IN SITU PCR AMPLIFICATION OF 16SRDNA AND *GYRB* GENE IN *ACINETOBACTER VENETIANUS* CELLS

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Abstract

The *in-situ* polymerase chain reaction (*in-situ* PCR) is a molecular technique combining the extreme sensitivity of the PCR with the *in-situ* hybridization. *In-situ* PCR is used to detect very low quantities of DNA in intact cells. We present a protocol, which has been used to amplify the 16S rDNA and the *gyrB* gene in *Escherichia coli* and in *Acinetobacter venetianus* cells. The latter has been isolated from the Venice Lagoon and is able to perform the efficient oxidation of *n*-hydrocarbons and could be used for bioremediation purposes in polluted areas.

Keywords: in-situ PCR, Acinetobacter venetianus, Escherichia coli, FISH.

In-situ PCR is a modification of PCR in which the amplification and the detection of specific target nucleic acid sequences are carried out inside intact cells rather than on extracted nucleic acid. The detection of the amplification signal is performed by fluorescent *in-situ* hybridization (FISH). In line of principle, individual genes, mRNA, and rRNA are all candidate targets for this technique. In this way genetic abilities and their expression, and taxonomic information are all potentially accessible on the individual cell level. This approach has been used successfully in eukaryotic cells, whereas very little is know about the possibility to amplify DNA or RNA sequences in whole prokaryotic cells. The application of this approach to microbial ecology should enable the *in-situ* analysis of gene content in bacteria, also including the tracking of conjugative plasmids or transposable elements. Cells might also be analyzed in their natural or experimental environment, as in the case of complex microbial communities (1). Up to now, few data are reported regarding this issue, and concern with *Escherichia coli* and *Pseudomonas* cells (1, 2, 3), but, in line of principle, this methodology could be applied to every (micro) organism. Therefore, this approach could be extremely useful for the analysis of natural microbial communities isolated from polluted areas.

In this study, we present the set up *in-situ* PCR for the detection of *A. venetianus* VE-C3 cells. This bacterium has been isolated, as a component of a microbial community, from polluted seawater of Venice Lagoon (5). It has been previously demonstrated that this strain is able to perform efficient oxidation of diesel-fuel (5), opening the possibility of its use as a biosensor. Therefore, the availability of a technique enabling the measurement of the distribution of this bacterium in polluted environments, and also the expression of genes involved in biodegradation of *n*-hydrocarbons would be of great importance.

The following experiments were carried out on pure cultures of *A. venetianus* VE-C3 and *E. coli* Xl1-blue cells. Cells of the two bacterial strains, cultured in LB medium, were fixed in 4% paraformaldehyde; cell wall permeabilization was then achieved by the use of lisozyme. PCR was performed on whole cells by using two primers allowing the amplification of nearly the entire 16S rDNA. During PCR, the amplified sequences were also labelled with digoxigenin11-dUTP. Samples were then spotted on slides and incubated with a hybridization solution containing alkaline phosphatase - labelled anti-DIG antibodies conjugate with FITC. Slides were analyzed under a fluorescence microscope and the target cells labelled with FITC were visualized under blue-green light excitation (488 nm). Results are shown in figures 1 and 2; data obtained showed the successful amplification of 16S rDNA in whole *A. venetianus* cells (Fig. 1), whereas no fluorescent signal was found in the control experiment (Fig. 2). The same results were obtained with *E. coli* Xl1-blue cells (not shown).

In order to check the possibility of using other target sequences for *in-situ*- amplification, we choose the *A. venetianus gyrB* sequence (encoding the b-subunit of DNA gyrase) as target in the further experiments. For this purpose, the VE-C3 *gyB* sequence was firstly amplified *via* PCR by using the primers previously described by Yamamoto et al (4). The amplification product was cloned, and its nucleotide sequence determined.

Two oligonucleotides were then designed on this sequence and used as primers in *in-situ* PCR experiments on whole *A. venetianus* VE-C3 cells. Data obtained (not reported) revealed the amplification of the *gyrB* sequence inside the *A. venetianus* cells.

This body of data suggested that in-situ PCR can be successfully used to amplify DNA sequences within *Acinetobater* cells and might represent a powerful tool for identification and monitoring of this bacterium in microbial communities.

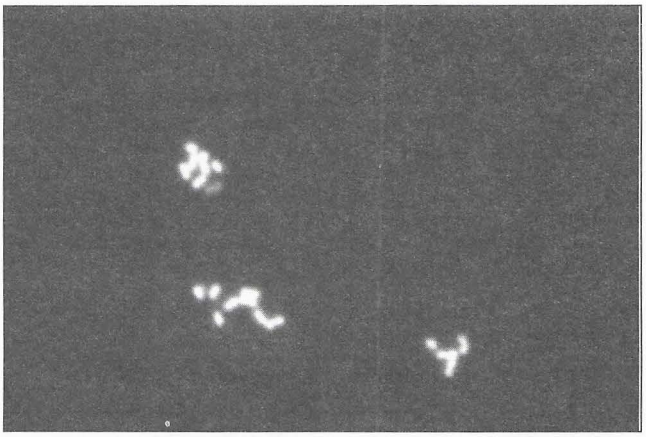

Fig. 1. *In-situ* PCR amplification of 16S rDNA sequence in VE-C3 *Acinetobacter venetianus* fixed cells.

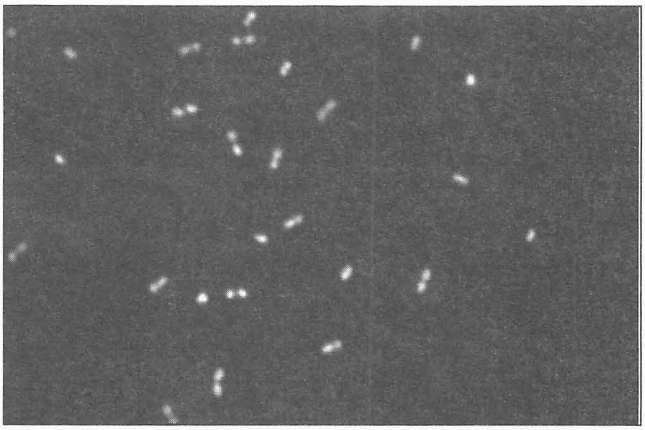

Fig. 2. *In-situ* PCR control reactions.

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