GREENHOUSE SCREENING OF RHIZOSPHERE BACTERIA FOR PLANT GROWTH PROMOTING RHIZOBACTERIA AND DELETERIOUS RHIZOBACTERIA OF MAIZE

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MISIR BİTKİSİNDE BİTKİ GELİŞMESİNİ TEŞVİK EDEN VE ENGELLEYEN BAKTERİLERİN SERADA RİZOSFER BAKTERİLERİ KULLANILARAK ORTAYA ÇIKARILMALARI

ÖZET: Mısır rizosferinden elde edilen 576 bakteri izolatı bitki büyümesini teşvik edici ve zararlı etkileri yönünden serada iki patojenin de bulunduğu topraklarda test edilmiştir. Seçilen izolatlar serada tekrar test edilmiştir. Çoğunlukla bakteri izolatları mısır bitkisinin boyu üzerinde istatistiki bakımdan önemli bir etki göstermemişlerdir. Bitki boyu ile ilgili izolat etkileri tutarsızlık göstermiştir. Tohumların bakteriyle inokulasyonu sonucu mısır bitki boyunda görülen yararlı veya zararlı etkiler başka topraklarda veya denemelerde genellikle tekrar edilememiştir.

SUMMARY: *A total of 576 bacterial isolates from the maize rhizosphere were tested in greenhouse experiments for growth promoting and deleterious activity towards maize seedlings in the presence of two pathogens. Selected isolates were further tested in the greenhouse. In most instances, the bacterial isolates had no statistically significant effect on maize plant height. Isolate effects on plant height were inconsistent. Beneficial or deleterious effects on maize plant height due to bacterial inoculation of the seed often were not repeatable in other soils or experiments.*

INTRODUCTION

One of the most important areas for plant microbe interactions is the rhizosphere. Recently, some research has been concentrated on bacteria-plant root interactions (SUSLOW et al., 1979; SCHROTH and HANCOCK, 1982). It is found that about 2 to 5 % of the bacterial strains isolated from root systems enhanced plant growth; 8-15 % of the isolates were deleterious to plant growth (SCHROTH and HANCOCK, 1981). Bacteria that promoted plant growth were mainly in the *Pseudomonas fluorescens* and *P. putida* group and they were termed plant-growth promoting rhizobacteria (PGPR) (SUSLOW et al., 1979; SCHROTH and HANCOCK, 1981; SCHROTH and HANCOCK, 1982). Some bacterial isolates reduced plant growth and they were termed deleterious rhizobacteria (DR) (SUSLOW and SCHROTH, 1982).

In this study a broad spectrum of rhizosphere bacteria isolated from maize roots were assayed for plant growth promoting and deleterious effects on maize (*Zea mays* L.) plants.

MATERIALS AND METHODS

Experiment 1

Isolation of bacteria from rhizosphere/rhizoplane

Twenty maize plants at silking stage and different locations on the Hinds Research farm of Iowa State University were sampled. Plants were dug, shaken vigorously to remove excess soil from the roots, and healthy root samples were put into 250 ml flasks containing 50 ml Ringer solution (WOLLUM, 1982). In the laboratory they were agitated 15 min at 100 rpm with a rotary shaker. Serial dilutions were made and samples plated using full strength and 0.2

strength Trypticase Soy Agar (TSA). Plates were incubated 2 days and 1000 colonies representing a wide spectrum of bacteria, based on colony morphology and pigmentation, were selected and transferred to TSA slants. They were tested for fluorescent pigment production on King's B medium (KING et al., 1954) and for *in vitro* antibiosis against maize root fungal pathogens *Helminthosporium pedicellatum* and *Fusarium graminearum*.

Detection of in vitro antibiosis against *H. pedicellatum* and *F. graminearum*.

The test fungus was seeded in the center of a petri dish containing King's B agar (KBA) and 4 different isolates of bacteria were placed equidistantly apart at the edge of each plate. In a second test, a test bacterial isolate was streaked in the middle of a KBA plate and the 2 test fungi were seeded at opposite edges of the dish, perpendicular to the bacterial streak. Inhibition zones greater than 2 mm were considered positive.

From the 1000 bacterial isolates, 576 were randomly selected for testing in the greenhouse for their effect on maize growth. Soil, collected from the Hinds farm (pH: 7.5), was infested with *H. pedicellatum* and *F. graminearum* for the tests.

Preparation of the fungal inoculum

Ten g of wheat bran and 20 ml tap water were placed in 60, 250 ml flasks. They were autoclaved for 1 h at 121°C on 3 successive days. The fungi were seeded into separate flasks and allowed to grow 30 days with occasional shaking. The cultures were mixed in a sterile V-blender. Soil was infested with both of these fungi, using a cement mixer, at a ratio of about 3500: 1 (w:w) for each isolate.

Experimental procedure

The 576 selected isolates were tested in seven sets with 131, 151, 74, 50, 48, 38 and 82 isolates tested in sets 1-7, respectively. Ringer solution was added to each tube and the tube mixed on a vortex mixer for 1 min or until a cloudy suspension was obtained. One ml of inoculum was placed on each maize seed by using a semiautomatic pipette. Control treatment in each set was Ringer solution. Each 11 cm square pot contained 3 seeds of Mo17 Tcms maize seeds treated with the same isolate. Three replicate pots were used. After inoculating, samples of inoculum suspensions were serial diluted and plated for approximate viable bacterial cell determinations. These ranged from 8.7×10^8 to 1.2×10^{10} cfu/ml ($\sim 10^9$ cfu/ml).

Experiment 2

The bacterial isolate treatments for each set in experiment 1 were ranked to select the 10 % "best" and 10 % "poorest" treatments in terms of maize plant height for each set. From about 57 "best" isolates, 25 were selected for further study; from about 57 "poorest" isolates, 24 isolates were also selected. The criteria for selection included relative ranking, viability and diversity of colony morphology. Two isolates H12 and 33, from previous experiments (KARAKAYA and MARTINSON, 1995) were included in each set.

These isolates were tested in the greenhouse as 2 tests to determine their effect on plant growth. Set 1 contained 23 isolates and set 2 contained 30 isolates. The isolates were seeded into 125 ml flasks containing 50 ml Trypticase Soy broth and cultured 3 days on a rotary shaker. The cells were concentrated by centrifugation and washed with Ringer solution. The concentration of cells was adjusted to a specific turbidity at 540 nm in Ringer solution using the Spectronic 20. Top soil (pH: 7.9) was used from the Veterinary Medicine farm and it had been cropped to alfalfa for an extended time. Inoculation, planting procedures, and seed were the same as in experiment 1. Ten replications were employed in a Randomized Complete Block Design. Four weeks later plant height data were taken.

Experimet 3

Experiment 3 employed the same conditions and procedures as in experiment 2,

Table 1. Calculated F values and P values for isolate effects from the analysis of variance. Experiment 1

Sets	Isolate values		
	F	df[a]	P
1	0.83	131,262	0.89
2	1.45	151,302	0.01****
3	1.20	74,148	0.18*
4	1.09	50,100	0.35
5	1.24	48,96	0.19*
6	1.00	38,76	0.49
7	1.06	82,64	0.37

[a] Degrees of freedom for isolates and error respectively
**** Denotes significance at 0.01 level
* Denotes significance at 0.20 level.

except Hids farm field soil was used and several of the bacterial isolates were omitted.

Statistical analyses of all experiments were done with Statistical Analysis System (SAS) at the Iowa State University Computation Center.

RESULTS AND DISCUSSION

Experiment 1 consisted of seven sets that included 576 isolates tested for an increase or inhibition of maize plant growth. Because of high positive correlations between height and other positive yield variables in previous experiments, (KARAKAYA, 1987) and the large number of isolates tested, only height data were taken. Bacterial isolate effects on plant height were statistically significant in set 2 ($P<0.01$)(Table 1). Sets 3 and 5 also showed significant isolate effects at the 0.20 level.

The frequency distributions of the mean plant height measurements approximated a normal curve for each set; however, there were some outlying values. In sets 3, 4, 5, 6 and 7, plant heights associated with some of the bacterial isolates tailed on either end of the normal distribution curve. Although isolate effects on plant height were usually insignificant those isolates on the upper and lower ends of the distribution curve warranted further study. The heights of the control plants were usually near the mean height of all of the treatments.

All of the bacterial isolates tested in Experiment 1 were also subjected to an *in vitro* antibiosis assay, using *Fusarium graminearum* and *Helminthosporium pedicellatum* as the test organisms. Twenty-eight isolates showed antibiosis to *F. graminearum*, 51 to *H. pedicellatum*, and nine to both organisms. Of those 28 isolates that showed antibiosis to *F. graminearum*, nine were among the best 20 % of the isolate treatments for plant height, 17 were among the best 50 % of the isolate treatments for plant height, and four were among the poorest treatments for plant height. The isolates that showed antibiosis to *H. pedicellatum* were randomly ranked among the isolates based on plant height. Of the nine isolates that exhibited antibiosis to both pathogens, four isolates were among the best 20 % for plant height responses. Although this may be a spurious association, it appears that good antibiosis may have resulted in PGPR activity.

Some of the bacterial isolates may have been PGPR or DR but the large screening experiment was not effective for identification of those with plant growth promoting or deleterious activities. However,

if any were PGPR, they would likely appear among the 10 % of the bacterial treatments associated with tallest maize plants. Similarly, the 10 % of the treatments with the shortest maize plants would likely contain some strains that could be identified as DR. The 10 % of the isolates associated with the tallest treatments and the 10 % associated with the shortest treatments were identified for each set. Those selected isolates showing *in vitro* antibiosis towards *F. graminearum* and/or *H. pedicellatum* were noted also; from the 88 isolates showing antibiosis, eight of the isolates were associated with the 10 % tallest maize treatments, five isolates showing antibiosis were associated with the 10 % shortest maize treatments.

Experiment 2. Significant ($P<0.01$) isolate effect on maize plant height were detected in both sets 1 and 2. Differences in maize plant height due to bacterial treatments were not large, yet differences among the treatments were significant. None of the isolates was plant growth promoting in this experiment, but some were deleterious.

Plant height responses due to bacterial treatment in this experiment did not agree with the plant height data in experiment 1. Twelve of the 21 isolates, which significantly decreased plant height and might be classified as DR, came from the best group based on increased plant growth responses in experiment 1. Isolate H12, which appeared to be a PGPR in a previous experiment (KARAKAYA and MARTINSON, 1995) was ineffective in this experiment. Those isolates that possibly reduced maize growth in experiment 1 did not necessarily reduce plant growth in this experiment. The isolates that decreased plant growth in both experiments should be tested again for DR activity.

Experiment 3. Experiment 3 was essentially a repeat of experiment 2, except the soil in experiment 3 was gathered freshly from Hinds farm in the early spring.

The isolate effects on plant height had P values of 0.21 and 0.13 in sets 1 and 2, respectively.

A comparison of the ranking of isolates between experiments 2 and 3 revealed little consistency. The order of ranking in experiment 3 seemed to have little relationship to the ranking in experiment 2. Correlation coefficients of the plant responses for the isolates between the two experiments was $r = -0.15$ for set 1 and $r = 0.14$ for set 2.

In most instances, the bacterial isolates had no statistically significant effect on maize plant height. Isolate effects on plant height due to bacterial inoculation of the seed often were not repeatable in other soils or experiments.

From these results it can be concluded that maize plant growth was not affected most of the time by bacteria inoculated on the seed. There were few instances where the isolate gave a statistically significant effect and the results were not consistent. Yield increase following seed bacterization was found to be low in maize (BROWN, 1974). Horticultural crops grown in organic matter rich soil seem to respond well to bacterial inoculation (MISHUSTIN and NAUMOVA, 1962; SCHROTH and HANCOCK, 1982). Soil type and cropping history of the soils might affect the plant response to inoculations (SUSLOW et al., 1979; BACKMAN et al, 1984). SUSLOW and SCHROTH (1982) found different plant responses to bacterial inoculation in different geographical regions.

Because of the low R:S ratio, maize may not be a strong supporter of rhizosphere microorganisms (WOLDENDORP, 1978). Maize plants that supposedly support a poor rhizosphere population may be inferior test plants to demonstrate PGPR activity or DR activity.

The Hinds farm soil that had been cropped to maize for 16 years may have been too severe of a test for the introduced bacterial strains. The two experiments that showed good isolate effects (KARAKAYA and MARTINSON, 1995 and experiment 2 in this experimental series) were the only two conducted in non-Hinds farm soils.

Past cropping history of the soils may be an important factor in the successful elucidation of plant growth promoting rhizobacteria and deleterious rhizobacteria.

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