soil)$^{-1}$; values followed by the same letter for each group of micro-organisms between 0 d and 3 d are not significantly different at $P = 0.05$ based on Student's t-test.

	Non-sterilized		Sterilized	
Type of micro-organism	0 d	3 d	0 d	3 d
Bacteria	$6.9 \times 10^7 a$	$6.9 \times 10^7 a$	$4.1 \times 10^5 a$	$5.5 \times 10^7 b$
Actinomycetes	$4.3 \times 10^6 a$	$2.3 \times 10^6 b$	$6.5 \times 10^4 a$	$1.7 \times 10^5 b$
Fungi	$5.9 \times 10^5 a$	$6.2 \times 10^5 a$	$2.8 \times 10^2 a$	$1.1 \times 10^4 b$

Table 5. *Percentage germination of fungal spores on nutrient agarose blocks preincubated on natural soil or synthetic soil containing non-antibiotic-producing micro-organisms*

Synthetic soil was inoculated with five isolates each of bacteria, actinomycetes and fungi, and incubated at 24 °C for at least 1 month before the experiment. Nutrient agarose blocks (0·8% SeaKem agarose, 100 μg glucose ml^{-1} and 100 μg peptone ml^{-1}) placed on polycarbonate membranes were incubated on surface of natural or synthetic soil at 24 °C for 24 h, then transferred to sterile Petri dishes and tested for spore germination. The data are from one of two experiments with similar results. More than 200 spores were counted in each treatment for each experiment.

Test fungus*	Natural soil	Synthetic soil with micro-organisms added	Synthetic soil with no micro-organisms added
N. tetrasperma	98	99	99
B. maydis	100	100	97
B. stenospila	96	97	97
E. rostratum	91	100	99
M. ramannianus	2	2	99
P. frequentans	1	4	100
P. funiculosum	1	5	100

* Ascospores of *N. tetrasperma* and conidia of the other species were used.

Table 6. *Percentage germination of fungal spores on nutrient agarose blocks preincubated on synthetic soils containing different groups of non-antibiotic-producing micro-organisms*

Synthetic soils were inoculated with (1) five isolates each of bacteria, actinomycetes and fungi, (2) five isolates of bacteria, (3) five isolates of actinomycetes and (4) five isolates of fungi, and incubated at 24 °C for at least 1 month before use. The final populations of micro-organisms in 1 g soil were: (1) 6.1×10^7 bacteria, 3.6×10^6 actinomycetes and 1.6×10^6 fungi; (2) 1.0×10^8 bacteria; (3) 8.1×10^6 actinomycetes; and (4) 4.5×10^6 fungi. Nutrient agarose blocks (0·8% SeaKem agarose, 100 μg glucose ml^{-1} and 100 μg peptone ml^{-1}) placed on polycarbonate membranes were incubated on the surface of synthetic soil at 24 °C for 24 h, then transferred to sterile Petri dishes and tested for spore germination. The data are from one of two experiments with similar results. More than 200 spores were counted in each treatment for each experiment.

	Micro-organisms added to synthetic soil:				
Test fungus*	Mixed	Bacteria	Actinomycetes	Fungi	None
N. tetrasperma	99	99	99	100	99
B. maydis	98	99	99	100	93
B. stenospila	99	100	100	99	99
E. rostratum	98	98	98	99	100
M. ramannianus	3	70	84	13	99
P. frequentans	4	68	58	61	99
P. funiculosum	8	36	17	20	100

* Ascospores of *N. tetrasperma* and conidia of the other species were used.

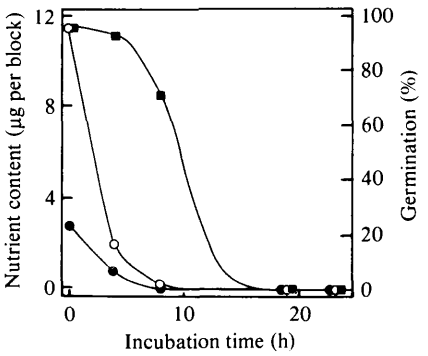

Fig. 3. Relationship between loss of soluble carbohydrates (○) and amino acids (●) from nutrient agarose blocks (0·8% SeaKem agarose, 800 μg glucose ml^{-1} and 250 μg asparagine ml^{-1}) incubated on synthetic soil at 24 °C and their ability to support spore germination of *P. frequentans* (■). The synthetic soil contained five isolates each of non-antibiotic-producing bacteria, actinomycetes and fungi.

(10 × 7·5 × 2 mm) were placed on polycarbonate membranes and incubated on natural soil or synthetic soil at 24 °C for 24 h, then transferred to sterile Petri plates and tested for spore germination as previously described. Both nutrient-independent and nutrient-dependent fungi germinated on nutrient agarose blocks preincubated on synthetic soil without micro-organisms added (Table 5). Preincubation on natural soil or synthetic soil inoculated with mixed micro-organisms rendered the nutrient agarose blocks incapable of supporting germination of nutrient-dependent fungi without reducing their ability to support germination of nutrient-independent fungi.

The same method was used to study the ability of different groups of micro-organisms to create a sink effect in soil. Although all the nutrient-independent fungi germinated completely, germination of nutrient-dependent fungi was reduced to various levels on nutrient agarose blocks preincubated on synthetic soils inoculated with bacteria, actinomycetes and fungi (Table 6). However, individual groups of micro-organisms were not as effective as mixed micro-organisms in causing diffusion of nutrients.

More than 80% of soluble carbohydrates and 70% of amino acids diffused from agarose blocks within 4 h and about 99% of each group of compounds was lost after 17 h (Fig. 3). The germination of *P. frequentans* on agarose blocks also decreased with increasing time of incubation on synthetic soil. No germination occurred on agarose blocks after 17 h incubation.

DISCUSSION

The inability of micro-organisms to multiply in natural soil could be due either to the presence of inhibitors or to the absence of nutrients required for microbial growth. In the study of fungistasis, inhibitors have been detected in certain soils but none has met the minimum criteria for being the cause of soil fungistasis (Ko & Lockwood, 1967; Lockwood, 1984). Ko & Lockwood (1967) proposed that soil fungistasis is a result of nutrient deprivation imposed by microbial activity in soil. Leaching systems were developed to demonstrate that a strong diffusion gradient of nutrients, presumed to be caused by microbial activity in soil, can inhibit germination of nutrient-independent fungi (Ko & Lockwood, 1967; Hsu & Lockwood, 1973). However, the ability of micro-organisms to create a 'nutrient sink' in soil cannot be tested in such systems. This problem was overcome by the development of a synthetic soil which contained micro-organisms from natural soil.

Several lines of evidence suggest the similarity of synthetic soil to natural soil. (i) The germination pattern of fungi on synthetic soil corresponds to that on natural soil. (ii) Agarose blocks become inhibitory to nutrient-dependent fungi but not to nutrient-independent ones after

incubation with either synthetic or natural soil. (iii) Like natural soil, synthetic soil becomes non-fungistatic after sterilization. (iv) Nutrients diffuse to both synthetic and natural soils from agarose blocks placed on these soils. (v) The germination patterns on natural and synthetic soils mixed with various amounts of silica sand are similar. (vi) Like natural soil, synthetic soil also induces lysis of fungal mycelia.

Each component of the synthetic soil was shown to be free from inhibitors and nutrients. The nutrient-independent fungi germinated freely, whereas the nutrient-dependent fungi germinated poorly in each separate component of synthetic soil. Before addition of micro-organisms, 1 g synthetic soil contained 88 bacteria and 71 actinomycetes but no fungi. The original micro-organisms were very few and insignificant in comparison with the numbers of micro-organisms added subsequently. It is very unlikely that the original micro-organisms had become prominent later, because the added micro-organisms maintained a relatively constant population in synthetic soil during the 3 month incubation. Since non-antibiotic-producing micro-organisms were used and agarose blocks preincubated with synthetic soil were inhibitory to nutrient-dependent but not nutrient-independent fungi, the synthetic soil was considered to be free of inhibitors of both abiotic and microbial origin. Conidia of *B. maydis, B. stenospila* and *E. rostratum* germinated completely in distilled water but failed to germinate on the synthetic soil. Agarose blocks incubated on synthetic soil rapidly lost nutrients and the ability to support germination of *P. frequentans* at the same time. These results strongly support the hypothesis that microbial activity of soil creates a strong diffusion gradient of nutrients which in turn causes the inhibition of germination of nutrient-independent fungal spores (Ko & Lockwood, 1967).

Non-antibiotic-producing micro-organisms were as effective as antibiotic-producing micro-organisms in inducing fungistasis in synthetic soil. This suggested that inhibitors of microbial origin do not play a significant role in fungistasis. If nutrient deprivation imposed by microbial activity is the main reason for the failure of nutrient-independent fungi to germinate in soil, germination should occur when the microbial population is decreased. This was exactly what happened when natural soils and synthetic soils were diluted with silica sand which was free of both nutrients and micro-organisms. The percentage germination of *B. maydis* and *E. rostratum* in both soils increased with increasing proportion of silica sand.

The characteristics of soil microbiostasis are similar to those of soil fungistasis, so nutrient deprivation inflicted by microbial activity has also been considered to be the main cause of soil microbiostasis (Ko & Chow, 1977; Ko, 1982; Lynch, 1982; Ko & Ho, 1984; Lockwood, 1984). This hypothesis is supported by the results of the present experiments with synthetic soil. The populations of bacteria, actinomycetes and fungi remained more or less unchanged in synthetic soil consisting of inhibitor-free abiotic components and non-antibiotic-producing micro-organisms. Moreover, microbiostasis disappeared when micro-organisms in synthetic soil were killed by sterilization.

The nutrient deficiency hypothesis permits a simple explanation for many aspects of soil microbiostasis, e.g. (i) its widespread occurrence in most soils tested (Ho & Ko, 1982); (ii) restoration of microbiostasis to sterilized soil by infestation with non-antibiotic-producing micro-organisms (Ho & Ko, 1982); (iii) differential effect of nutrients on bacteriostasis, actinostasis and fungistasis (Ho & Ko, 1982; Ko, 1982); (iv) annulment of microbiostasis by freezing treatment (Ho & Ko, 1985).

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