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AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

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MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose VO

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following

AID/SCI Progress Report No. C5-107
Sept 85 - Dec 86

Attachment

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PD-AAW-282

SCIENTIFIC REPORT

SEPTEMBER 1985 - DECEMBER 1986

U.S. - ISRAEL CDR PROJECT NO. C5-107

BIOLOGICAL CONTROL OF PLANT DISEASES USING MICROORGANISMS GROWN ON AGRICULTURAL FORESTRY WASTES

BY

Professor I. Chet, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel

Dr. A. Ofusu-Asiedu, Forest Products Research Institute, Kumasi, Ghana

SUBMITTED TO: THE UNITED STATES AGENCY FOR INTERNATIONAL DEVELOPMENT (AID)

1987

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S C I E N T I F I C R E P O R T

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Project Title: Biological Control of Plant Diseases Using Microorganisms Grown on Agricultural and Forestry Wastes.

The objectives of the project are as follows:

1. To isolate potential biocontrol agents such as *Trichoderma* spp. or bacteria from local soils and to test their antagonistic ability against plant pathogenic fungi.

To grow the biocontrol agents on various agricultural wastes such as baggase or cassava, wood waste such as sawdust, chips and bark fortified with nitrogen and phosphorus.

To determine the most economic and appropriate methods of application of these antagonists either to soil or on seeds of the plant.

To scale up the system in a semi-solid fermentation and to test the spectrum of effectiveness under field conditions against the most common plant diseases.

STUDIES UNDERTAKEN IN GHANA

Isolation of potential biocontrol agents

Soil samples were sent to Israel for isolation of biocontrol agents. Four strins of *Trichoderma* spp. and one bacterium were isolated from these soils.

Work started in our laboratory for the quantitative estimation of Trichoderma spp. in some cultivated soils in Ghana. So far isolations have been made from soils on which tomato, cowpeas and garden eggs have been cultivated. The Trichoderma selective medium (TSM) is being used for the isolations. Isolates obtained from this study will be screened for antagonistic properties against plant pathogenic fungi.

Incidence of Rhizoctonia solani and Sclerotium rolfsi Diseases in selected crops

A study was initiated to determine the incidence of R. solani and S. rolfsi diseases in tomato, cowpea and garden eggs in some nurseries and fields.

The investigation will give information on the magnitude of the problem and also provide strins of the fungi for the study of antagonism between them Trichoderma spp. and other biocontrol agents.

Antagonism between Trichoderma spp. and some soil borne plant pathogens

Work started on the antogonism between Ghanaian and the Israel isolatesof Trichoderma spp. and a strain of Sclerotium rolfsi isolated from cowpeas. The test is being conducted using tomato as the indicator crop.

STUDIES UNDERTAKEN IN ISRAEL

THE ROLE OF COMPETITION FOR NUTRIENTS IN BIOCONTROL OF *PYTHIUM* DAMPING OFF BY BACTERIA

ABSTRACT

Of the 130 bacteria isolated from the rhizoshpere of *Pythium* infested plants, 6 were found to be efficient biocontrol agents of this pathogen under greenhouse conditions. No lytic enzymes were involved in *in vitro* interactions between the bacteria and *P. aphanidermatum*. Substances inhibiting growth of the fungus were produced by both the biocontrol agents and other rhizobacteria. On the other hand, competition for nutrients between germinating *P. aphanidermatum* oospores and bacteria, which was unique to the

biocontrol agents, significantly correlated with suppression of disease in the greenhouse. Oospore germination in rhizospheres of wheat, tomato, cucumber, melon, bean and cotton plants was decreased in the presence of these bacteria. Broadcast application or seed coating with these bacteria reduced disease incidence in cucumbers planted in peat vermiculite mixture or soil by 60-75%. P. ultimum damping off in cucumbers was reduced by up to 94%. Integrating low amounts of bacteria with the fungicide Previcur resulted in an additive effect. Bacteria applied to cucumber seeds became established along the roots. Populations were 100-fold higher at root tips than at their upper parts. On the other hand, except for the root tips, the total population of bacteria was decreased in the presence of the beneficial ones. Disease control was achieved in bean, pepper, melon, tomato and cotton plants as well.

INTRODUCTION

Rhizobacteria, by their interactions with various pathogens, play a major role in the biological equilibrium among microorganisms in the rhizosphere (23). Pythium spp. have a broad host range and cause pre- and post emergence damping off and root rots followed by considerable yield losses in many important crops.

Biological control, by means of bacteria, of this pathogen is not well developed. Mitchell and Hurvitz (15) protected tomato seedlings against damping off caused by P. debaryanum, with a lytic strain of Arthrobacter. Howell and Stipanovic (9) isolated a strain of Pseudomonas fluorescens, which produces an antibiotic inhibiting growth of P. ultimum on seeds.

Rhizobacteria have been the subject of several reports (11,12,16). It was suggested, that the antibiotics produced by these bacteria displace deleterious microorganisms, presumably minor pathogens (12). In such a case, competition with the native microflora should also be considered. The objectives of the present work were to isolate potential antagonistic rhizobacteria, apply them against Pythium damping off and to investigate their antagonistic mechanism.

MATERIALS AND METHODS

Isolation and characterization of bacteria. About 130 bacteria were isolated from roots of bean, cotton, radish, cucumber and melon plants, grown in Pythium spp. infested soils or peat vermiculite growth media. Isolations were made on nutrient agar (NA) (Becton-Dickinson Corp. Cockeysville, MD 21030) or King's Agar (KB) (10). Bacteria were identified by the API system (La Balme les Grottes 38390, Montalieu Vercieu, France). The following isolates were: Pseudomonas putida (805,310), P. Cepacia (808, 814, Y11) and Alcaligens sp. (Rh2) and Pseudomonas sp. (AA4, SS3 and 806). Cultures were maintained on NA for daily use. Pure cultures were grown on NA for 24 hr, washed from the agar surface with 10% skimmed milk and freeze dried.

Antagonistic properties of selected bacteria. The cell wall degrading enzymes: Exo 1,3-β-D-glucosidase (β-1,3-glucanase, EC 3.2.1.58), Exo-1,4-β-D-glucosidase (Cellulase, EC 3.2.1.74) and b-N-acetyl-D-glucosaminidase (Chitinase, E.C. 3.2.1.30), were tested in filtrates of bacteria, grown on either laminarin (Sigma Chemical Co., St. Louis, MO 63178), chitin (Sigma), cell walls or mycelium of

Pythium aphanidermatum, as a sole carbon source. All enzymatic tests and preparation of fungal cell walls or mycelium were carried out according to previous publications (4,5).

For production of inhibitory substances, bacteria were grown for 40 hr in a potato dextrose and nutrient broth (each 15 g/l) medium (PDNB). Bacterial cells were separated from the medium by centrifugation at 3000 g after which the supernatant was sieved through a 0.45 u millipore. The cell free medium, diluted with fresh, double concentration of PDNB, was inoculated with a mycelium disk taken from a 48 hr old culture of P. aphanidermatum. After 48 hr incubation at 28 C, the mycelium was dried for 4 days at 60 C and weighed.

Mycelium mats removed from P. aphanidermatum cultures, grown for 48 hr in petri plates containing liquid PDNB, were transferred into a fresh medium to check their growth in the presence of bacteria. Dry weight, gained by the fungus during the interaction period, was measured 48 hr later and compared with the average dry weight of the fungus before incubation with the bacteria.

Enrichment of soil with oospores. Roux bottles, each containing 100 ml of carrot medium (18), were inoculated with mycelial disks, taken from the margin of a 48 hr culture of P. aphanidermatum, and incubated for two months at 30 C. Mycelial mats, containing oospores, were placed on four layers of cheesecloth, washed with running distilled water for 5 min to remove the medium, suspended in tap water and homogenized in a Waring blender for 5 min. Oospores, separated on a 40 um Nitex nylon screen (Tetko Inc., Elmsford NY 10513), were resuspended in 100 ml of H_2O and added to 1 kg air-dried,

well-mixed loamy sand soil whose characteristics have been described by Hadar et al., (6). The soil was then placed in plastic bags, incubated for 2 weeks at 30 C, sieved through a 2 mm screen and stored at 4 C until needed. This soil contained an average of 4.5 x 10 colony forming units (cfu) of P. aphanidermatum per gram, as determined on a selective medium according to Schmitthenner (22). Colonies originated predominantly from oospores.

Oospore germination tests in soil. Bacterial isolates, grown on NA plates for 24 hr, were collected, washed twice and resuspended in distilled water to the desired concentration. Oospore-enriched soil samples (5 g each) were placed in a 10 ml test tube. Aliquots (0.1 ml) of glucose and asparagine solutions (to induce germination. 19) and bacterial suspensions were added to give the desired concentrations at 15% (-0.3 bar) water content. Soil was thoroughly mixed and incubated at 30 C for 24 hr. Three 0.5 g subsamples of each treatment were transferred to test tubes for processing, staining with calcofluor New M2R (American Cyanamide Company, Bound Brook, NJ 08805) according to Scher and Baker (20) and viewing at x 160 under a UV light microscope (Zeiss, W. Germany) for fluorescence observation. One hundred oospores were counted for each of the three replicates per treatment.

Oospore germination in the rhizosphere was tested using the method developed by Elad and Baker (3). Oospore enriched soil was mixed with bacteria and placed between two glass slides along with roots of pregerminated plants. Test plants were: Cucumber (Cucumis sativus L cv 'shimshon'), tomato (Lycopersicum esculentum L cv 'Marmand Rehovot'), pepper (Capiscum annum L cv 'Maor'), melon

(Cucumis melon L cv 'Galia'), bean (Phaseolus vulgaris L 'Brittlewax'), wheat (Triticum aestivum L 'scout') and cotton (Gossypium herbaceum L Pima U. SJ2). Root exudates induced germination of oospores.

Rhizosphere soil was separated from the roots after 48 hr of incubation and oospsore germination was assessed according to the procedure mentioned above.

Infestation of greenhouse growth media: P. aphanidermatum or P. ultimum were isolated from diseased cucumber seedlings on a selective medium for Pythium species (22) and identified according to Middleton (14) and Waterhouse (25). Growth medium contained 75% Canadian peat and 25% vermiculite no. 2. P. aphanidermatum oospores were added to peat vermiculite medium (PVM), after adjusting their concentration by diluting the suspension with water to the desired level, using a hemacytometer.

In most experiments with both Pythium spp., infested peat was used as inoculum, after replantings were carried out to give 100% of diseased cucumbers. Inoculum was suspended in 500 ml H_2O to allow fine mix with the growth medium. Average level of Pythium, as counted on Schmitthenner's selective medium (22), ranged from $5 \times 10^3 - 5 \times 10^4$ cfu/g dried infested medium.

Bacteria were applied either as water suspensions mixed with the total growth medium or by dipping seeds into a suspension of the tested isolates, drying at room temperature and planting in the greenhouse.

Polypropylene boxes (7 x 19 x 14 cm) were filled with the infested media; planted with 10 seeds each, irrigated once a day and

the number of diseased seedlings was recorded. All experiments were conducted under greenhouse conditions at 28 - 32 C, consisted of six replicates and were repeated at least twice. Severity of disese symptoms in cotton was calculated according to an index ranging from 0 for healthy symptomless plants to 4 for plants covered with Pythium scars over more than 80% of their hypocotile. Preemergence damping-off was calculated by deducting the rate of emerged plants in a certain treatment from the average emergence in Pythium free medium.

The fungicide prothiocarb (Previcur, S-ethyl N-(3-dimethylaminopropyl) thiocarbonate (50%) (Schering, A.G., Berlin, W. Germany) was mixed with PVM alone or combined with bacteria suspensions to control diseases caused by P. aphanidermatum in cucumbers.

Density counts and bacterial populations. Strains of the biocontrol agents resistant to Rifampicin (3-(4-methylpiperazinyl-minomerhyl) rifamycin 5v) and nalidixic acid (Sigma) were derived from parent cultures by planting cell suspensions on NA containing 150 µg/ml Rifampicin and nalidixic acid and incubating them for 48 hr at 30 C.

Counts of bacteria from soil or peat were carried out by plating the appropriate dilutions, using the drop plate method (17), on agar medium. Results were expressed as colony forming units per g dry soil (cfu/g). Rhizosphere populations were assessed by separating the soil, adhering to the specific root segments, in water, after shaking them in a rotary shaker for 30 min at 100 rpm. Average dry weight of rhizosphere soil was measured in 20 samples of each root segment according to the distance from the root neck. Population dynamics

along cucumber roots were tested by the rhizosphere competence technique of Scher et al (21) as modified by Jaleed and Baker (personal communication). Two longitudinal halves of a 50 ml conical polypropylene tube (Falcon Div. Becton Dickinson and Co., Oxnerd, CA 17030) were filled with wet sandy loam soil (15% - 0.3% bar), and one with bacteria coated seeds was placed between both parts, 1 cm away from the top. The two parts were sealed with 2 rubber bands and incubated in a polyethylene plastic bag for 5 days at 30 C under artificial light without additional watering. At the end of the incubation period, the tube halves were separated and the plant gently removed to assess the rhizosphere population as mentioned above.

RESULTS

Isolation of potentially antagonistic bacteria

Bacteria were isolated from roots of bean, cotton, radish, cucumber and melon plants. Selected plants were uprooted from soils naturally infested with *Pythium aphanidermatum*. Of the 130 bacterial isolates tested for their ability to suppress damping-off in cucumbers, the six which were superior, reducing disease incidence by 17-67% (Fig. 1), were selected for further experiments.

Antagonistic activity of bacteria in culture

Bacteria were tested for the following antagonistic activities: Lytic enzymes - All bacterial isolates produced neither β-1,3-glucanase, chitinase, nor cellululase when grown on laminarin, chitin, cellulose or cell walls of *P. aphanidermatum*. Similarly, when

inoculated into liquid medium containing a mycelium mat of P. aphanidermatum as a sole carbon source, none of the bacteria degraded the mycelium.

Inhibitory substances - Bacteria were separated from the potato dextrose nutrient broth (PDNB) medium, in which they were grown, after 40 hr. The cell free media, mixed with a double concentration of PDNB, inoculated with P. aphanidermatum and incubated for 48 hr for dry weight determination. Dry weight of mycelium grown in non-amended control medium was 84.4 mg per plate. Bacterial Isolates No. 805, 808 Rh2 and 310 inhibited P. aphanidermatum growth by 87.9, 85.4, 49.8 and 39.0% respectively, while isolates AA4, 806 and SS3, which are not biocontrol agents of Pythium, reduced it by 76.5, 89.6 and 70.4%, respectively, as compared with the control (dry weight 84.4 mg/plate), grown in non-amended medium. P. aphanidermatum growth was inhibited by only 5.7% when grown in a medium which previously supported growth of the same fungus.

Competition - Competitive ability of both the bacteria and Pythium mycelium was tested in dual cultures. P. aphanidermatum was grown in liquid PDNB for 48 hr and transferred into fresh medium along with different bacterial isolates. Isolates 805, 808, Y11, Rh2, 310 and 814 inhibited fungal growth by 50,45,60,98, 14 and 62%, respectively, compared with the control (dry weight 63.5 mg/plate), grown in bacteria-free medium. However, other bacteria when grown together in liquid culture also inhibited P. aphanidermatum growth.

Inhibition of oospore germination by bacteria.

Bacteria were introduced into raw soil enriched with oospores of P. aphanidermatum. Oospore germination, determined after 24 h of incubation, was inhibited by up to 57% by the biocontrol agents (2 x 10^8 cfu/g soil) compared with only 13-20% inhibition by bacteria not effective in disease control under greenhouse conditions (Fig. 2). These results reduced by 20-50% by lowering the initial population level of the bacteria to 2 x 10^7 cfu/g soil.

Significant correlation at P = 0.05 or 0.01 was found between the ability of the various bacteria (applied by broadcast application or seed coating) to inhibit oospore germination and reduce Pythium damping-off in cucumbers grown under greenhouse conditions in soil enriched with 150 or 250 ug/g glucose or PVM.

To determine whether the bacteria produce inhibitory substances affecting oospore germination, cell-free supernatant of the different bacteria cultures were added to the oospore enriched soil. No significant reduction in oospore germinability was observed unless bacterial cells were present in the soil.

Germination of oospores in rhizospheres of wheat, tomato, melon, cucumber, bean or cotton were compared with their germination in untreated control (Table 1). Average inhibitions of germination in all plant crops by isolates 805 and 808 were 72.3% and 66.4% whereas isolate AA4 which is not capable of controlling Pythium in the greenhouse inhibited germination by only 3.3%.

Biological control of damping-off in cucumbers

Effect of bacterial concentrations. Bacteria were suspended in water and mixed with PVM to give two high initial population levels: 10^7 and

10^8 cfu/cm^3 Isolates 805 and 808 were the most effective in Pythium control when applied in concentrations of 10^2 cfu/cm^3 (Table 2). Relatively high percentages of healthy cucumber plants up to 62% reduction of disease were obtained when bacteria were applied in concentrations of 10^7 cfu/cm^3. Cells of bacterial isolates 805 and 808 were suspended in three low concentrations to give final counts of 4×10^7, 8×10^6 and 2×10^6 cfu/cm^3 of PVM. As shown in fig. 3 concentration of cells of isolate 808 influenced the efficacy of disease control whereas differences between concentrations of applied isolate 805 were not reflected in marked differences in disease control. Preemergence damping off in untreated control was 71.6% whereas it was reduced to 21.1.-26.3 and 2.1 - 10.5% by treatments with isolates 808 and 805, respectively.

Effect of bacterial seed coating and intergrated control - Cucumber seeds were dipped in bacterial suspensions (8×10^9 cfu/ml) and planted in PVM. Damping off caused by P. aphanidermatum, was recorded during 12 days post sowing. The most efficient isolates - 805, 808 and Rh2, reduced disease incidence by 57-67% compared with 38-42% by isolates 814 and 310 (Fig. 4). Preemergence damping off, 27.5% in the untreated control, was reduced to 0-12.5% by the different bacterial isolates.

Bacterial isolates, at the low rate of 3×10^6 cfu/cm^3 of PVM, were combined with Previcur, at the rate of 0.25 ml per liter PVM. Isolates 805 and 808, alone, decreased Pythium damping-off by 9-15%, due to the low bacterial level in the growth medium (Table 3). A significant decrease in disease incidence, 34%, was obtained by the chemical treatment. However, the combination of Previcur with isolate

808 was signficantly superior, yielding an 80% reduction in disease incidence.

Effect of bacteria on disease build up during successive replanting - Cucumber seeds were planted five times at 10 day intervals in PVM mixed with suspensions of isolates 805 or 808, and the percentages of healthy plants were recorded at the end of each growth cycle. In the untreated control, *Pythium* disease incidence which was 93% at the end of the first cycle, stabilized on 50% between the third and fifth growth cycles (Fig. 5). Disease incidence in bacteria-treated media was 12-45% during the five successive plantings.

Biological control of *P. ultimum* damping-off in cucumbers - Control of *P. ultimum* in cucumbers was tested in PVM amended with each of the bacterial isolates. Disease incidence was drastically reduced by all isolates on the first growth cycle (Fig. 7). However, after two weeks of the second growth cycle there were 7, 18, 51, 42, 4, 71, and 17% healthy plants in the untreated control and treatments with isolates 808, 805, 310, 814, Rh2 and Y11, respectively.

Biological control of *Pythium* damping off in pepper, melon, bean tomato and cotton.

Isolates 805 and 808 were mixed with PVM and their influence on *Pythium* disease in several crops was examined in the greenhouse. Both isolates reduced disease incidence in melons, tomatoes, peppers and beans (Fig. 6). Disease indexes in cotton, calculated according to root coverage by *Pythium* scars were 2.17, 0.83 and 1.0, respectively in the untreated control and in treatments with isolates 805 and 808.

The respective percentages of disease incidence were 92, 50 and 62. Disease incidence was decreased by 19-53% when pepper, bean and tomato plants were replanted in the original growth mixture.

Competitive root colonization by antibiotic resistant biocontrol agents, general populations of bacteria and *Pythium* - Isolates 805 and 808, selected for their tolerance to 150 ug/ml of the antibiotics rifampicin and nalidixic acids, were applied to soil. Both decreased *Pythium* in cucumbers by 50%. Efficient recovery was obtained on medium supplemented with both antibiotics at a rate of 150 µg/ml.

Cucumber seeds were coated with suspensions of isolates 805, 808, 814, Y11 or Rh2. Two longitudinal halves of plastic tubes were filled with *Pythium* infested soil and sealed together with one coated seed in each tube. The roots, exposed after 5 days, were cut into segments 0-1, 2-3, 4-5 and 6-7 cm from the root neck. Serial dilutions were performed for assessment of bacterial population in the rhizosphere. General population, along the untreated roots, graduated between 2.0×10^6 - 2.2×10^5 cfu/g soil in the 0-1 and 6-7 cm segments (Fig. 8). Application of isolates 814, Y11 and Rh2 to the seeds decreased the general bacterial population to 4×10^3 - 2.3×10^5 cfu/g soil in segments 0-1 - 4-5cm, whereas isolates 805 and 808 decreased it to 10 cfu/g soil in segments 2-3 and 4-5 cm. However the general population of the tips of treated roots did not differ from that of untreated ones.

The specific population of bacteria applied to seeds before incubation was 2.2×10^1 - 2.0×10^6 cfu/g of rhizosphere soil (Fig. 8). Except for isolate Y11, bacterial counts at root tips were up to

100 times higher than at the upper root parts. There was a positive correlation between the level of general population and that of the biocontrol agents in all the root segments, except for the tips.

Colonization of cucumber roots by P. aphanidermatum was reduced by 50-100% in the presence of the biocontrol agents.

DISCUSSION

A major objective of the present study was to isolate potential bacteria and characterize their activity in controlling Pythium spp. The ability of six bacteria applied into peat vermiculite mixture or soil, to reduce the incidence of damping-off in the greenhouse was tested. About 60-90% of disease reduction was obtained under growth conditions prevailing in our experiments. Although cucumber was the major test plant throughout this work, disease control was also obtained in other crops, susceptible to P. aphanidermatum, e.g. pepper, melon, bean, tomato and cotton (Fig. 6). Moreover, the bacteria were most effective in controlling P. ultimum (Fig. 7). One application of the biocontrol agents was sufficient to prevent disease build up in replanted cucumbers (Fig. 5). The additive control achieved by combining bacterial application, at a rate too low to achieve disease control, with the fungicide previcur (commonly used against Pythium), reduced disease incidence by 80% (Table 3).

Our results indicate that the presence of bacteria along roots of susceptible hosts reduced the establishment of the pathogen in its potential sites of attack.

Damping-off, induced by Pythium spp., was successfully controlled by coating seeds with a variety of antagonistic microorganisms (7,8,9,13,24). In this work, seed coating was as effective as direct application of bacteria into the soil (Fig. 4). Nevertheless, seed treatment is an attractive method for introducing bacteria to the

soil-plant environment, since in this way, the bacteria have an opportunity of being the first colonizers of roots (Fig. 8). It was evident that bacteria, introduced as seed treatments, moved along the roots. Weller (26) showed the distribution of Pseduomonas fluorescens introduced via seeds on seminal roots of winter wheat . Similarly, we have counted higher populations near the seed or at root tips. Moreover, the lower counts of endogenous bacteria obtained in wheat (26) and cucumber (Fig. 8), in the presence of the introduced strains indicate the obvious competition between them. Intense competition for resources such as nutrients or space between rhizosphere bacteria is very probable. Seed coating places the bacteria where they are needed the most, at the most important site of interaction with the plant rotting Pythium spp. The ability of a bacterial strain to colonize or establish a large population in the rhizosphere is a crucial factor which determines the importance of rhizobacteria as root associates.

Competition for carbon or nitrogen between the efffective bacteria and germinating oospores of P. aphanidermatum may be the mechanism involved in disease control. Significant positive correlation was observed between inhibition of oospore germination and disease reduction, induced by the same bacteria. Moreover, inhibition of germination of oospores in the rhizosphere of several plants was achieved by the biocontrol agents.

Oospore germination has already been shown to be affected by exogenous nutrients (9). Nutrients supplied by root exudates play the same role, as exogenous glucose, on oospore germinability in the rhizosphere. It, therefore, appears that bacteria compete with

germinating oospores for available carbon or nitrogen sources and by eliminating these resources, the bacteria reduce the percent of oospore germination. Similar results were recently obtained with chlamydospores of Fusarium spp. (3). Moreover the reduction in the general poulation of bacteria along the roots, caused by the biocontrol agents, is probably due to competition (Fig. 8).

Although lysis was suggested as a mechanism in the control of Pythium sp. (15) when cell wall lytic bacteria are added to soil, such heterolytic activity was not confirmed in vitro in our studies. Production of inhibitory substances may be a potential means by which the bacteria affect the plant pathogen. Pythium is, indeed, known to be sensitive to such inhibitory effects. However, our in vitro experiments have shown that bacteria, unable to induce suppressiveness in soil, effectively inhibited mycelium of the pathogen.

According to Baker (1), bacteriazation is not a very successful method of biological control in non sterilized soils. One of the reasons for this is the fact that microorganisms present around roots and showing antibiosis in vitro, were not necessarily those effecting biological control in soil. Broadbent et al. (2) added B. subtilis to soil treated by aerated steam. P. ultimum was controlled on Antirrhinum possibly by hindering infection rather than by antibiosis. Hadar et al (7) protected seedlings from Pythium disease by application of Enterobacter cloacae. Howell and Stipanovic (9) concluded that a strain of P. fluorescence was antagonistic to P. ultimum due to the production of an antibiotic - pyoluteorin - by the bacterium.

We have shown here the potential of bacteria to control Pythium. The bacteria applied as a seed coating or mixed with the growth medium

compete with the pathogen along the roots. Further research is needed to develop the formulation of bacterial biocontrol agents and exploit them in agriculture.

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Table 1: Inhibition of oospore germination (%) by bacteria in rhizosphere of various crops*

Bacterium Isolate	CROP					
	Wheat	Tomato	Melon	Cucumber	Bean	Cotton
805	89a[y]	71a	83a	66a	68a	59a
808	52b	70a	74a	67a	71a	67a
AA4	36c	5b	11b	20b	12b	25b
Percent of germination in untreated control	64	37	46	57	65	36

* Seeds of the various crops were germinated in moist bags and placed on soil enriched with oospores, between two glass slides.

Germination was calculated, after 3 days of incubation, by the calcofluor staining method.

y Numbers in each column followed by the same letters are not significantly,different according to Duncan's multiple range test (P=0.05).

All percentages of germination in treatments of isolates 805 or 808 differed significantly from the non-amended control.

Table 2: Influence of bacterial concentration, in peat vermiculite medium, on their ability to control P. aphanidermatum in cucumber

Bacterium isolate	Emergence (%) 7 days after planting		Healthy plants (%) 11 days after planting	
	10^7 cfu/cm^3	10^8 cfu/cm^3	10^7 cfu/cm^3	10^7 cfu/cm^3
Untreated control	25		16	
Rh2	18	42	12	27
805	60	83	48	70
Y11	37	48	18	37
808	77	72	60	68
814	37	51	10	32
310	42	53	19	41

Suspensions of 24 hr old bacteria were mixed with artificially infested peat vermiculite container medium to give 10^7 or 10^8 cfu/cm^3.

Table 3: Effect of low population of bacteria combined with previcur on percentage of damping-off of cucumbers, induced by *P. aphanidermatum*.

Previcur treatment*	Bacterial isolate		
	None	805	808
-	89a[y]	81a	76a
+	59b	48b	18c

* Previcur was applied at a dose of 0.25 ml/l PVM.

Bacteria were applied at a rate of 3×10^6 cfu/cm^3 PVM.

y Numbers followed by a common letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

LEGENDS TO FIGURES

Fig. 1. The influence of bacterial suspensions on the percentage of healthy cucumber plants planted in P. aphanidermatum-infested PVM. Treatments were: control (o) and bacterial isolates 805 (▲), 808 (△), 310 (▽), Rh2 (●), Y11 (■) and 814 (□).

Fig. 2. The effect of suspensions of bacterial biocontrol agents 805 (▲), 808 (△), 814 (□), Rh2 (●) and Y11 (■), and other isolates: AA4 (△) and SS3 (▲) on the percentage of reduction of oospore germination in soil amended with glucose and asparagine (5:1).

Reduction was calculated according to the following formula: $(1-\frac{A}{B})\ 100$ where B = percent of germination in the control without bacteria and A = percent of germination in bacterial treatments.

Fig. 3. The effect of isolates 805 (A) and 808 (B), applied at concentrations of -.- 2×10^6, -.- " 8×10^6 and -- 4 x 10 cfu/cm on percentage of healthy cucumber plants planted in PVM infested with P. aphanidermatum.

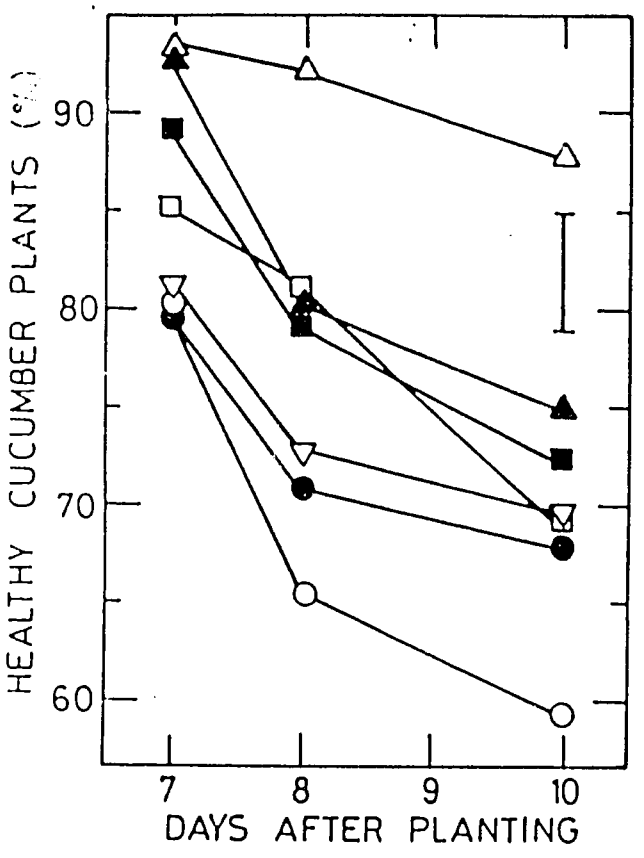
Fig. 4. Influence of seed coating with bacterial isolates 808 (△) 805 (▲), Rh2 (●), Y11 (■), 310 (▽) and 814 (□) on the percentage of healthy cucumber seedlings planted in P. aphanidermatum-infested PVM.

Fig. 5. Stand of cucumber plants, 14 days following each of 5 successive replants in PVM infested with P. aphanidermatum and treated once with isolates 805 (▲), 808 (Δ) or non-treated (O).

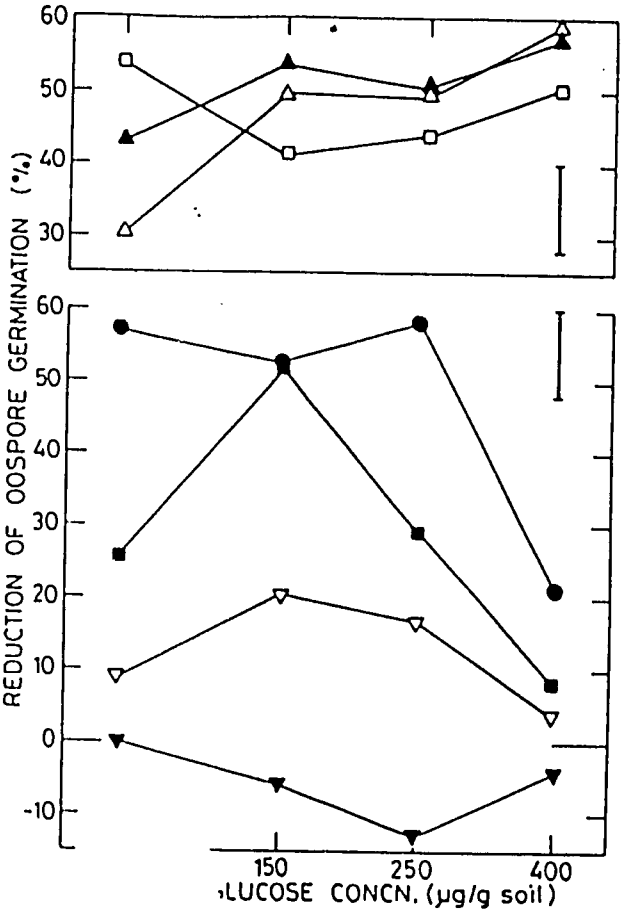
Fig. 6. Percent of healthy plants in PVM infested with P. aphanidermatum and treated with isolates 805 (▲), 808 (Δ) or not treated (O).

Fig. 7. Stand of cucumber plants in P. ultimum infested PVM treated with isolates 805 (▲), 808 (Δ), Rh2 (●) Y11 (■), 310 (▽), 814 (□) or non treated (O).

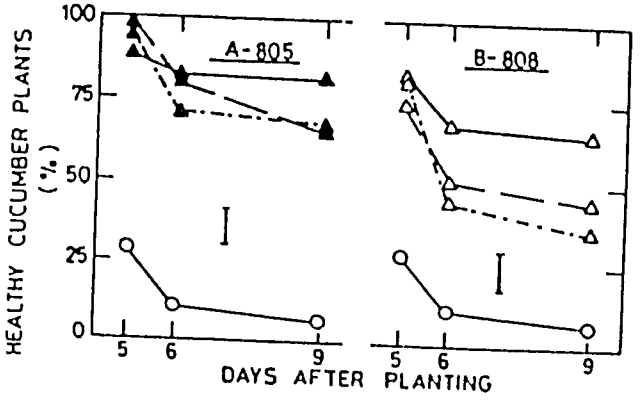
Fig. 8. Densities of general bacteria (A) and biocontrol agents (B) in the rhizosphere of 6 day old cucumber plants. Isolate 805 (▲), 808 (Δ), Rh2 (●), Y11 (■) and 814 (□) were applied to seeds planted between two longitudinal halves of plastic tubes filled with soil . (O - control).


HEALTHY CUCUMBER PLANTS (%)
90
80
70
60
7
8
9
10
DAYS AFTER PLANTING

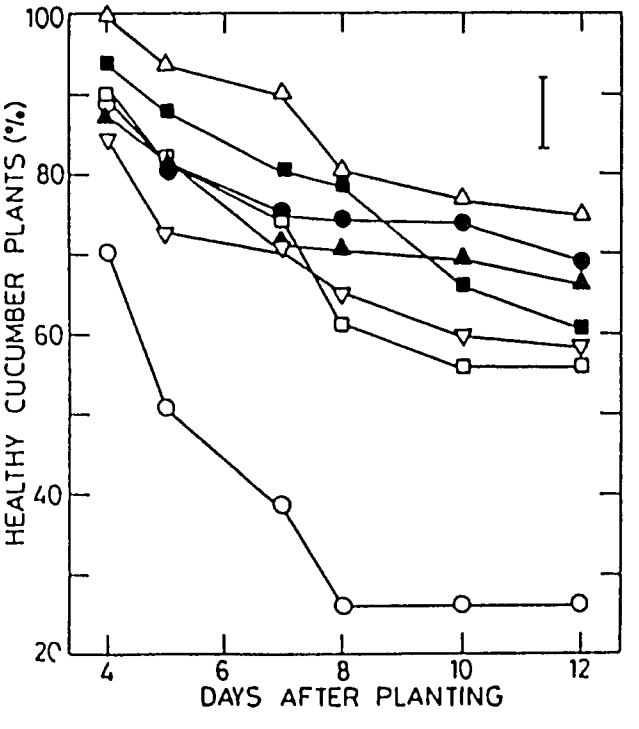
2


REDUCTION OF OOSPORE GERMINATION (%)
60
50
40
30
60
50
40
30
20
10
0
-10
150
250
400
GLUCOSE CONCN. (µg/g soil)

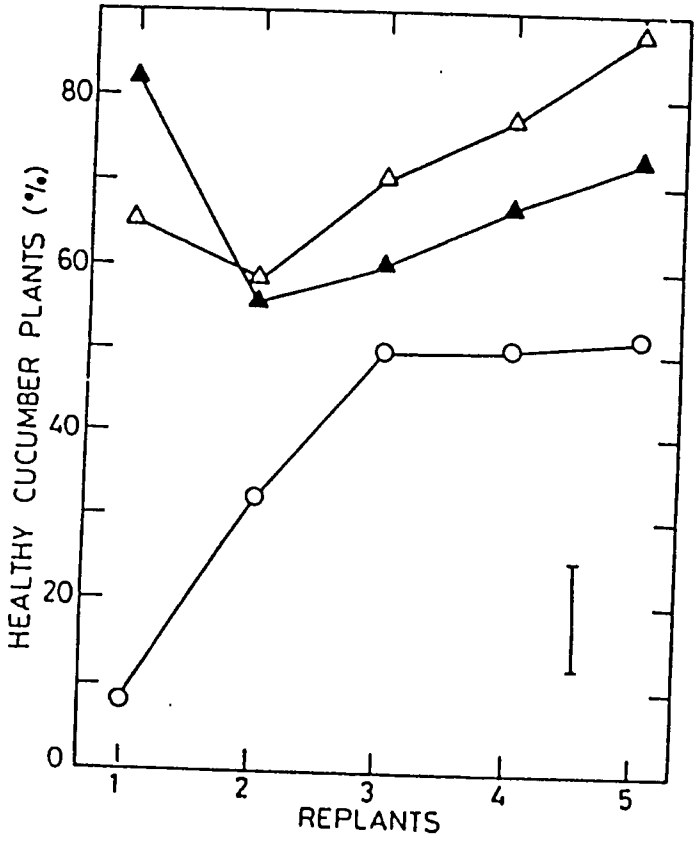
3


HEALTHY CUCUMBER PLANTS (%)
100
75
50
25
0
A-805
B-808
5
6
9
5
6
9
DAYS AFTER PLANTING

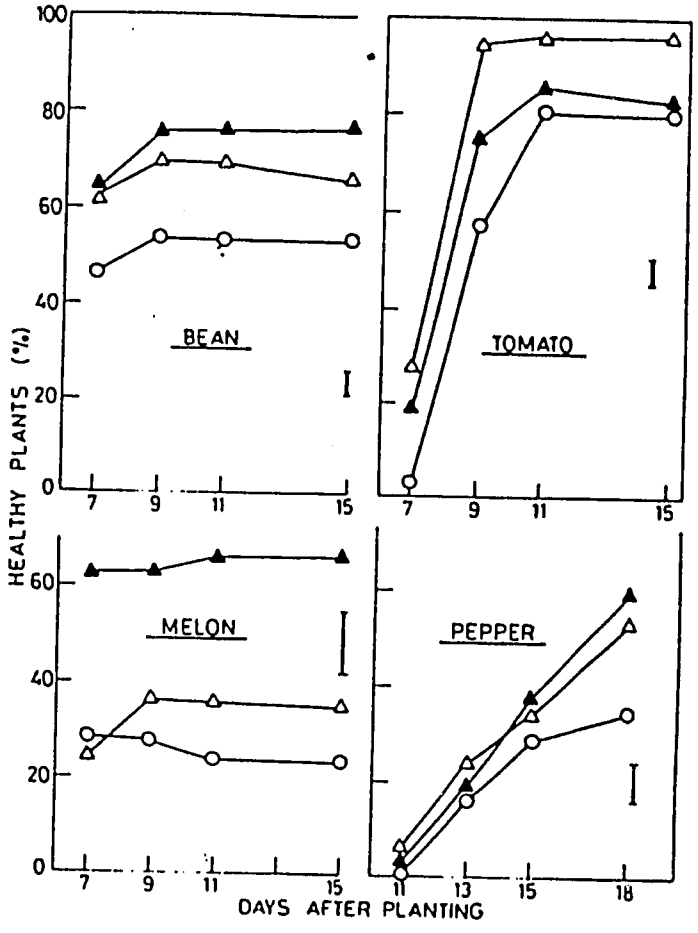
4


HEALTHY CUCUMBER PLANTS (%)
100
80
60
40
20
4
6
8
10
12
DAYS AFTER PLANTING

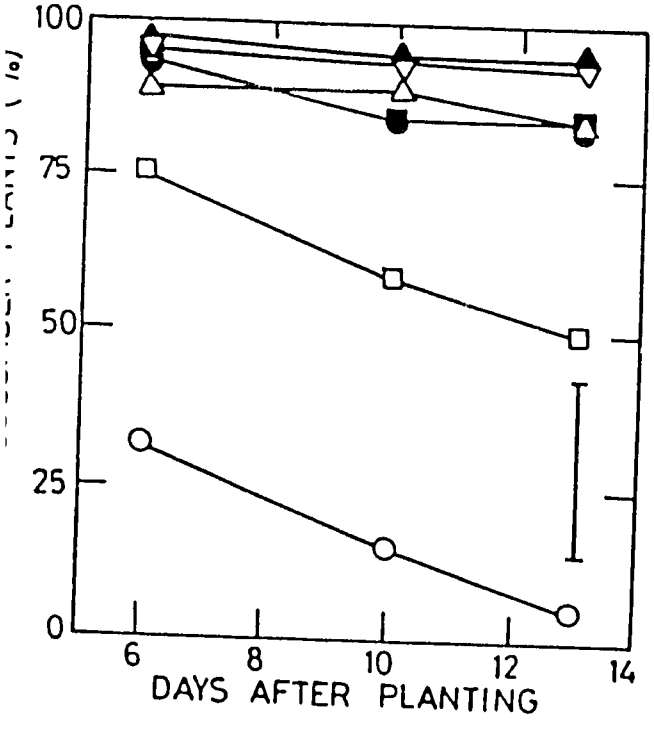
5


HEALTHY CUCUMBER PLANTS (%)
REPLANTS

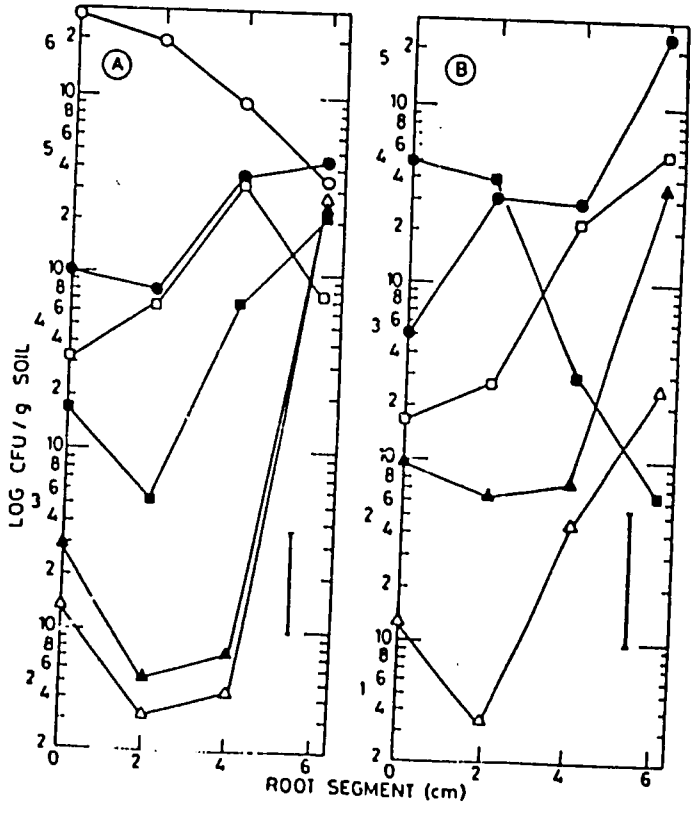
6


HEALTHY PLANTS (%)
BEAN
TOMATO
MELON
PEPPER
DAYS AFTER PLANTING

7


DAYS AFTER PLANTING

8


A
B
LOG CFU/g SOIL
ROOT SEGMENT (cm)

Visits

Prof. Ilan Chet paid a visit to Ghana from 4th to 10th April 1986.

During the period he gave a seminar to final year B.Sc. students in Biological Sciences, Agriculture, Forestry and Biochemistry on Opportunities in Agricultural Biotechnology A case study of Biocontrol using Trichoderma spp.

He held discussions with the Ghanaian team on programing, control, financing and procurement of equipment and supplies for the joint project.

He also gave a final seminar to the staff of the Forest Products Research Institute, the Institute for Renewable Natural Resources and Faculty of Agriculture on financing of research in Israel.

Dr. Ofosu-Asiedu paid a reciprocal visit to the Israel laboratory from November 14th to December 3rd 1986.

He discussed with Prof. Chet's team procedures and methodologies for conducting experiments in biological control. He was taken through methods of screening antagonistic micro-organisms, isolation of antagonists and preparation of bulk inoculum of Trichoderma spp. for laboratory and field experiments in biocontrol of plant pathogenic fungi.

Dr. Ofosu-Asiedu gave a seminar on "Nutrient Changes in Soils planted to Pure Culture inoculated Pines" to graduate students and staff of the Plant Pathology and Microbiology Department of the Faculty of Agriculture.

Prof. Chet and Dr. Ofosu-Asiedu held discussions again on the budget and procurement of equipment and supplies for the project.

He was shown laboratory experiments in progress and visited many places of interest in Israel.