Quantification of microbial gene expression by RT-quantitative PCR

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Handbook of Methods used in Rhizosphere Research

4.4. Gene Expression of Single Species and Communities

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Parameter	**Quantification of microbial gene expression by RT-quantitative PCR**
System	Microbial culture
Method	**RT-quantitative PCR**
Method description	Monitoring of the expression of selected microbial genes in response to different stresses. This method allows the rapid and reliable quantification of the expression of several genes, focusing a specific microbial activity (catabolism of xenobiotics,…). Total mRNA are reverse transcripted using specific reverse primer. The cDNA target is then amplified by quantitative PCR using specific primer pairs in presence of SYBR Green. Positive controls consisting of several dilutions of standards are also amplified to check for quantitative PCR efficiency. Negative control consisting of quantitative PCR conducted directly on total RNA are also realised to check for any DNA contaminations. The determination of the cycle threshold (Ct) allowed the determination of mRNA copy number using the calibration curve reporting the mRNA copy number as a function of the Ct.
Do's, don'ts, potential limitations, untested possibilities	• The RT-quantitative PCR allows the quantification of the level of expression of gene in less than 45 min. The impact of different stresses on gene expression can easily be compared. • This technique allows the quantification of lowly expressed gene since up to 1 copy can be detected by quantitative PCR from a complex cDNA template. In addition, the expression of genes belonging to multifamily can be differentiated by using TaqMan® RT-quantitative PCR based on the use of specific probe labelled with fluorescent quencher and reporter.
References	Devers, M.; Soulas, G.; Martin-Laurent, F. 2004. Real time reverse transcription PCR analysis of gene expression of atrazine catabolism genes in two bacterial strains. J. Microb. Methods 56: 3-15.
Additional information	A more detailed protocol for use of this approach is available upon request from the author.