Volume 58(1):73-94, 2014
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Ecophysiological characterisation of a biocontrol *Bacillus subtilis* strain

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As conventional chemical pesticides considerably increase the environmental load of agricultural areas, serious efforts are made to find and develop effective biocontrol agents with no ecotoxicological risks. Good extracellular enzyme and antibiotic producing microorganisms could be excellent antagonists of phytopathogenic fungi and bacteria. *Bacillus subtilis* is a Gram positive, aerobic, endospore-forming, soil bacterium, which is able to produce various antibiotics and a broad spectrum of extracellular enzymes. This bacterium may produce various non-ribosomal oligopeptides, such as iturin, surfactin and fengycin. These cyclic lipopeptides have both antifungal and antibacterial effects. Previously, elevated protease and -amylase secretion was reported by Kurosawa et al. (2006) from streptomycin resistant *B. subtilis*. This phenomenon appeared in connection with spontaneous mutations in the *rpsL* gene encoding the ribosomal protein S12. The aims of our study were *(1)* to make an ecophysiological characterization of the isolated *B. subtilis* strain and *(2)* to prove the effectiveness of the simple approach of Kurosawa (2006) for generating a series of biocontrol strains without the need for induced genetic modification of the original bacterium.

After isolating several bacteria from soil samples and rhizosphere of tomato, the isolates were identified based on the partial sequencing of the *gyrA* gene. Sequence of the whole genome of one strain (B23), which showed the best biocontrol abilities, was determined and compared with the that of the *B. subtilis* type strain (DSM-10). Antibiotic production of the two strains was also compared by TLC analysis. By sequence analysis, several single-nucleotide polymorphisms were found in various genes involved in the antibiotic production. These changes are suggested to be responsible for the enhanced antibiotic production of the newly isolated strain.

From the B23 isolate, spontaneous streptomycin resistant colonies were selected. Chymotrypsin-type protease activity in the ferment broths of the streptomycin resistant strains were determined and compared with the B23 strain. From the 20 tested mutants the K2 strain was outstanding with its fourfold chymotrypsin producing activity. Among the spontaneous streptomycin resistant mutants, six showed significantly enhanced tyrosine-containing antibiotic production. *In vitro* antagonism of the B23 strain and its streptomycin resistant mutants against phytopathogenic microorganisms and some mycotoxin producing fungi were characterized. Elevated inhibition zones were detected in case of some important pathogens. Effect of metal ions (*i.e.* cadmium, copper, manganese, nickel and iron) and pesticides (*i.e.* 2,4-dichlorophenoxyacetic acid, carbendazim, chlortoluron and linuron) to the enzyme production and activity were also examined. Manganese had positive effect on the enzyme production, while the presence of pesticides had no inhibitory effect. Analysis of the antibiotic profiles in the presence of metal ions and pesticides produced very similar results. Effect of the carbon and nitrogen sources on the production of antibiotics was tested. Saccharose, glycerol, cellobiose, starch, Na-nitrate and proline elevated the production rate of the tyrosine containing antibiotics.

Kurosawa K, Hosaka T, Tamehiro N et al. (2006) Appl Environ Microbiol 72:71-77.

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Investigation of redox homeostasis and elements of abiotic stress responses in *Arabidopsis* model plant

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The growth and yield of plants are highly dependent on environmental factors. The extremes of these conditions act as stressors leading to the formation of reactive compounds in the cells and to the imbalance of redox homeostasis. Adequate stress responses may restore the redox balance. Growing evidence suggests a model for redox homeostasis in which the reactive oxygen species (ROS)-antioxidant interaction acts as a metabolic interface for signals derived from metabolism and from the environment during stress.

The aim of my research was to explore the elements of defence mechanism, with focus on the redox re-establishment of redox homeostasis. In this work, the effect of salicylic acid (SA) and salt stress were investigated using *Arabidopsis thaliana* L. Columbia ecotype (wild type), glutathione reductase (*gr*) and dehydroascorbate reductase (*dhar*) mutant lines which grown in hydroponics. In order to increase salinity tolerance, as a priming effect, plants were pretreated with 10^{-9}-10^{-4} M SA followed by 100 mM NaCl in long-term experiments.

The stress induces serious metabolic perturbations in plants, as it generates ROS which disturb the cellular redox system. In this study we examined the viability of cells and ROS level and its derivatives by fluorescent dyes. We determined the levels of antioxidants and the activities of some antioxidant enzyme such as total ascorbate (Asc) and reduced (GSH) and oxidized (GSSG) glutathione, glutathion reductase (GR) and dehydroascorbate reductase (DHAR), which are protecting plants against ROS damages. The amounts of Asc and GSH increased under stress conditions mainly at 10^{-7}-10^{-5} M SA concentrations also at mutants lines. In addition, maintaining a high ratio of GSH/GSSG showed to play an important role in SA and salt tolerance of *Arabidopsis* wild type and mutants. The activities of GR and