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Application of the cDNA-AFLP method for studying gene expression in *Fibrobacter* succinogenes S85 exposed to 134.2 kHz electromagnetic field

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ABSTRACT

Many biological effects related to the exposure of cells and tissues to electromagnetic fields have been reported in the literature, including those influencing DNAs and RNAs structure and functions. Rumen bacteria metabolism was shown to be affected by a 48-h exposure to 134.2 kHz electromagnetic (EM) field induced by the transponder reading system used for animal electronic identification. In this study, we report a preliminary investigation on growth and gene expression in *Fibrobacter succinogenes* cells, exposed *in vitro* to this EM field, with the aim of detecting possible induced changes in gene expression using a modified cDNA-AFLP procedure.

Pure cultures of *F. succinogenes* S85 were grown anaerobically at 37.5 °C in Hungate tubes, in liquid synthetic medium. The tubes were randomly allocated to control and treated group, the latter continuously exposed to a 134.2 kHz electromagnetic field generated by the transponder reading system. Samples were harvested from both groups after 6, 8, 10, and 12 h incubation. Total RNA was isolated by TRIzol reagent according to the manufacturer's instructions and cDNA synthesis was carried out using a standard procedure, starting from 500 ng of RNA after a treatment with RNase-free DNase.

Restriction of cDNA was carried out using EcoRI and MseI as rare and frequent cutter as derived from restriction map analysis carried out with the in-house developed software Virtual AFLP. The respective adapters were ligated to obtained fragments. Successively, the AFLP procedure was performed according to standard protocols. Reaction products were separated on a 6% denaturing polyacrylamide gel. Differentially expressed mRNA fragments were then eluted, cloned into a pCR4-TOPO and sequenced with ABI PRISM 377. Similarities for all cDNA clones were sought in the NCBI with the BLAST applications. The reproducibility of the technique, starting from four extractions of RNA from the same sample, was also tested. All samples showed the same pattern on polyacrylamide gel. cDNA-AFLP were carried out on control and treated samples of *F. succinogenes* obtained at the four incubation times. Specific transcripts with qualitative (presence vs absence) and quantitative expression differences between control and treated cultures were detected. Six transcript-derived fragments, detected using 8 primer combinations, resulted clearly up or down modulated in treated samples. The obtained sequences did not show similarity with genes related to a possible answer to EM field. In conclusion, the technique can be successfully used to study the gene expression in *F. succinogenes* under *in vitro* conditions. However, other primer combinations have to be tested to understand the effects of the EM field on this microorganism.