

EXTRACELLULAR ENZYME SYSTEMS OF ANTAGONISTIC *BACILLUS* STRAINS ISOLATED FROM TOMATO RHIZOSPHERE.

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ABSTRACT - Extracellular enzyme systems of antagonistic *Bacillus* strains isolated from tomato rhizosphere.

Chitinolytic, proteolytic and lipolytic enzymes could be important in the biological control of soil borne plant-pathogenic microorganisms by antagonistic microbes and in this way besides antibiotic production, the secretion of certain extracellular enzymes also could have great importance in the effectiveness of biocontrol bacilli. Some of these enzymes could directly promote the inhibition processes, while others could help the competition of the bacilli against other rhizosphere microbes. In case of five *Bacillus* strains, which showed excellent antagonistic effects against phytopathogenic fungi and bacteria, the secretion of some components of the protease, chitinase, cellulase, lipase and β -1,3-glucanase enzyme systems were investigated under inductive and non-inductive circumstances. Enzyme activities were measured both with classical methods and with other experimental approaches based on chromogenic enzyme substrates. The best biocontrol strains constitutively secreted chymotrypsin-like proteases and/or trypsin-like proteases, and lipases. On the contrary, the chitinase components were only secreted in chitin containing media. Cellulases and β -1,3-glucanases were produced only at very low level either in inductive media.

Keywords: *Bacillus*, extracellular enzymes, antagonism

INTRODUCTION

Antagonistic bacteria are able to reduce the population density or disease-causing activities of the pathogens through one or more of the following mechanisms: antibiosis, competition and hyperparasitism (PAULITZ and BELANGER, 2001; WHIPPS, 2001). Among them, hyperparasitism and in some cases antibiosis relies on lytic enzymes capable to degrade of cell walls and membranes of pathogenic fungi and bacteria. First of all, chitinolytic, proteolytic and lipolytic enzymes have been considered important in the biological control of soilborne plant-pathogenic microorganisms (BERKELEY et al., 1973; ORTIZ et al., 1973; PRIEST, 1977; PLEBAN et al., 1997).

Besides the antibiotic production, the secretion of certain extracellular enzymes also could have great importance in the effectiveness of biocontrol bacilli. Some of these enzymes, first of all β -1,3-glucanase, chitinases, lipases and proteases could directly promote the inhibition processes, while others, such as cellulases and xylanases could help the competition of the bacilli against other rhizosphere microbes.

MATERIAL AND METHOD

The used non-inductive medium was YEG (glucose 0.2%, yeast extract 0.2%) the corresponding inducing media contained YEG supplemented with the inducer at 1 mg/ml concentration. The used inducers were: carboxymethylcellulose for cellulase, laminarin for β -1,3-glucanase, casein for proteases, colloid chitin for chitinases and trybutyrin for lipase.

Enzyme activities were measured both with the classical dinitrosalicylic method of MILLER (1959) in the case of β -1,3-glucanase, endocellulase and xylanase, and with chromogenic enzyme substrates in the case of lipase, proteases and chitinases (KREDICS et al., 2001). The taxonomical positions of the isolates with best antagonism ability were determined by partial sequencing the 16S ribosomal RNA genes. For PCR reaction standard conditions were applied with the following primers: Eub-341f (5'-CCTACGGGAGGC AGCAG-3') and UP-765r (5'-CTGTTTGCTCCCCACGCTTC-3') (MUYZER et al., 1993).

RESULTS

Numerous isolates (350 strains) deriving from tomato rhizosphere were investigated in the *in vitro* antagonism tests (Fig. 1). The species identity of the strains with highest antagonistic potential was determined by partially sequencing of their 16S ribosomal genes.

According this, the 5 best performing isolates were the following: B5= *Bacillus mojavensis*, B12= *B. subtilis*, B23= *B. subtilis*, B73= *B. amyloliquefaciens* and B83= *B. amyloliquefaciens*.

In the case of these *Bacillus* strains, which all displays excellent antagonistic effects against both phytopathogenic fungi and bacteria, the secretion of some components of the protease, chitinase, cellulase, lipase and β -1,3-glucanase enzyme systems were investigated under inductive and non-inductive circumstances.

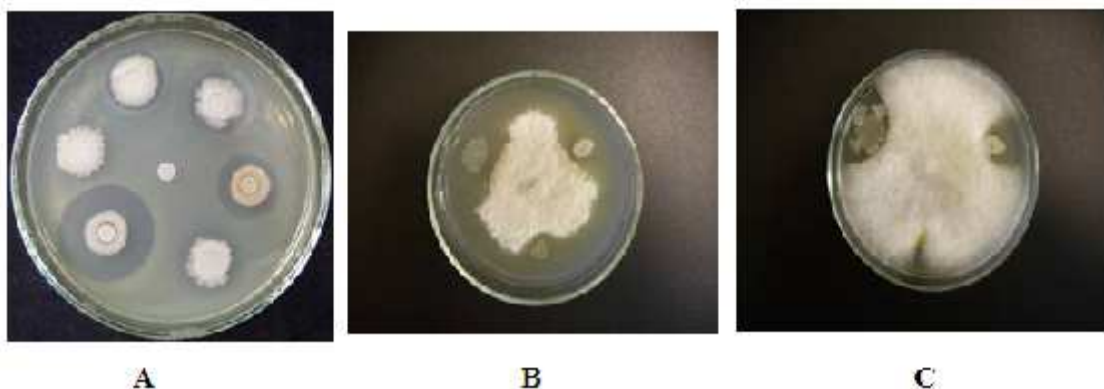


Figure 1: *In vitro* antagonism of some *Bacillus* strains against *Clavibacter michiganensis* (A), *Botrytis cinerea* (B) and *Phytophthora infestans* (C).

The best biocontrol strains constitutively secreted chymotrypsin-like proteases and/or trypsin-like proteases (Fig. 2.), and lipases (Fig. 3.). On the contrary, the chitinase components, NAG-ase (N-acetylglucosaminidase) and chitobiosidase were only secreted in chitin containing media on inductive manner (Fig. 4.). Cellulases, xylanases and β -1,3-glucanases were produced only at very low level either in inductive media (results are not shown).

Relative enzyme activities

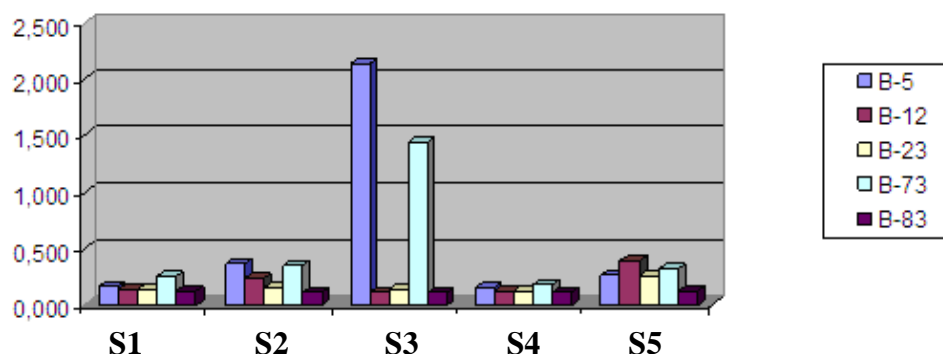


Figure 2: Constitutive secretion of distinct proteases by five *Bacillus* strains as detected with five (S1-5) chromogenic protease substrates.
 S1: Bz-Arg-pNA, S2: Bz-Phe-Val-Arg-pNA, S3: Suc-Ala-Ala-Pro-Phe-pNA, S4: N-acetyl-L-Leu-pNA, S5: CBZ-Ala-Ala-Leu-pNA.

Relative enzyme activities

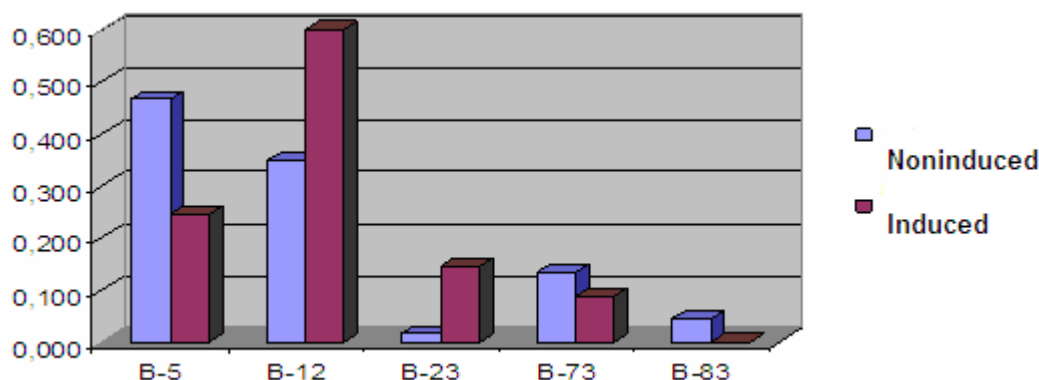


Figure 3: Production of p-nitrophenyl-palmitate splitting enzymes in yeast extract glucose medium (YEG, non-induced) and YEG+ tributyrin medium (induced).

Relative enzyme activities

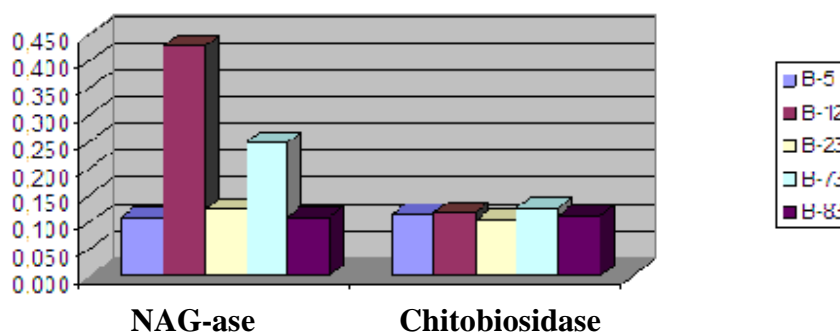


Figure 4: Secretion of chitinases in YEG + colloid chitin medium.

CONCLUSIONS

Besides producing distinct antibiotics the secretion of cell wall and membrane degradation enzymes could also be very important factors of the biocontrol *Bacillus* strains as regards their effectiveness against distinct phytopathogenic fungi and bacteria. In some cases the proteases and lipases are secreted constitutively by the best biocontrol *Bacillus* strains.

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