THE INFLUENCE OF CONDITIONING TYPE UPON THE BIOLOGICAL FEATURES OF MICROBIAL INOCULATIONS

INFLUENȚA FORMEI DE CONDIȚIONARE ASUPRA CARACTERISTICILOR BIOLOGICE A INOCULANȚILOR MICROBIENI

Narcisa BĂBEANU*, O. POPA*, A. VAMANU*, Marina PAMFIL, Oana LIVADARIU*, E. VAMANU***

**University of Agronomical Sciences and Veterinary Medicine, Bucureşti, România*
***Chemical Pharmaceutical R & D Institute, Bucureşti, România*
Corresponding author: Narcisa BABEANU, e-mail: bnarcisa@yahoo.com

Abstract: *The research carried out by our team between 2004 and 2006 aimed the obtaining of microbial inoculants having with biostimulation activity in order to promote them in agricultural technologies. A bacterial (Bacillus subtilis) and fungal (Trichoderma sp.) biomass has been obtained for which several conditioning modes were studied: polysaccharides like pullulan, alginate. The bioproducts thus obtained have been tested for their resistance to thermic and hydric stress. The best results have been obtained in the case of cells alginate immobilized (for thermic stress), and in pullulan immobilized cells (for hydric stress). We also studied the influence of the immobilized biomass on wheat in laboratory conditions. The use of microbial inoculants had a positive effect on seeds germination (plus 10-17.00% comparative the control). Research continues in order to determine the best methods to apply the microbial agents; to assess their behaviour in the ecologic niche where they are introduced; to develop and to optimize the implementation technologies and to integrate them with the conventional technologies, as well as to quantify the influence of microbial agents on the quality of agricultural production.*

Rezumat: *Cercetările efectuate de colectivul nostru în perioada 2004-2006 au urmărit obținerea unor inoculați microbieni cu activitate de biostimulare în scopul introducerii acestora în tehnologiile agricole. În acest scop a fost obținută biomasă bacteriană (Bacillus subtilis) şi fungică (Trichoderma sp.) pentru care s-au studiat mai multe variante de condiționare: polizaharid de tip pululan, alginat. Bioprodusele obținute au fost testate pentru rezistența în condiții de stres termic şi hidric. Cele mai bune rezultate au fost obținute cu celule imobilizate în alginat (pentru stresul termic), respectiv pululan (pentru stres hidric). S-a studiat influența biomasei imobilizate pe semințe de grâu şi fasole la nivel de laborator. Utilizarea inoculanților microbieni a avut un efect pozitiv asupra germinației semințelor (plus - 10-17.00% față de martor). Cercetările continuă pentru determinarea celor mai bune metode de aplicare a agenților microbieni; evaluarea comportamentului acestora în condițiile nişelor ecologice în care sunt introduşi; dezvoltarea şi optimizarea tehnologiilor de aplicare şi integrarea cu tehnologiile convenționale, precum şi cuantificarea influenței agenților microbieni aplicați asupra calității producției agricole.*

Key words: *microbial inoculations, immobilization, Trichoderma, Bacillus*
Cuvinte cheie: *inoculanți microbieni, imobilizare, Trichoderma, Bacillus*

INTRODUCTION

The use of microbial agents in sustainable agriculture is part of the environment protection policy in agricultural sector and converges to both with Romanian agriculture for 2007 –2012 as well as to European Union policies.

Achieving a sustainable agriculture is the major objective of the strategies elaborated worldwide as well as at national level (on a medium term, of regional development, for developing areas).

Biostimulating / biofertilizing microbial agents are a main alternative source for plant nourishment. Bioproducts based on bacterial, algae or fungi are used in agricultural technologies with the purpose of increasing and improving the agricultural production and in the same time maintaining the ecological balance.

The critical factors influencing the efficacy of any effectiveness bioproduct are: compatibility between the microbial species and the target plants, identifying the strains which are the most suitable for the agricultural ecological system (this efficiency is influenced especially by the soil humidity), the characteristics of the carrier agent used in formulation which influences the viability and the bioavailability of the biological agent etc. (*NOGUCHI K, 2004)*

MATERIAL AND METHODS

The microbial biomass (4 strains of *Bacillus subtilis* and one strains of *Trichoderma* sp.) has been obtained by researchers from the Chemical Pharmaceutical R & D Institute Bucharest. It has been separated by means of sedimentation (*Bacillus subtilis*) or filtration (*Trichoderma.* sp). The wet biomass has been suspended in sterile water.

For the immobilization in alginate gel, it was used a 2.00% (w/v) sodium alginate solution with low viscosity. The obtained suspension has been gently stirred for 90 minutes.

The seeds have been sterilized by immersing them into 2.50% (w/v) sodium hypochlorite solution for three minutes, and then they have been washed in sterile water for three minutes and finally dried with air. The seeds have been further immersed in the microbial alginate suspension for 30 seconds. Shortly after, they were introduced in the $CaCl_2$ solution (0.1 M) for 30 seconds. After being washed in distilled water, the seeds are air dried ($t=40^0C$). The microbial titre obtained has been of 5×10^7 - 2×10^8 UFC per seed.

For the immobilization in a pullulan like polysaccharide, the microbial suspension has been mixed with the polysaccharide so that the final solution to reach a 5.00% w/v polysaccharide concentration. The seeds are immersed for 30-45 seconds in the microbial suspension and then air dried.

For the investigation of the resistance at lower temperatures both, *B. subtilis* spore suspension and the seeds coated by alginate calcium or pullulan have been maintained for 16 days at -22^0C.

In order to quantify the influence the lack of humidity has on the viability of *B. subtilis* spores, seeds covered by the immobilized biomass have been maintained at room temperature, in sealed vessels containing $CaCl_2$ anhydrous.

During the experiment, withdrawn seeds have been immersed in sterile water. The serial dilutions have been made from the resulted solution.

Lab experiments have been set in 17x17x14 cm pots. For testing wheat seeds products, 20 seeds per pot have been used, and for the beans 5 seeds/ pot. The influence that the microbial biomass immobilized on seed has on the germination, was also observed.

EXPERIMENTAL RESULTS

As it can be seen in figure 1, the highest rate of survival has been obtained in the case of immobilization in calcium alginate. After 16 days the survival rate was 89.00%. The biomass immobilized in pullulan had a lower rate of survival (68.00%). It must be pointed out that both forms of conditioning demonstrated a good protection ability of the microorganisms. Microbial suspension had a survival rate of only 47.00%. All four strains of *Bacillus subtilis* had a similar behaviour (see figure 2). The presented data represent the average of 5 replicates.

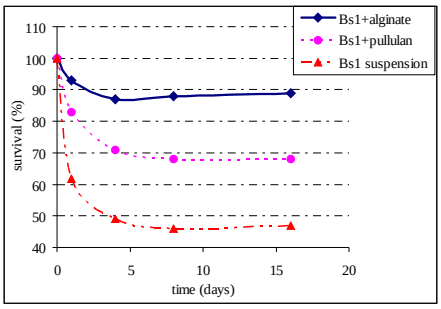

Figure 1. Influence of immobilization manner on the survival rate of *Bacillus subtilis* spores at –22°C.

Regarding the biomass resistance to hydric stress conditions, pullulan immobilization has proved to be superior to alginate immobilization. The survival percentage after 270 days has been in both cases over 70.00%. We note that during the first 60 days, there were no significant variations of bacterial titre.

In dryness conditions, the pullulan immobilization of *Trichoderma* sp. strain lead to better results than alginate immobilization (see figure 4).

Although the resistance to dryness of *Trichoderma* biomass is lower to that proved by the *Bacillus* strains, it can be seen that in both cases, the pullulan immobilization has as result a higher rate of survival by 15.00%, 7 .00% respectively.

The obtained bioproducts have been tested in the laboratory, observing the germination time (figure 5). The best results have been obtained by the mixture of *Bacillus* with *Trichoderma* (a positive difference of 17.00%), most of the tested products having significantly lower period of vegetation compared with the control.

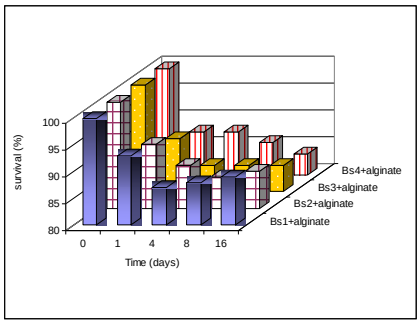

Figure 2. Comparison between the lower temperatures resistance of *Bacillus subtilis* strains.

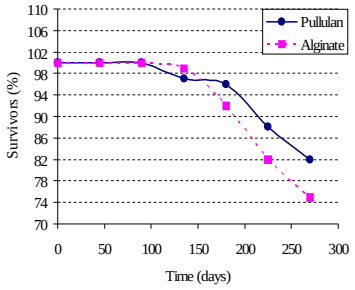

Figure 3. Comparing *Bacillus subtilis* biomass immobilized in alginate versus pullulan under dryness conditions.

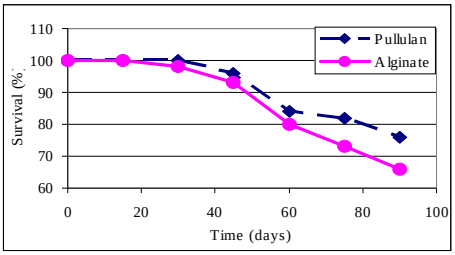

Figure 4. Comparing *Trichoderma* sp. biomass immobilized in alginate versus pullulan under dryness conditions.

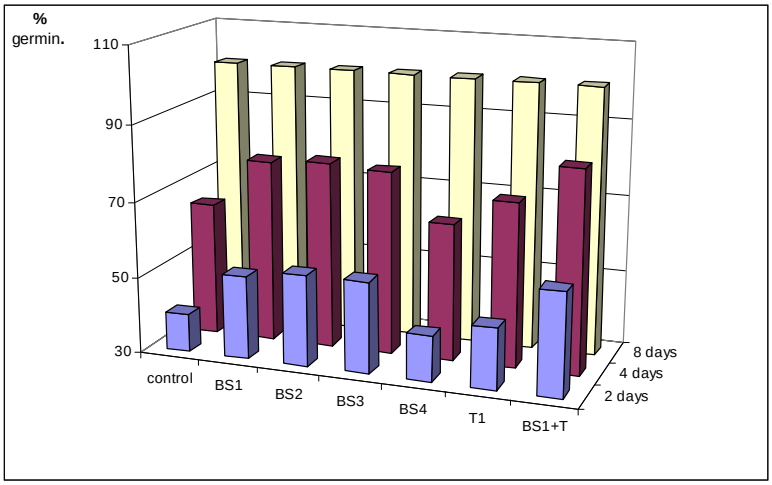

Figure 5. Treatment's influence on wheat seeds germination.

CONCLUSIONS

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

1. biomass immobilization strengthens both the resistance in thermic stress conditions and in hydric stress conditions;
2. in thermic stress conditions, immobilization in alginate has the most protector effect;
3. in hydric stress conditions, the immobilization in pullulan is superior to alginate immobilization;
4. the results have the same trend both in the case of *Bacillus* strains and in the case of *Trichoderma* strains;
5. by testing the products at lab level, it has been shown that strains Bs1, Bs2, *Bs3* and *Trichoderma* shortens the germination time with over 10.00%; Bs1+ *Thrichoderma* mix leads to the best results (with over 17.00%).

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