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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

DHAR enzymes also contributed and helped to maintain the cell balance under stress conditions.

Most importantly, antioxidants provide essential information on cellular redox state and they influence gene expression associated with abiotic stress responses to maximize defense. We analyzed also the expression levels of GR and DHAR genes by real-time-PCR with focus on the role of GR and DHAR izoenzymes and our data also showed changes in their transcript levels under stress conditions and in the acclimatization process.

Redox reactions are the fundamental metabolic processes through which cells convert and distribute the energy that necessary for growth and maintenance. *Arabidopsis* plants transformed with a redox-sensitive GFP (roGFP) targeted to the cytosol (c-roGFP1) were used for monitoring the real-time redox status of the cytosol in SA and salt stressed plants. Utilization a flourometer to detect redox-related changes of roGFP has been demonstrated. The utilization of a fluorimeter enables the processing of many samples and it averages the whole tissue rather than only few cells within a tissue, as in the case of confocal imaging.

It is concluded that constitutively high level of reduced GSH are advantageous to act as a strong buffer against ROS but would make the system less responsive to changes in redox potential that may be needed to upregulate the inducible defence components. In this study we have adapted fluorometer reading and compared this assay with confocal imaging. Nevertheless, the data showed that roGFP is redox sensitive in plant cells and that sensor makes it possible to monitor, in real time, dynamic changes in redox homeostasis *in vivo*. During long-term experiments, we were able to apply this technology in combination with many aspects of the antioxidant defence system measurements to the analysis of redox changes in response to stresses or to various mutants.

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Protecting roles of 27 kDa heat shock protein Hsp27

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Hsp27 belongs to the small heat shock protein family, which are ATP-independent chaperones. The most important function of Hsp27 is based on its ability to bind non-native proteins and inhibit the aggregation of incorrectly folded proteins maintaining them in a refolding-competent state. Additionally, it has anti-apoptotic and antioxidant activities.

Several studies have shown cytoprotective effects of Hsp27 against reactive oxygen species. Doxorubicin is a widely used chemotherapeutic agent against several types of cancer. Beside it's cytostatic properties, doxorubicin has a severe cardiotoxic side effect. To study the cardioprotective effect of Hsp27 *in vivo*, a transgenic FVB mouse strain overexpressing the human Hsp27 protein was established. Transgenic mice and their wild type littermates were injected with a single dose of doxorubicin, control animals were treated with saline. We detected significant level of apoptosis in cardiac tissues of doxorubicin treated wild-type mice using caspase-3 immunhistochemistry and TUNEL (terminal deoxynucleotidyl dUTP nick end labelling) assay. However, the number of apoptotic cells were substantially reduced in Hsp27 overexpressing transgenic hearts. Caspase-3 western blot analysis also confirmed the cardioprotective effect of Hsp27 against doxorubicin. Using qPCR analysis, we found significant increase in the expression of proteasomal genes in wild-type hearts after doxorubicin treatment. mRNAs of proteasome subunit 3, Psmc3 interacting subunit and ubiquitin conjugase 4 showed the most remarkable increases. However, overexpression of Hsp27 did not repressed the expression of these genes, suggesting that cytoprotective effect of Hsp27 is not directly linked to proteasome function.

Hsp27 has well known neuroprotective effect as well. Previously, using APPxPSe1xHsp27 triple transgenic mice we have shown that overexpression of Hsp27 protein ameliorates certain symptoms of Alzheimer's disease. Alzheimer's disease (AD) model mice overexpressing Hsp27 showed reduced number of amyloid plaques and improved presynaptic and cognitive functions. In order to clarify the molecular role of Hsp27 in amyloid plaque number reduction, we monitored the gene expression of several genes potentially involved in ß-amyloid metabolism such as APP, ApoA1, ApoD, ApoE, LDLr, Lrp1, Lrp2, Hsp90, and neurodegeneration (NOS1 and NOS2) in the cortex of Hsp27 transgenic mice using qPCR. Expression levels of ApoD and Lrp2 were slightly increased (128% and 128%, respectively), in the brain of Hsp27 transgenic mice compared to wild type controls (100%), whereas there was no change in the mRNA level of APP, ApoE, LDLr, Lrp1, Hsp90, NOS1, and NOS3. Rather surprisingly, cortical expression of ApoA1 was reduced by half in Hsp27 transgenics versus wild type mice. Decreased ApoA1 expression in Hsp27 transgenic mice was further confirmed using western blotting. ApoA1 protein level was reduced in Hsp27 transgenic mice (61.1%), but slightly elevated in AD model mice (126.7%) compared to wild types (100%). However, AD mice overexpressing human Hsp27 protein possessed similar ApoA1 protein level than wild type mice, indicating that Hsp27 influenced ApoA1 expression .

A less studied aspect of Hsp27 mediated cell protection is its possible role in DNA repair mechanisms. Heat shock protein 27 have been reported to be overexpressed in various cancers and to associated with poor prognosis for survival in patients with cancer. Association of Hsp27 with UV light- and radiosensitivity in cancer cells was also shown by several studies. Phosphorylated Hsp27 can stimulate pentose phosphate pathway (PPP) via binding and activating glucose-6-phosphate dehydrogenase (G6PD). PPP is responsible for producing nucleotide precursors for DNA repair, and G6PD-deficient cells are impaired for DNA double strand break (DSB) repair. To study the possible