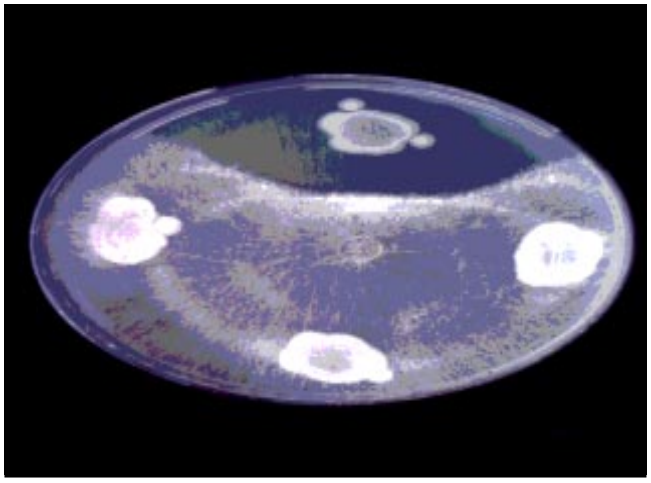

***In vitro* antibiosis test. One out of the four bacterial strains tested is strongly inhibitory to the plant pathogenic fungus.**

Suppression of Root Diseases of Ornamentals by Plant Health-Promoting Rhizobacteria

J.O. Becker and U. Schuch

Horticultural production of plants in most parts of the world is affected by poor root health. Root diseases and root deterioration are a constant problem in warmer climates, such as California or in greenhouse production (Forsberg, 1975, Fletcher, 1983). They are frequently the result of complex interrelationships of soilborne pathogens, plant parasitic nematodes, other deleterious microorganisms and predisposing abiotic stress. Especially with potting mixes, steam-treated substrates or rockwoll which have typically poor or no natural biological buffering capacity, pathogens may cause catastrophic crop losses.

In the past, soil pesticides offered relatively effective, although often only short-term, solutions for suppression of plant disease causing organisms. The future of soil pesticides appears questionable because of environmental and economic considerations. Along the same line, the current cost of more than $100 million for developing a new pesticide is prohibitive for many diseases and minor crops (Bellus, 1991). Finally, increasing numbers of consumers demand pesticide-free ornamentals. The horticultural industry is therefore in need of reliable, safe and economic alternatives to traditional root disease control.

In recent years, plant health-promoting rhizobacteria (PHPR) have been identified as a biological control alternative to pesticide use by achieving disease suppression without negative effects on user, consumer or the environment (Linderman, 1986, Weller, 1988). These beneficial bacteria are found on many subterrenial plant surfaces and thrive by consumption of mainly plant-derived nutrients. They are capable of suppressing microorganisms or nematodes that cause root damage (Sikora, 1991). However, in most plant production systems the population of beneficial rhizobacteria is too small to have a significant impact on root health. By introducing selected PHPR strains during sowing or planting, the rhizosphere can be enriched with beneficial bacteria which create a biological barrier for plant pathogens and pests deleterious to root health (Cook and Baker, 1983).

Screening for plant beneficial microorganisms is likely to be a number game, very much like the discovery of a new plant protection chemical. It was therefore proposed to develop a screening program for beneficial rhizobacteria which utilized a combination of *in vitro* and *in vivo* methods. A large number of strains was initially tested for antibiosis. The biological active strains were then subjected to a greenhouse screening test which would offer the opportunity to select the best strains in the presence of a plant host and target pathogen.

This research program was initiated to identify and develop root-colonizing bacteria with the potential to control soil-borne seedling diseases in ornamental plant production. During the past two years, more than 7000 rhizobacteria have been isolated from the roots of trap plants which grew in soil samples collected from 120 different locations in California representing a wide range of geographic and ecological niches. Approximately 10% of the bacterial strains inhibited in vitro the main target pathogen, Rhizoctonia *solani*. About 1% of the total number of strains tested expressed activity against both *R. solani* and *Pythium ultimum*. A growth chamber assay was developed which allowed mass screening of the in vitro active strains against post-emergence damping off. In this screen, 653 bacterial strains were tested against *R. solani* . Thirty-three strains demonstrated significant biocontrol activity. Furthermore, eleven strains dramatically inhibited or strongly reduced the germination and further development of the test plants. Five others strains enhanced disease symptom development.

Results

Collection of Soil Samples and Isolation of Rhizobacteria. Root and soil samples from 120 locations were collected representing various crop growing areas, different soil types and climatic zones in California. Special attention was given to soils in locations known to be suppressive to soil-borne diseases. Areas sampled included the San Joaquin Valley, Napa Valley, Coastal Range, Coachella, Palo Verde and Imperial Valley. The samples were potted and sown with sweet peas, watermelons, sugar beet, or cotton which were used as trap crops. The seedlings were harvested after 2-4 weeks and processed to obtain microorganisms from the root surface or endo-rhizosphere. Suspensions obtained from various fractions of root and rhizosphere were also exposed to 10-minute heat treatments at 80C to allow for selective recovery of spore-producing microorganisms. Bacteria were isolated on various general or semi-selective media such as 5% tryptic soy agar, 10% potato dextrose agar, and King's medium B. After purification they were stored at -80C in 50% glycerol. Typically 8-12 strains were isolated from each medium.

***In Vitro* Antibiosis Test**. Despite the well documented observation that *in vitro* antifungal activity does not necessary imply *in vivo* biocontrol activity, most of the in scientific literature described superior biocontrol strains are active *in vitro*. It seemed therefore opportune to select a large number of strains first for their ability to inhibit the main target pathogen in a simple dual culture system. *R.solani* was used as the primary screening organism due to its importance and difficulty to control. More than 700 bacterial strains demonstrated in vitro activity in dual culture against this fungus. In a second pathogen/antagonist test, 68 strains exhibited also activity against *P.ultimum* which was considered another target pathogen. This is noteworthy because currently available pesticides tend to protect only against one but not the other fungal pathogen.

***In Vivo* Biocontrol Trials**. Testing a large number of active isolates required the development of a quick and sensitive bioassay. After preliminary tests with a number of different plants, we chose radish as the test plant because of its high germination rate (~98%), fast growth, convenient plant size, and high susceptibility against both *R. solani* and *P. ultimum*. Trials were conducted in seedling trays with LP5 growth substrate, a medium often used in the production of ornamentals. Each cell was seeded with 8 radish seeds at the perimeter and inoculated with a *R. solani*-infested millet in the center. This method allowed precise placement of the inoculum and caused moderate to severe disease symptoms (slight eye spot lesions, severe cavities or even post emergence damping off) in approximately 75% of the non-treated control plants. The soil was drenched with candidate bacteria at approximately 10^7 colony-forming units/cc substrate. This rate has been proven in the past effective for screening beneficial rhizobacteria (Kempf and Becker, 1992). The trays were incubated in a growth chamber at 21C and 14 hours light period.

Seedling germination and damping-off was recorded after 6 days. After another 6 days, roots were carefully cleaned off the substrate and rated for disease symptoms. Each individual trial was designed as a complete randomized block experiment with four replications. In addition to the bacteria treatments, a pathogen-free control and a pathogen-infested but non-treated control completed each trial set up.

In total, 653 strains have been tested in this system. Thirty-three strains have shown excellent suppression of Rhizoctonia disease without affecting the germination or growth of the test plant. In a few cases, the plant growth seemed slightly stimulated but potential growth-promotion activity needs to be confirmed in further tests in pathogen-free soil. The majority of the tested strains did not seem to influence the pathogen or the host. However, with at least five strains the disease symptoms appeared to be more pronounced. The disease ratings in these cases were significantly higher than in the controls. It seems that the presence of the bacteria or some of their metabolites may accelerate disease expression.

Eleven strains were isolated which inhibited or delayed the germination of the radish seed. The latter ones never recovered fully, but remained stunted or deformed. Often, they were obviously weakened in terms of defense against the fungal attack and were rapidly killed by *Rhizoctonia*. Both types of plant-deleterious strains are of considerable interest for further investigations because they may explain poor germination of certain crops in the absence of major pathogens.

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