IDENTIFICATION OF THE COMPONENTS OF A NOVEL PRPE PHOSPHATASE-DEPENDENT REGULATORY PATHWAY CONTROLLING SPORE GERMINATION IN BACILLUS SUBTILIS

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Background and aim: Sporulation in Bacillus subtilis, an ubiquitous Gram-positive endospore former, is one of the most extensively studied developmental programs in terms of molecular and cell biology. However the picture of the signal transduction processes occurring during sporulation and spore germination in this bacterium is still unclear. Recent studies revealed that the B. subtilis protein phosphatase PrpE is an important component of a novel regulatory pathway controlling, in a direct or indirect way, the expression of genes coding for germination receptors. Germination of spores produced by the strain lacking PrpE is impaired in the presence of L-alanine. The aim of the project is to identify other components of the PrpE-dependent regulatory pathway controlling spore germination in B. subtilis.

Methods: Random transposon mutagenesis was used to create a library of mutants of the

B. subtilis strain that is deleted for prpE. To recognize mutants with a reversion of the phenotype displayed by the strain lacking PrpE, a fast screening assay was developed. Transposon insertion sites from the selected mutants were mapped with the use of inverted PCR.

Results: Of the approximately 1100 mutants that have already been screened, 12 produced spores exhibiting a germination phenotype similar to that of spores of the wild-type strain. In most cases, the selected mutants were found to carry transposon insertions in genes whose function is unknown.

Conclusions: The identified genes are potentially involved in the PrpE-dependent spore germination in B. subtilis. Further plans involve the construction of E. coli strains overproducing the candidate proteins, followed by the in vitro analysis of the proteins as potential PrpE substrates.