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Tightly induced genetic stress response to phenolic acids in Gram-positive bacteria and its use to develop tools for gene expression

Jean-François CAVIN*, Jérôme GURY, Hélène SERAUT, Phuong-Ngoc TRAN, Min Ngoc TA, Véronique DARTOIS, Lise BARTHELMEBS

Laboratoire de Microbiologie UMR UB/INRA 1232, ENSBANA Université de Bourgogne 1 esplanade Erasme 21000 Dijon, France. *Correspondence to: cavinjf@u-bourgogne.fr

Microorganisms are naturally and constitutively adapted to the physical, chemical and biological characteristics of their natural and usual environment. Moreover, they can survive rapid shifts of these characteristics by expressing genes encoding stress proteins or enzymes, which allow them to adapt to the new conditions. Phenolic acids, like *p*-coumaric and ferulic acids, are abundant compounds in the plant kingdom because they are involved in the structure of plant cell walls, and are present in the vacuole. In plant-soil ecosystems, but also in the digestive tract of some animals, they are released as free acid by hemicellulases produced by several fungi or bacteria and in this form display antimicrobial activity. By using reverse genetic and random transposon mutagenesis, we have shown that free phenolic acids are able to induce a specific chemical stress response in several Gram (+) bacterial species. This response involves a substrate inducible *padA* gene encoding a phenolic acid decarboxylase enzyme (PadA) and a *padR* gene encoding PadR, the negative transcriptional regulator of *padA* gene expression. The PadA enzyme degrades the phenolic acids into vinyl phenol derivatives, which are not toxic for these bacteria. PadR, which, in the repressive form, tightly binds to the *padA* promoter, is released from this promoter soon after the addition of phenolic acid to the medium. PadR is the first member of a now fast growing family of transcriptional regulators (Pfam PF03551), which are being identified in genome sequencing projects. However, except for PadR, only AphA, which activates a virulence cascade in *Vibrio cholerae*, and LmrCD, a major determinant of both acquired and intrinsic drug resistance in *Lactococcus lactis*, have been well characterized. We have characterized the *padA-padR* system in *Bacillus subtilis* and several lactic acid bacteria (LAB), especially in *Lactobacillus plantarum.* This bacterium, which is a model of LAB from the plant kingdom and a starter for the elaboration of many fermented foods, is now considered as a valuable probiotic for animals and humans. Although gene organization differs from one species to another, the expression of the *padA* gene, which is not detectable in cells growing without phenolic acid, is tightly induced by a factor of about 8,000 only a few minutes after the addition of phenolic acid to the growth medium. DNA mobility shift assays and DNAseI foot printing demonstrate that this expression results from the inactivation of PadR by phenolic acid through a mechanism which is not yet completely elucidated in spite of the implementation of various experimental strategies (random mutagenesis, knockout, heterologous expression, biochemistry…). In *padR* knockout *L. plantarum and B. subtilis* mutants, the PadA enzyme is the main protein produced in the bacteria. In the wild strain of *B. subtilis*, cloning an *L. plantarum* gene under the *padA* gene promoteur into both non-integrative and integrative vectors generated an inducible expression of this gene. In the *padR* knockout mutant *B. subtilis* strain, a constitutive over-expression of this gene was obtained with the same plasmids. This kind of work is currently under progress in *L. plantarum*. The *padA-padR* system has shown itself to be an effective tool to express either constitutively, or with induction, genes of interest for basic research and biotechnology.