

STUDIES ON THE INFLUENCE OF SEVERAL ABIOTIC FACTORS ON SOME NEWLY ISOLATED ANTAGONISTIC STRAINS

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Abstract

The microbial antagonists represent natural means to combat the phytopathogens in order to obtain healthy crops. The abiotic factors such as temperature, pH, NaCl concentration in the growth medium and medium composition have decisive influence on the existence and development of microorganisms. Here are presented the results of the influence of these factors on development of *Pseudomonas* sp. and *Bacillus* sp. strains. Two of these strains, B1 and Bm, identified as *Pseudomonas putida* respectively *Bacillus mycoides* were recently isolated and showed remarkable activity in *in vitro* biocontrol of the phytopathogens *Erwinia carotovora* and *Xanthomonas campestris*. During experiments were tested, in the same conditions, two other strains *Bacillus subtilis* ICCF 84 and *Pseudomonas putida* ICCF 391 from Culture Collection of Industrial Importance Microorganisms (CMII). To study the influence of abiotic factors were chosen five values of temperature, five for the pH and five values for concentration of NaCl from medium. The best results regarding microbial growth were obtained for temperature values of 28 and 32°C, NaCl concentration in the culture medium between 0.5-4% and the pH between 5 and 8. Regarding the composition of the culture medium, of the four types of media, the best for the antagonists growth and development proved to be the medium M44 containing yeast extract, peptone and glycerol.

Key words: abiotic factors, antagonists, biocontrol, phytopathogens.

INTRODUCTION

Biological control of phytopathogenic microorganisms that can induce plants diseases and cause important economic losses can be accomplished by using microbial antagonists (Pamfil et al., 2008).

In order to be able to exercise their action in combating the phytopathogens, the microbial antagonists or beneficial microorganisms require certain conditions for growth and development (Davies and Whitbread, 1989). The abiotic factors such as temperature, pH, NaCl concentration in the growth medium and medium composition have decisive influence on the existence and development of microorganisms (Strickland and Rousk, 2010; Gao et al., 2012). In this situation is needed a good knowledge of abiotic factors which favors or unfavors the biocontrol agents (Ownley, 2003) in supporting the health and growth of the plants (Nihorimbere et al., 2011).

Here we investigated several factors that have decisive influence in development of some strains from the genera *Pseudomonas* and *Bacillus* that showed remarkable antagonistic activity in *in vitro* experiments.

MATERIALS AND METHODS

Growth media and cultivation conditions

Studies on the influence of abiotic factors such as temperature, pH, NaCl concentration in the growth medium and medium composition, were performed in laboratory conditions.

For temperature were chosen the values: 5°C, 16°C, 28°C, 32°C and 37°C.

The influence of pH on strains development was studied for values between 5 and 9. The NaCl concentration in the growth medium had next values: 0.5%, 1.0%, 2.0%, 4.0%, and 8.0%. Regarding the composition of the medium were investigated four recipes, detailed in CMII catalog:

- NA (0.5 g % peptone, 0.3 g % yeast extract, 1.5 g % agar, 0.5 g % NaCl),
- YMPG (yeast extract 0.3 g%, malt extract 0.3 g %, peptone 0.5 g %, glucose 1.0 g %, agar 2.0 g %),
- PDA (potato infusion 20.0 g %, dextrose 2.0 g %, agar 2.0 g %),
- M44 (yeast extract 1.00%, bacteriological peptone 1.00%, glycerol 5.00%, agar 2.00%).

A broth medium with the same composition was used for submerged fermentation. Specific parameters like optical density, pH and the biomass amounts were regularly controlled.

All culture media were prepared with distilled water, pH adjusted and sterilized for 15 minutes at 121°C.

Biologic material

For experiments were used four microbial strains (that showed remarkable antagonistic activity in *in vitro* experiments) as it follows:

- B1 and Bm, newly isolated and identified as *Pseudomonas putida* respectively *Bacillus mycoides*.

- *Bacillus subtilis* ICCF 84 and *Pseudomonas putida* ICCF 391 from Culture Collection of Industrial Importance Microorganisms (CMII) of the National Institute for Chemical-Pharmaceutical Research and Development, Bucharest.

For studying the influence of the temperature, pH and NaCl concentration, the bacterial strains were developed for 48-72 hours on NA medium. When the strains development on different recipes of medium was studied, the microorganisms were allowed to grow for 48-72 hours at 30°C.

RESULTS AND DISCUSSIONS

All the abiotic factors included in this study are very important for microbial growth and development. For best action in controlling the phytopathogenous microorganisms, the biocontrol agents should have certain characteristics like: resistance under extreme temperature (frost or heat), development at different values of pH and of NaCl concentrations, versatility to metabolize the nutrients from the environment.

As it shows in table 1, the optimal temperatures for the growth of the antagonists are situated

between 28-32°C, but there have grown as well at temperatures below 28°C, and also at 37°C.

Table 1. The influence of *temperature* on growth and development of the microbial antagonists

Microbial strain	Temperature				
	5°C	16°C	28°C	32°C	37°C
B1	+	++	+++	+++	+++
Bm	+	++	+++	+++	+++
<i>P. putida</i>	+	++	+++	+++	++
<i>B. subtilis</i>	+	++	+++	+++	+++

Legend: "+"=minimal growth; "++"=good growth; "+++ "=very good growth.

For all five values of pH, the strains Bm and *B. subtilis* ICCF 84 were very good developed (see table 2).

Table 2. The influence of *pH* on growth and development of the microbial antagonists

Microbial strain	pH				
	5	6	7	8	9
B1	+	++	++	+	-
Bm	+++	+++	+++	+++	+++
<i>P. putida</i>	+	++	+++	++	++
<i>B. subtilis</i>	+++	+++	+++	+++	+++

Legend: "+"=minimal growth; "++"=good growth; "+++ "=very good growth; "-" =no growth.

At concentrations of NaCl in the growth medium between 0.5-4.0%, all the strains were developed well (see table 3), but none was developed at the 8.0% concentration.

Table 3. The influence of *NaCl concentration* on growth and development of the microbial antagonists

Microbial strain	NaCl concentration				
	0.5%	1%	2%	4%	8%
B1	++	++	++	+	-
Bm	+++	+++	+++	+++	-
<i>P. putida</i>	+++	+++	+++	++	-
<i>B. subtilis</i>	+++	+++	+++	+++	+++

Legend: "+"=minimal growth; "++"=good growth; "+++ "=very good growth; "-" =no growth.

Regarding the composition of the culture medium (see table 4), of the four types of

media (NA, YMPG, PDA, M44), the best for the antagonists growth and development proved to be the medium M44 containing yeast extract, peptone and glycerol. On this medium all the strains grew in less than 48 h.

A broth medium with the same composition (as M44) was used for submerged fermentation (data not shown). Interestingly those after 62 h the values of optical density were still growing, and at the end of the bioprocess were obtained significant biomass amounts.

Table 3. The influence of the *culture medium* on growth and development of the microbial antagonists

Microbial strain	Culture medium			
	NA	YMPG	PDA	M44
B1	+	++	+	+++
Bm	+++	+++	+++	+++
<i>P. putida</i>	++	++	++	+++
<i>B. subtilis</i>	+++	+++	+++	+++

Legend: “+”=minimal growth; “++”=good growth; “+++”=very good growth.

CONCLUSIONS

The results of the influence of abiotic factors on the development of strains B1 and Bm, identified as *Pseudomonas putida* respectively *Bacillus mycoides*, were very good in terms of adaptability at different values of temperature, pH, concentration of NaCl. During experiments were tested, in the same conditions, two other strains *Bacillus subtilis* ICCF 84 and *Pseudomonas putida* ICCF 391 for which were obtained similar results.

The best results regarding microbial growth were obtained for temperature values of 28 and 32°C, NaCl concentration in the culture medium between 0.5-4% and the pH between 5 and 8. Regarding the composition of the culture medium, of the four types of media, the best for

the antagonists growth and development proved to be the medium M44 containing yeast extract, peptone and glycerol. These results encourages us to affirm that all strains used in this study could be suitable as antagonists as they can resist in such conditions of the environment similar to those studied here.

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