Use of bacterial bioproduct for plant growth stimulation and protection against phytopathogenic fungi

Voaides Catalina 1,2*, Cornea Petruta 1,2, Tezel Rodica 1, Babeanu Narcisa 1

1University of Agronomic Sciences and Veterinary Medicine Bucharest, Faculty of Biotechnologies, 59 Marasti blvd., sect. 1, Bucharest, 2Center of Applied Biochemistry and Biotechnology, 59 Marasti blvd., sect. 1, Bucharest

Corresponding author. Email: catalinavoaides@gmail.com

Abstract Our study emphasizes the use of a bioproduct based on a Bacillus sp. strain, tested "in vitro" and "in vivo" conditions, in order to prove its antifungal potential (against some Alternaria tenuis strains) and also both germination of seeds and plants growth promotion. The application of the treatment was performed before, simultaneously and after the infection in order to establish the optimum moment of application. The plant growth stimulation action of bioproduct was observed both on sterile and non-sterile soils.

The interest of the scientists in the potential uses of plant associated bacteria as agents stimulating plant growth and managing plant health is proved by the numerous articles published in the last 10 years (2, 3, 1, 4, 6). The beneficial actions are due to the synthesis of various compounds (7). But, despite the achievements in this area, the isolation of new bacterial strains from natural sources remains a goal for scientist working in this field. This was the aim of our study in order to use a bioproduct based on a new local (Romanian) bacterial isolate, with increased antifungal and plant growth promoting activity.

Experimental variants of the bioproduct based on Bacillus sp. BW were obtained and tested in vivo, using pepper, cucumber and tomato seeds. The bacterial strain was selected in previous experiments and it was efficient in the in vitro growth inhibition of the phytopathogen Alternaria tenuis as well as against other pathogens. The bacterial strain was isolated from soil being identified using Biolog System and molecular techniques (5, 8). Starting from these aspects it has been obtained a bioproducts for phytosanitary use, by conditioning the bacterial strain with high fungicide potential.

Biological Material and Method

In our experiments were used a bacterial strain Bacillus sp. BW, with antifungal properties, isolated and characterized in previous experiments and two phytopathogenic fungi: Alternaria alternata (tenuis) and Botrytis cinerea.

1. Bioproduct based on antagonistic bacteria. In order to assess both the inhibition potential of the bioproduct against fungi and the stimulation of the germination and growth of the biological material, the testing was performed by comparison with two chemical fungicides: iprodione (conditioning as Rovral Aqua Flo) and procymidon (conditioning as Sumilex 50 WP), recommended against phytopathogenic fungi Alternaria tenuis. Biological material used for in vivo testing was represented by pepper seeds (Capsicum annuum, Export variety), tomato seeds (Lycopersicum esculentum, Rio Grande variety) and cucumber seeds (Cucumis sativus, Cornichon Paris variety). Bacillus sp. BW biomass was conditioned as granules with controlled release.

Antagonistic bacteria was inoculated on a specific media (glucose 10 g/l, yeast plasmosilyzed (supplemented with Fe) 5 g/l, KH2PO4 1 g/l; Mg2SO4x7H2O 0,5 g/l, adjust with water for 1 l). After centrifugation, the biomass was mixed with 5% sodium alginate, yeast plasmosilyzed, kaolin and skimmed milk powder (5:2:4:2). The homogenized mix was dropped into a solution of 0,25 M CaCl2. The obtained granules were air-dried at room temperature (18-20°C).

2. Testing the antifungal potential of the bioproduct. In vivo testing of the bacterial bioproduct was performed using two different experimental models. Thus, in the first variant, the bioproduct was used dissolved in water and applied into the soil using a micropipette. The experiments were performed in plastic boxes, using 450 g of previously sterilized soil. In the second experimental variant, the bioproduct was used in its granular form and has been applied in soil together with seeds. Testing was conducted in plastic cups, using 100 g regular soil and soil from greenhouse, in sterile and non-sterile version.

The bioproduct potential, both antifungal and the stimulation of germination and growth processes of
biological material, was tested in comparison with two chemical fungicides, iprodione and procymidon. In both cases, the seeds of tomatoes, peppers and cucumbers have been sterilized with 5% sodium hypochlorite (w/v) and then were rinsed with sterile distilled water before sowing.

The spore suspension of *Alternaria tenuis* was applied at a concentration of $10^5$ spores/ml (using Thoma slide). Findings antifungal potential of bioproduct is achieved by calculating the percentage of germination of seeds of tomato, pepper or cucumber every two days, for two weeks.

**Results and Discussions**

After the determinations, it was found out that after four days of cultivation, it can be obtained a rich biomass, no longer being necessary the 5th day of cultivation. After four days of cultivation, the produced biomass had a concentration of approximately $10^{10}$ CFU/ml and a level of at least 90% spores. Thus the biomass was incorporated into the mixture of precipitation and was well homogenized by vigorous shaking.

The granules obtained were immediately separated from CaCl$_2$ and were dried at room temperature on filter paper. From morphological point of view, the granules were characterized by gray-white colour, spherical shape, measuring 2-3 mm in diameter. Although the antifungal ability of the bioproduct proved to be very promising, its practical use in order to combat fungal diseases can only be made after tests that confirm this activity *in vivo*, too.

In a first experimental version was tested the effect of the bioproduct based on *Bacillus sp.* BW on pepper seed germination (*Capsicum anuum*, Export). In this case was use only sterilized soil. The infection with *Alternaria* spore suspension was achieved by mixing it into soil. Simultaneously with sowing of seeds (which were previously sterilized) was applied the bioproduct, dissolved in sterile distilled water. Monitoring germination percentage was achieved during two weeks. After only one week of cultivation, significant differences were observed between seeds treated with bacterial bioproduct and control seeds infected with *Alternaria tenuis* (Fig. 1).

![Fig. 1. *In vivo* antifungal potential of the bacterial bioproduct against phytopathogenic *A. tenuis* a1 (left), compared with the uninfected control (right), two weeks after sowing.](image)

Thus, ten days after sowing, the seed of pepper treated with the bacterial bioproduct has reached 99% germination rate, while the control sample infected with *Alternaria* reached a rate of only 23% (Fig. 2).

Monitoring of the pepper seedling variants was done only during germination (two weeks) because the small size of test-vessels did not allow tracking the experiments longer period of time. At the end of the experiment, the differences between variants were clear. Although promising results were obtained in this experiment, they are not decisive to recommend the use of the tested bioproduct in practice.

Another version of experiments, in order to confirm *in vivo* efficacy of the obtained bioproduct, involved the use of two types of soil: sterilized (SS) and unsterilized (NS).
This time, seeds from three species of horticultural plants: pepper (*Capsicum annuum*, Export), tomato (*Lycopersicum esculentum*, Rio Grande) and cucumber (*Cucumis sativus*, Cornichon Paris) were used for testing. Another aspect of differences between this experiment and the previous one was represented by the application method of the bioproduct, granules being used (fig. 3).

![Fig. 3. Aspect of the bioproduct granules.](image)

Also, the antifungal potential of the bioproduct was compared with that of chemical fungicides Rovral Aqua Flo and Sumilex 50 WP. When both sterilized and unsterilized soil was used, three different experiment variants were prepared:

A - application of the bioproduct granules in the soil infected with spores of *Alternaria*, three days after sowing;

B - application of the bioproduct granules simultaneously both with sowing and infection with fungal spores;

C - application of the bioproduct granules simultaneously with sowing, the infection with fungal spores being done after three days.

The samples obtained in these variants were examined every two days, for two weeks. A first observation was that, no matter what the type of seeds was used for testing, no significant differences between seed germination in sterilized soil (SS) and unsterilized soil (NS) were noted. However, several differences were observed, firstly between the test variants (A, B, C) and secondly, for the same variant, between the tested bioproduct, the uninfected control and the chemical products (Rovral Aqua Flo and Sumilex 50 WP).

When testing the effect of the bioproduct on pepper seed germination, in A experimental variant, four days after starting the experiment, has been observed the emergence of fungal mycelium on soil surface. After two more days, the mycelium continued to grow in infected control, while in the case of the samples treated with this bioproduct it decreased gradually.

In the case of B variant, when the granules of the bioproducts were applied simultaneously with infection, the germination of seeds treated with bacterial bioproduct was achieved faster with approximately a day and a half (fig. 4), while the best results were obtained in the experimental variant C, the germination being achieved three days faster, compared to variant A.
When different experimental variant was performed, the seeds treated with the bioproduct had a higher germination percentage compared to those treated with chemical fungicides, an abundant mycelium being observed on soil surface of the latter.

Although, 10 days after sowing, the size of the pepper plantlets from seed treated with those two chemical fungicides used as standard, was almost similar to those treated with the biological product (fig. 5), the germination percentage was significantly higher for the bioproduct.

Moreover, because the fungal mycelium grew on the soil surface and spread to the plantlets, their growth was seriously affected. Thus, in the 14th day after sowing, the difference between the variants treated with bioproduct and those treated with chemical fungicides was evident, taking into account not only the percentage of germination but the length of the obtained plantlets and their force to grow.

When testing the effect of the bioproducts on tomato seeds, obtained results were similar to those reported for pepper seeds, the difference between the three experimental variants A, B and C being visible. For the first experimental variant, because of the fungal mycelium growth before the application of the bioproducts, chemical fungicides respectively, the results were not very promising. Thus, after ten days from sowing, although the percentage of germination of seeds treated with bioproduct was not very high, however, it was observed the stopping of the development of fungal mycelium, even its disappearance, the same aspect being noted for seeds treated with fungicide Rovral Aqua Flo, too.

For seeds treated with fungicide Sumilex 50 WP, although they germinated synchronously with the other ones, covering ground mycelium extended to the end of the experiment to the stem and leaves of the rises plantlets.

For the experimental B variant, six days after sowing, there was a certain uniformity of the germination percentage for seeds treated with bioproduct and uninfected control, however, differences being observed for control infected with Alternaria.

As the experiment progressed, there was clearly a superior germination percentage of seeds treated with bioproduct, compared to chemical fungicides and especially the infected control. Taken together, the results were better than those obtained in variant A.

In the third experimental variant, only one week after sowing, the germination percentage of

---

**Fig. 4.** The bioproduct influence on pepper seeds germination, in the experimental variants.

**Fig. 5.** *In vivo* action of the bioproduct (3) on pepper seed germination, compared with chemical fungicides Rovral Aqua Flo (1) and Sumilex 50 WP (2), in the experimental variant C, 10 days after sowing.
tomato seeds treated with bioproduct was up to approximately 100%.

Comparing the time for germination of tomato seeds treated with bacterial bioproduct, in all three experimental variants, revealed the effectiveness of using the bioproduct before the fungal infections (fig. 6).

Fig. 6. The action of the bioproduct on tomato seeds germination, in experimental variants A, B and C.

Thus, it can be noticed that the results obtained for in vivo tests are particularly promising in all experimental variants, with real economic implications.

Conclusions

The results obtained in our experiments allow following conclusions:

1. Regarding the application method of the bioproduct (dissolved in water, granules or as it lies), there have been obtained almost similar results.

2. For in vivo testing, although there have been obtained good experimental results; it has been observed that the treatment with the bioproduct is preferably done preventively. This offer clear protection against the phytopatogen fungi, and, in the same time, it stimulates the growth of the treated plants.

3. The good antifungal action of tested bacterial strain observed in vitro experiments was also observed in vivo conditions. Moreover, plant growth promoting action of the bioprodct based on bacterial strain Bacillus sp. BW was observed both on sterile and non sterile soil.

Acknowledgments

This work was funded by 52/2006 CEEX Research Project (acronym BIOCOMB) and 1099 CNCSIS grant.

References
