THE EFFECT OF SOME RHIZOSPHERE BACTERIA ON DEVELOPMENT OF MAIZE

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BAZI RİZOSFER BAKTERİLERİNİN MISIRIN GELİŞİMİNE ETKİSİ

ÖZET: Onbeş bakteri izolatı mısır bitkisi büyümesini geliştirici ve zararlı etkileri yönünden Iowa Eyalet Üniversitesi'nin Hinds ve Curtiss araştırma çiftliklerinde test edildi. Mısır bitkisinin çıkış hızı, boyu ve püsküllleri üzerindeki izolat etkileri Curtiss çiftliğinde görülebilmesine karşılık Hinds çiftliğinde genellikle görülemedi. Tarlada eskiden yetiştirilen ürünler, bitki büyümesini geliştirici ve zararlı bakterilerin ortaya çıkarılmasında önemli olabilir.

SUMMARY: *Fifteen bacterial isolates were tested for their plant growth promoting and deleterious effects towards maize at both the Hinds and Curtiss Research farms of Iowa State University. Isolate effects on maize plant speed of emergence, height and silking were detectable at the Curtiss farm, but were generally lacking at the Hinds farm. Past cropping history of the soils may be an important factor in the successful elucidation of plant growth promoting rhizobacteria and deleterious rhizobacteria.*

INTRODUCTION

Plants are associated with microorganisms at all times. Microorganisms are an important component of the ecosystem in which the plant grows.

Inoculation of the seed with certain bacterial strains has enhanced plant growth. Early work involved the use of *Azotobacter chroococcum* and *Bacillus megaterium* and these bacterial strains were called bacterial fertilizers (Mishustin and Naumova, 1962). Strains of *Pseudomonas, Clostridium, Bacillus,* and *Beijerinkia* species were also used to enhance plant growth (Brown, 1974).

It was found that selected strains of bacteria applied to seed consistently increased plant growth and crop yields in certain environments. These bacteria were termed plant growth promoting rhizobacteria (PGPR) (Schroth and Hancock, 1981; Schroth and Hancock, 1982; Suslow et al., 1979). Most of the PGPR belong to *Pseudomonas fluorescens* and *P. putida. Bacillus subtilis* (Brosten, 1987) and *Azospirillum* sp. were also reported to enhance plant growth (Okon, 1982). The effect of PGPR appears to be related to the displacement of the harmful microflora of roots (Schroth and Hancock, 1982).

Some bacterial isolates reduced plant growth and they were termed deleterious rhizobacteria (DR). DR included species of *Klebsiella, Citrobacter, Flavobacterium, Achromobacter, Arthrobacter,* and *Pseudomonas* (Suslow and Schroth, 1982b).

In this study fifteen bacterial isolates selected from initial greenhouse experiments were tested for their plant growth promoting or deleterious effects towards maize (*Zea mays* L.) at the Hinds and Curtiss research farms of Iowa State University.

MATERIALS AND METHODS

Bacterial isolates used in these experiments were obtained from an initial greenhouse screening of 83 isolates. The origin of these isolates was maize rhizosphere.

Preparation of the inoculum. For the Hinds farm experiment, isolates were grown on petri dishes containing Trypticase Soy agar (TSA). After 3-days growth, the bacteria were scraped from the agar surface, put in cold Ringer solution

(Wollum, 1982) and adjusted to a specific turbidity using the Spectronic 20.

For the Curtiss farm experiment, isolates were grown in shake culture for 3 days in 250- ml flasks containing 50 ml Trypticase Soy Broth. The bacterial cells were harvested by centrifugation, washed with cold Ringer solution, and adjusted to a specific turbidity using the Spectronic 20.

The inoculum was transported to the field in plastic bottles in an ice chest. Immediately after planting, the residual inoculum was serially diluted in sterile water and plated on TSA to determine the viable population density of the bacterial isolates.

Field plot design and procedure. A randomized complete block design was employed with five replications. A plot consisted of 15 seeds in a 3 meter row. Plant spacing between rows was 76 cm, and was 20 cm within rows.

The Hinds farm field (Spilville loam soil) had been cropped to maize for 16 consecutive years. The Curtis farm field (Clarion loam soil) had been in a maize - soybean rotation for more than 20 years. Fields were moldboard plowed the previous autumn and no P and K was applied because of excessive applications the previous years. Nitrogen as NH_3 was applied (225 kg N/hectare) previous to spring tillage and at least 30 days previous to planting. Atrazine, metolachlor, and fonofos were pre-plant incorporated at the Hinds farm and cyanazine and alachlor were pre-plant incorporated at the Curtiss farm.

Planting was done with the aid of a 9- cm wide lumber template 3- meter long, with 2.5 cm holes spaced at 20 cm intervals. The template was placed over the row and 4 cm deep holes were made into the soil beneath the template with a 1.5 cm diameter steel rod pushed through the template holes. After removal of the template, one seed of the maize hybrid A632Ht x H99Ht was placed in each hole. Two ml of bacterial inoculum (Ringer solution for control) was squirted over the seed with a repetitive syringe. The seed was covered immediately and the soil firmed over the seed. Planting at the Hinds farm was done on May 23, 1986, and at the Curtiss farm on June 2, 1986.

Weeds were removed by hoeing as needed. No cultivation was employed.

When about 50 % of the seedlings were emerged, the percentage emergence was determined (7 days after planting). Plant heights were measured 3 and 5 weeks after planting. In addition, percentage of maize plants silked 60 days after planting were determined.

RESULTS AND DISCUSSION

At the Hinds farm the effect of isolates on the speed of emergence was significant ($P \leq 0.01$) (Table 1).

Table 1. Calculated F statistics from analysis of variance for isolate effects

Location	Variables			
	Emergence	1st height	2nd height	% silking[d]
Hinds[e]	2.76****	0.92	0.62	0.52
Curtiss[e]	1.87***	2.45****	1.65**	1.56*
Overall[f]	1.34*	1.78***	1.11	1.12

[a] Coleoptiles emerged 1 week after planting; [b] Height 3 weeks after planting

[c] Height 5 weeks after planting; [d] silking 60 days after planting

[e] Degrees of freedom are 15, 60; [f] Degrees of freedom are 15, 135

**** Significant at $P \leq 0.01$; ** Significant at $P \leq 0.10$

*** Significant at $P \leq 0.05$; * Significant at $P \leq 0.20$

The isolates had no significant effect on height or silking. At the Curtiss farm, significant effects of the bacterial isolate inoculations were observed for speed of emergence, plant height 3 weeks after planting, plant height 5 weeks after planting and percentage of plants silked 60 days after planting (Table 1). With a combined analysis of variance only speed of emergence (seeds with coleoptiles emerged 7 days after planting ($P \leq 0.20$) and the first height measurement ($P \leq 0.05$) showed significant isolate effects (Table 1). It was difficult to find any consistency among the isolates for the plant traits measured at the two fields; however, plants treated with isolate H12 were always the top responders. It was the only isolate that could be a PGPR. Isolate H12 was identified as a *Bacillus* sp. based on colony morphology, gram stain, and microscopic observations. It could be *Bacillus mycoides* (John G. Holt, Department of Microbiology, Iowa State University, personal communication). However, some biochemical tests are needed to confirm this.

Plants from seeds treated with isolates 33, 36, 53 and 75 were inhibited in growth somewhat as these isolate treatments were usually among the lowest values, often significantly lower than the control treatment. The isolate effect was more obvious at Curtiss farm (Table 1). Each farm had different soil types and different cropping histories. In this experiment, some bacterial isolates were applied at slightly higher concentrations at the Curtiss farm than at the Hinds farm. The viable number of cells of bacterial isolates used at Hinds farm ranged from 1.12×10^8 cfu/ml to 6.07×10^9 cfu/ml; the bacteria concentrations used at the Curtiss farm were between 2.00×10^8 cfu/ml and 2.04×10^{10} cfu/ml. The concentration of the inoculum reported to be important (Suslow et al., 1979; Bashan, 1986). The isolate effect at Curtiss farm might be explained partly by the use of higher inoculum; however, other factors like weather, soil type, planting date, and cropping history could have affected the results. Suslow et al., (1979) pointed out that soil type was important for the effectiveness of bacterial inoculants. Backman et al., (1984) reported that responses to inoculations of peanut with *Bacillus subtilis* differed with cropping history of the soils and planting time. The Curtiss farm experiment was started 10 days after the Hinds farm experiment and this could have affected the results.

In a review of the bacterization studies Brown (1974) reported that the claims of the yield increase with seed bacterization were the lowest with maize. The effect of bacterial fertilizers was greatest with horticultural crops that were grown in soils rich in organic matter (Mishustin and Naumova, 1962; Brown, 1974). Other factors like soil type and inoculation time might greatly affect the results. For example, Suslow and Schroth (1982a) found different plant responses to bacterial inoculation in different geographical regions. Microbial interactions in the rhizosphere can greatly affect the growth and survival of introduced bacteria.

The Rhizosphere: Soil (R:S) ratio for maize was found to be relatively lower than the R:S ratio for other plant species (Woldendorp, 1978). This suggests that maize may not be a strong supporter of rhizosphere microorganisms. However, more work needs to be done to support this hypothesis.

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. Curtiss farm, which has been in a maize-soybean rotation for at least 20 years, showed good statistical isolate effects. At this site, significant isolate effects were detectable in the maize through silking. The Hinds farm soil probably contained high populations of numerous minor pathogens of maize whereas the Curtiss farm soil possessed lower inoculum potential and fewer potential pathogens. The potentially beneficial bacterial isolates probably were overwhelmed in the Hinds soil and, likewise, the DR were not detectable in the same soil with all of the other DR and pathogens.

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