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# OPTIMISATION OF BIOMASS PRODUCTION OF ANTAGONISTIC BACTERIA FOR CONTROL OF PHYTOPATHOGENIC FUNGI

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**Abstract:** The phytopathogenic fungi attacking the seedlings of forest trees, in forest nurseries produced important damages of transplantation and reforrestation plant material. One of the most modern and important methods is to use alternative environmental friendly antagonistic bacteria and fungi for the pathogens. The mass growing of antagonists means to find new optimized method of growing including new type of growth culture media assuring a high yield of biomass and conserve the antagonistic properties of the strains. In the laboratory of bacteriology from RDIPP, were designed and tested improved culture media in order to select the most appropriate ones from scientific and economic point of view for this biotechnological process, in order to obtain a better biomass yield.

**Key words:** *forest nurseries, biomass production antagonistic bacteria, phytopathogens, culture media*

## INTRODUCTION

One of the most important problem for the successful cultivation of seedlings of forest trees in nurseries, are the control of the fungal pathogens and pests which can destroy and produce losses and damages in the cultures and of course economical damage. The treatments for prevention and control of the pathogenic fungi from soil is time, money and manual work consuming and consist in application of three treatment per year with chemical substances potential dangerous for environment , animals and humans.

There are known that the soil microbiota in the rhizosphere contains many species of bacteria each having a role to play in this specific habitat. The microbiota associated with plant roots are very important for plants health (Berendsen et al, 2012).The same authors citing different sources, shown that there are about 30.000 species of prokaryotes with a high number of about $10^{11}$ cells, and the effect is the apparition of disease suppressive soils and to improve the plant resistance to pathogens attack and compete with pathogens presence in soils.In the same time, each plant species is able to shape their own root microbiome by its root exudates.

The use of fungal pathogens antagonists can reduce considerable the damages. There are many experiments in all the world in order to reduce chemicals applied as fungicides in agriculture and replace partially or totally with antagonists as for example control of *Fusarium oxysporum* to banana (*Musa sativa*) cultures using a *Bacillus subtilis* strain KY 21 (Sun et al., 2011). The same methods were applied to many temperate region plants for maize cultures using some isolated pseudomonads (Djuric et al, 2011). *Pseudomonas putida* is a plant endophytic bacterial strain with abilities of PGPB and in the same time are able to inhibit growth and enzymes produced by plant pathogens (Weyens et al, 2012).

Based on previous experience and on the data from literature, we designed experimental culture media (taking into account the costs) with cheap ingredients for developing further the

technology of antagonists' biomass production in order to apply it in forest nurseries. The strains were isolated in the laboratory of bacteriology from our institute (Constantinescu et al, 2010).

## MATERIALS AND METHOD

The experimental growth media were composed using the previous experience (Oancea et al., 2012) and tacking into account the needs of the strains used in experiments. Cheap ingredient were selected like molasses, glucose, corn flour, yeast extracts according to their biochemical composition. The M1 and M2 culture media contained 5 g/l and 10g/l molasses respectively. The culture media M3 and M5 contained glucose in concentrations of 10 g/l and 7 g/l respectively. The culture medium M 4 contained corn flour 10g/l. All the culture media (M1- M5) excepting M6, contained yeast extract (7g/l) and some organic substances salts of Mg, Mn, CaCO3. The pH of all media was 7. The M6 was the cuture medium Luria Bertani (LB) use as standard reference without any modification. The media were prepared after consulting the suggestion from literature for biotechnological purpose growth of Bacillus subtilis strains (Oancea and colaborators 2011; Ubalua, 2014)

The culture media were distributed in Erlenmayer (fig 1) 200 ml flask (47,5 ml medium and 2,5 ml bacterian inoculum each of OD equivalent and incubated (Thermoshake, Gerhard) at 28°C and 150 rpm (18 culture). The OD was measured at a spectrophotometer (Spectronic 21, Milton Roy Company GB), and 1 ml of culture were sampled in weighted Eppendorf microtubes for centrifuge. Following centrifugation at 5000 rpm 10 minutes (Allegra X-12, Beckman Coulter) and discharge of liquid, the samples were weighted to analytical balance for determination of wet cell biomass.

## RESULTS AND DISCUSSIONS

The culture media M 1 and M 2 contained molasses in concentration of 5 and 10 g/l respectively and stimulated the growth of microorganisms with the exception of the strain Bce2 in M 1, were the growth was not so great maybe for the characteristics of the bacterial strain or by reduction of pH by forming some volatile organic acids in specially in the last part of the experiment.

The culture media M 3 and M 5, with glucose content 10 g/ l and 7 g/l enhanced very much the growth, in M3 culture medium the optical density was 1.30-1.40 depending of the strain. too (fig. 2) and a correlated with the cell biomass content (fig. 3).

The culture medium M4 with a content of corn flour 10g/l stimulated the growth with a higher OD (optical density), to all the strains obtining a consistent biomass to all the strains. This fromulation was most favourable for the strain Fsb1. It is recommended to use this culture medium by using an corn flour extract instead the corn flour itself because is needed a better clarified medium for a accurate OD measurements and for efficient separation of bacterial biomass.

The control (M 6) was in fact the well known Luria Broth and this allowed a moderate growth of the bacterial biomass.The differences between this and the improved culture media was considerable.

The cfus obtained were consistent with the OD being between at the highest OD in the range of $5x\ 10^8$ at the strain Fsb1 in the M3 culture medium.

The M2 culture media with a molasse content of 10g /l and M 3 with glucose were the most efficient in cultivation of all the strains the maximum of growth was demsonstrated by the strain Fsb 1 from points of view of optical density and of the wet bacterial biomass obtained . The number of cfu s, according to the plating results were in the range of $10^8$ cells /ml that is consistent with the results obtained by other specialists (Korsten et Cook 1996). This specialists practically used other culture media –the potato containing medium being the best of all for obtaining high yield of cfu s of the *Bacillus subtilis* strains thats means in fact a better biomass content. The media containing molasses enhanced the growth of the strains, but the media is lowering the pH during growth, due to formation of some organic acids formation.

## CONCLUSIONS

The improved culture media M3 with 10% glucose was the best to obtain a superior biomass, but the results were variable according to the specific of each used strain. The most prolific strain was Fsb1 in this culture medium.

The culture medium M4 must be considerably improved.

Due to the characteristics of the strains acording to our results, new studies must be performed in order to obtain a better sporulation. Sporulation can be enhanced by using special culture media because the spores have a better shelf life, and can be easy managed but this will be the subject of another experiment.

## AKNOWLEDGMENTS

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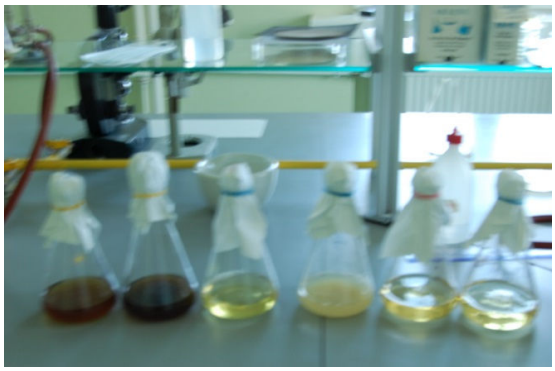

**Fig 1. Improved culture media for bacterial antagonists strains cultivation**

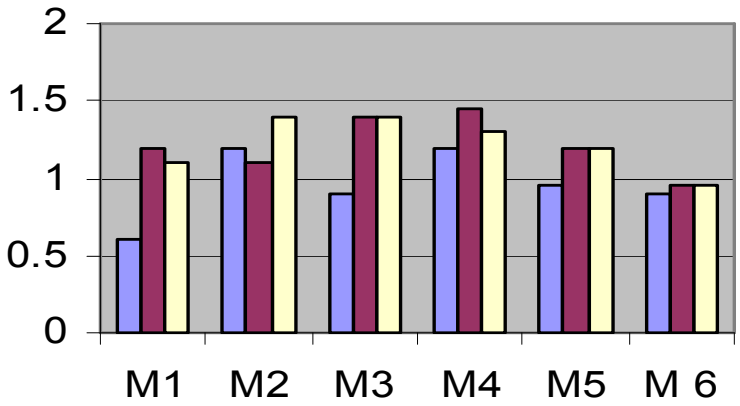


**Fig. 2. The optical density of the bacterial strains biomass resulted by cultivation in improved culture media**

blue-strain Bce2 ;red-strain Fsb1; yellow-strain 83,2s ; M1-M6 the culture media used in the experiment;M6-control culture medium (LB) On vertical line-Optical density (OD).

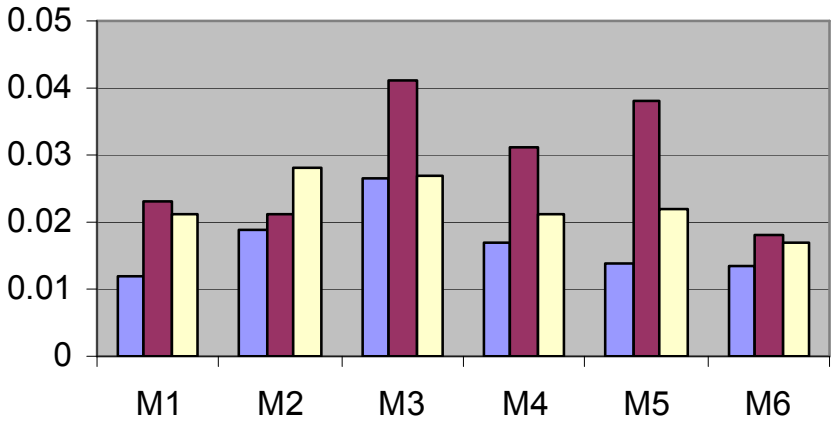


**Fig.3. The quantity of biomass obtained with the improved culture media**
blue-strain Bce2; red-strain FsB1; yellow- strain 83,2s; M1-M5 the culture media used in experiment; M6-control medium (LB). On vertical line-Wet weight Biomass (g/ml ).