The influence of various microbial bio-products, for bean crop, on biological activity of the red preluvosol soil

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Abstract

The aim of our work between 2005 and 2007 was to obtain and to test various microbial bio-products with bio-stimulation / bio-fertilization activity, in order to implement them in the agricultural technologies. Bacterial biomass obtained from Bacillus subtilis strains, Azotobacter chroococcum strains and Rhizobium phaseoli strains as well as fungus biomass from Trichoderma spp. strains were conditioned and applied to the bean crop on red preluvosol soil.

The characterization of these bio-products wouldn't be complete without the analysis of the influence of them on the biological activity of the soil, evaluated through the modular indicators (IPAV % and IPAE %) and synthetic indicators (ISB %) of the soil fertility.

The bio-products which emphasize the best soil stimulation activity were: Rhizobium phaseoli and the mix Bacillus sp. 1 + Bacillus sp. 2 + Trichoderma sp. Thus, the application of the bio-products increased the biological activity of the red preluvosol soil, using ISB% indicator, with 8,6% - 34,98%.

Key words: *Bacillus spp., Trichoderma spp., Rhizobium spp., Azotobacter spp.,* bio-products, vital activity, enzymatic activity, indicators of soil fertility

Introduction

Biological activity is an important feature of the soil, thus making possible the recycling of matter in the environment. The recycling of matter represents the transformation between the organic component and the mineral component, in both ways, achieving two important objectives: to avoid the accumulation of organic matter (biodegradation) and to maintain biological activity (to supply the nourishing substances for the vegetation cover – the base of the terrestrial ecosystem). The main objective of agricultural systems was and still is the application of the technical methods to improve some segments from the biogeochemical cycle of elements. The manner of approaching this objective is very important. Thus, the applied technical methods have to be not aggressive to nature. Such a manner is the inclusion of the microbial bio-product in agricultural technologies.

The application of bacterial bio-products represents an ecological measure meant to improve plant nutrition, without the long-term modification of the physical, chemical and biological properties of the soil, which happens when chemical fertilizer is applied. The characterization of these bio-products would not be complete without analyzing their influence on soil activity as well as on the physiological state of the soil, which is emphasized through enzymatic activity. This represents the start of all energy producing processes, followed by several chemical transformations allowing nourishing substances to get to the plant.

Material and Methods

25 soil samples from fields cultivated with wheat were collected in order to isolate *Bacillus* spp and *Trichoderma* spp. In each field, 500 g of soil samples were taken from five locations, within an area of 30 x 30 m and put together in plastic containers. These soil samples were later used for isolating *Bacillus* spp. and *Trichoderma* spp.

Bacillus spp. strains were isolated using a soil dilution plate method by adding 1 g of soil sample aseptically to 9 mL of sterile distilled water in each test tube. These tubes were then heat shocked at 80^{o}C for 20 min. and the soil suspension, serially diluted to 10^{-5} and 1 mL aliquots was dispensed on nutrient agar plates. The plates were then incubated at 32^{o} C for 7 days. A single colony of the bacteria grown on nutrient agar was collected by transferring it to agar slants in test tubes for further testing. Isolates of *Bacillus* spp. were subsequently identified using physiological and biochemical tests. The results of the tests were compared to identification schemes based on MacFaddin [1].

Trichoderma spp. strains were isolated from the same soil samples inoculated onto potato dextrose agar (PDA, MERCK) and malt extract agar (MEA; MERCK) and incubated at 28^{o}C for 5 days. After an incubation period, colonies determined to be *Trichoderma* spp., according to Rifai [2], were purified.

Fermentation conditions: cultures were grown at 31°C in a working volume of 7 liter in a 15-liter New Brunswick bioreactor equipped with pH, dissolved-oxygen, temperature, and foam probes. The medium contained (per liter) 25 g of glucose, 10 g of corn steep liquor (51% D.M.W.), 2 g of Na NO_3, 0.5 g of $MgSO_4$ x 7 H_2O, 0.2 $MnSO_4xH_2O$, $CaCl_2x2H_2O$; initial pH: 6.6, aeration: 0.5 v.v.m, agitation: 300-350 r.p.m.(*Bacillus subtilis*).

For *Trichoderma harzianum* cultures were grown at 27°C, in a medium with the following composition (g per liter): 17 g of glucose, 15 g of corn steep liquor (51% D.M.W.), 2 g of KH_2PO_4, 1 g of $MgSO_4x7$ H_2O, 1 g of $FeSO_4$ x7 H_2O. We used the same bioreactor with the following parameters: pH 4.8, agitation 150-200 r.p.m., aeration 0,5 v.v.m.

During cultivation, growth was monitored at various time intervals, by measuring optical density at 660 nm for bacterial cultures (1 unit O.D. = 0.32 D.C.W.) and by dry cell weight measurements for fungal cultures. Glucose was enzymatically estimated using F-kit Glucose (Boehringer Mannheim Co.Ltd, Mannheim, Germany).

In order to obtain *Rhizobium phaseoli* biomass, the media had the following composition: mannitol 10 g/l; NaCl 0.1 g/l; K_2HPO_4 40.5 g/l; $MgSO_4x7H_2O$ 0.2 g/l; $CaCO_3$ 0.2 g/l; yeast extract 1-1.5 g/l (pH 7-7.2). The cultivation conditions were: temperature 28^oC; aeration 0,5 v.v.m.; agitation 400 r.p.m. The inoculum's volume was 4% v/v.

Three *Azotobacter chroococcum* strains were isolated from soil samples on Burk media, using mannitol as carbon source. The cultivation conditions in bioreactor were: temperature 28^oC; aeration 0,5 v.v.m.; agitation 100 r.p.m.; volume of the inoculum 5,5%.

In 2007 there were performed tests in the field, in a farm near Racari, Dambovita county, placed on a field red preluvosol soil, with pH -6.2, Ct. -1.05, Nt -0.153, P Al -17 ppm, K Al – 180 ppm.

Bacillus spp. and *Trichoderma spp.* biomass was immobilized on seeds using pullulan like polysaccharide ($5x10^7$ – $2x10^8$CFU/seed). *Rhizobium* biomass was immobilized on agar ($1,3x10^9$ CFU/mL) and applied on seed before sowing time and *Azotobacter* biomass was applied on soil before sowing time as solution with $1x10^9$ CFU/mL concentration, pH = 7,2 (Babeanu et al., 2007).

The experimental variants were: V1 - N0P0, V2 - AT1 500 mL, V3 - AT1 1000 mL, V4 – RT1, V5 - $N_{60}P_{30}$, V6 - Bs1+Bs2+T.

Soil samples were collected after harvesting the bean crop, from a depth of 20 cm. The samples were conditioned in the laboratory at 40 – 60% humidity from CC.

The life level of the soil was evaluated by means of two biotic tests: respiration (mg CO_2/100 g soil d.s.) and cellulosolytic activity (g enzymatically hydrolyzed cellulose/100 g soil d.s.). Three enzymes were selected from the pedo-enzymes assortment (adsorbed enzymes on the organic-minerals components) with an important role in the cycle of the elements in the nature: saccharidase activity (mg enzymatically hydrolyzed saccharide/100 g soil d.s.; carbon cycle), urease activity (mg NH_4^+/100 g soil d.s.; nitrogen cycle) and phosphatase activity (mg P/100 g soil d.s.; phosphorous cycle). Catalase soil activity was added to the others (cm^3 O_2/100 g soil d.s.), this analysis offering information regarding the oxidation processes from the soil.

Based on both biotic and enzymatic tests, were calculated two modular indicators: VPAI% (Vital Potential Activity Indicator) and EPAI% (Enzymatic Potential Activity Indicator) and one synthetic indicator BSI% (Biological Synthetic Indicator). The calculation methodology for these indicators represents a numerical taxonomy variant, which recognizes an equal importance of each test for fertility soil level (Stefanic et al., 1999).

The experimental results were statistically analyzed using the analysis of the variant.

Results and Discussions

1. Bio-products obtaining

The development of the *Bacillus spp.* strains is shown in fig. 1.

From Fig. 1 it can be observed that the maximum concentration is 16 g/L DCW. During experiments, there were not large differences between growth rate and final biomass concentration for *Bacillus spp.* tested strains.

For *Trichoderma spp.* Strains, the fermentation profile, shown in Fig. 2, presents a maximum biomass concentration (13,5 g/l) after 76 h of cultivation.

For *Rhizobium spp.* tested strains, the maximum concentration was obtained after 84 h of cultivation, but the optimum process standing is 72 h of cultivation because between 72 h and 84 h of cultivation O.D. remained invariable ($1,3x10^9$ CFU/mL) (Fig. 3).

For *Azotobacter spp.* Strains, the final concentration was obtained at 72 h of cultivation ($1,0 – 1,3x10^9$ CFU/mL). No large differences were observed in this case, either (Fig. 4).

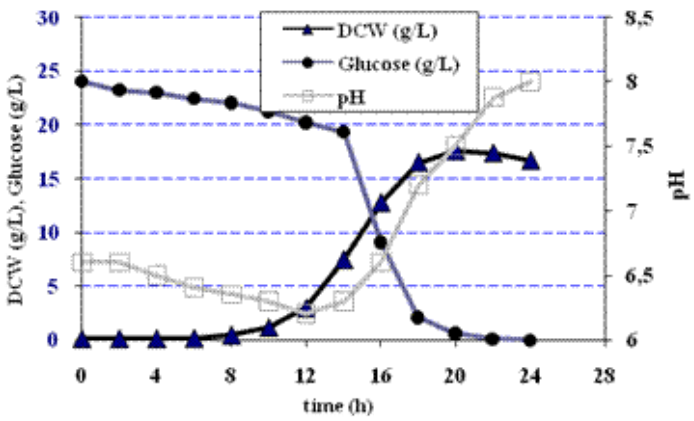

Figure 1. Fermentation profile for *Bacillus subtilis*

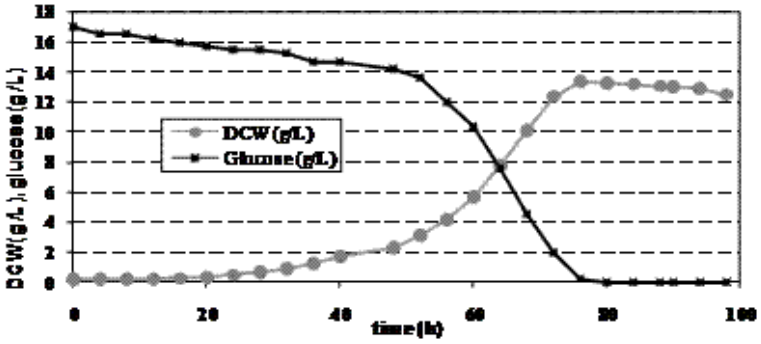

Figure 2. Fermentation profile for *Trichoderma harzianum*

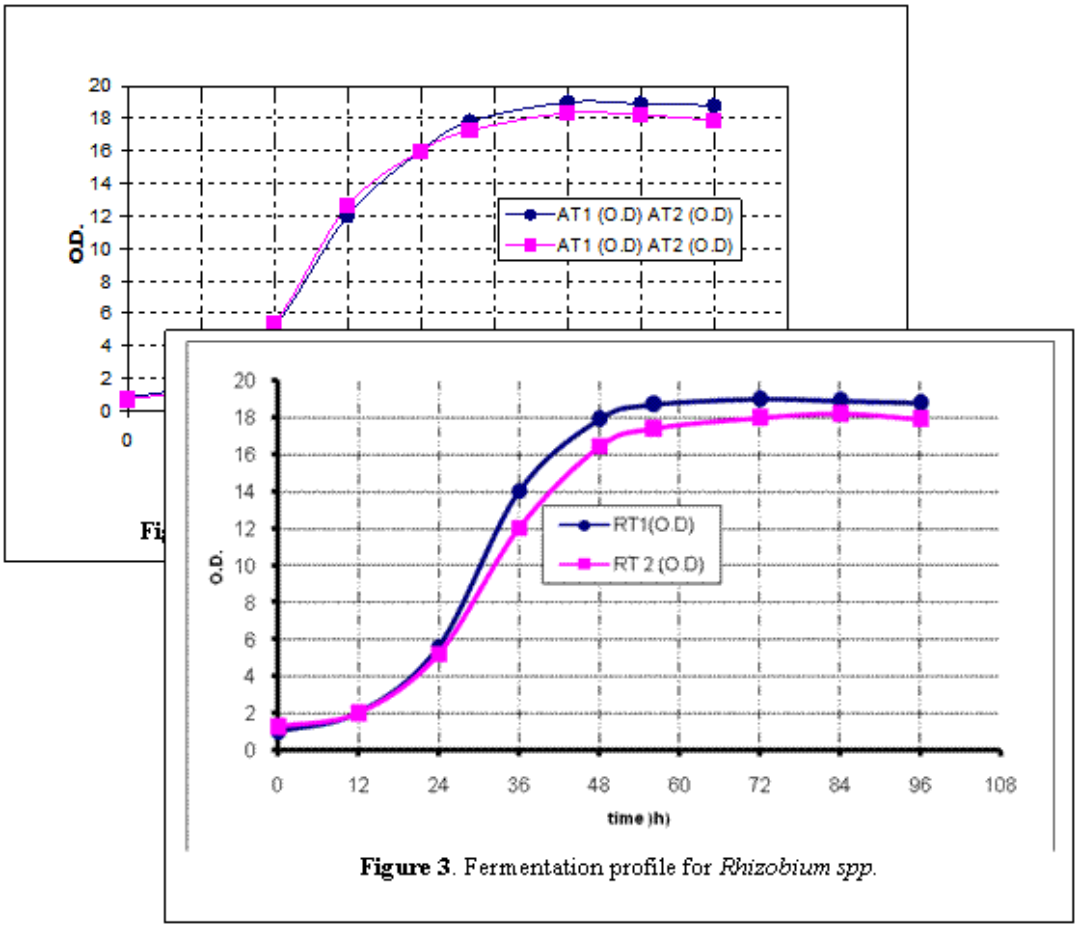

Figure 3. Fermentation profile for *Rhizobium spp.*

For field-testing, several bio-products were used: *Bacillus spp.* + *Bacillus spp.* + *Trichoderma spp.* (Bs1+Bs2+T), *Azotobacter chroococcum* (AT1) and *Rhizobium phaseoli* (RT2).

2. The influence of the bio-products on soil vital activity

2.a. Vital Potential Activity Indicator is shown in Table 1. The application of both bacterial bio-products and chemical fertilizer to the bean crop increased the life level from the soil, comparing with control sample (without bacteria and without fertilizer). There was only one exception, for the variant with AT1 500 mL bio-product, when it was observed that the life level it is the same for both sample and control sample. The increasing range was 11,73 – 30,98%.

Table 1. The influence of bacteria strains and nitrogen fertilizers on soil vital activity, using Vital Potential Activity Indicator (VPAI%)

Treatment	Test		IPAV %
	Respiration mg CO_2/100g soil DCW	Cellulolysis g cellulose. /100 g soil DCW	
Control sample – without bacteria, without fertilizer	7,32	36,88	c 20,88
Azotobacter chroococcum 500	14,03	31,23	c 20,29
Azotobacter chroococcum 1000	17,65	34,90	b 23,33
RT1	18,52	41,93	a 27,14
NPK	12,69	38,36	b 23,41
Bs1+Bs2+T	12,40	46,45	a 27,35
Maximum Empiric Value	150	100	DL 5% -2,398* DL 1% - 3,409 DL 0,1% - 4,936

The highest stimulation level of the soil vital activity it was obtained using RT2 and Bs1+Bs2+T bio-products.

Using a double dose, from 500 mL to 1000 mL of *Azotobacter* bio-product, we observed an increase in the soil vital activity to 14,98%.

2.b. Enzymatic Potential Activity Indicator is shown in Table 2. This indicator offers information regarding the activity of soil accumulated enzymes (pedo-enzymes). The main source for pedo-enzymes is soil micro-flora.

Table 2. The influence of bacteria strains and nitrogen fertilizers on soil enzymatic activity, using Enzymatic Potential Activity Indicator (EPAI%)

Treatment	Test				IPAE %
	Catalase cm3 O_2/ 100 g soil DCW	Urease mg NH_4/100 g soil DCW	Saccharase mg sucrose/100 g soil DCW	Total phosphatase mg P/100 g soil DCW	
Control sample – without bacteria, without fertilizer	146	45,93	1090	1,71	f 19,09
Azotobacter chroococcum 500	188	49,47	1385	2,63	d 23,11
Azotobacter chroococcum 1000	201	55,34	1439	2,59	c 24,60
RT1	192	56,37	1752	2,50	a 26,81
NPK	171	48,69	1466	2,17	e 22,89
Bs1+Bs2+T	187	45,07	1847	2,37	b 25,41
Maximum Empiric Value	2000	150	3500	25	DL 5% - 0,496* DL 1% - 0,706 DL 0,1% -1,022

This activity is supplemented by root filaments, plant's roots after vegetation cycle, vegetal rubbish, animals from soil, stable garbage, green manure etc., which lead to the various enzymes production, through autolysis and the decay of the cells and tissues. This explanatory note is to understand that the enzymatic activity has not to follow the same direction as soil vital activity.

The application of bacteria strains as well as of nitrogen fertilizers increases the accumulation of the soil enzymes. The values of EPAI% increase between 19,90% (chemical manure) and 40,44% (*Rhizobium spp.*).

Among the bacteria strains, first place in increasing enzymatic accumulation is for *Rhizobium phaseoli* (26,81%), followed by *Bacillus spp. + Bacillus spp. + Trichoderma spp.* (25,41%), *Azotobacter chroococcum* 1000 (24,60%) and *Azotobacter chroococcum* 500 (23,11%).

2.c. Biological Synthetic Indicator is shown in Table 3. The Biological Synthetic Indicator includes both soil vital activity (VPAI %) and soil enzymatic activity (EPAI %).

The application of bacteria strains as well as nitrogen fertilizers led to the improvement of the soil biological activity, estimated using BSI%. The increase is between 8,6% and 34,98%, in comparison to the control sample (without bacteria and without fertilizer).

The best results were obtained using two bio-products variants, RT1 and Bs1+Bs2+T, a statistically difference being impossible to obtain.

Azotobacter chroococcum based bio-product could represent an alternative due to its increasing biological activity in comparison with the control sample.

Thus, with those variants where microbial bio-products were applied, the biological nitrogen fixation on symbiotic path as well as on un-symbiotic path could increase, the solubilization of an increased phosphorous quantity from organic forms and a decreasing of the pathogen fungus action.

Table 3. The influence of bacteria strains and nitrogen fertilizers on soil biological activity, using Biological Synthetic Indicator (BSI %)

Treatment	BSI%
Control sample – without bacteria, without fertilizer	d 19,98
Azotobacter chroococcum 500	c 21,70
Azotobacter chroococcum 1000	b 23,97

RT1	a 26,97
NPK	b 23,15
Bs1+Bs2+T	a 26,38
DL 5% - 1,369* DL 1% - 1,946 DL 0,1% - 2,818	

Conclusions

As a result of the experimental research, it has been shown that:

1. The application of the microbial bio-products determines the stimulation of vital activity (11,73% - 30,98%) as well as enzymatic activity (19,90% - 40,44%) of the red preluvosol soil.
2. The biological activity of red preluvosol soil, using BSI%, increased by 8,6% - 34,98%, due to the application of the bacteria strains; the best results were obtained by using two bio-product variants, RT1 and *Bacillus spp* 1 + *Bacillus spp.* 2 + *Trichoderma spp*;
3. The best results for bean crop were obtained using inoculums such as *Rhizobium phaseoli* and *Bacillus spp* 1 + *Bacillus spp.* 2 + *Trichoderma spp.*
4. Due to its superior effect by comparison to the control sample (without bacteria and without fertilizer), the *Azotobacter chroococcum* based bio-product could represent a technological alternative.

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