Journal of Ecotechnology Research 8(2), 250-251 (2002)

POLYMERASE CHAIN REACTION TECHNIQUE FOR MICROBIAL AND ENVIRONMENTAL INVESTIGATIONS: PRELIMINARY REPORT ON CONTAMINATED LAGOON SEDIMENT#

HASSEN Abdennaceur *, CHERIF Ameur *, BELGUITH Khaoula*, SAIDI Neila*, CHERIF Hanène* and YOSHIDA Mitsuo**

* Laboratoire Eau & Environnement, Institut National de Recherche Scientifique et Technique (INRST), B.P.95, Hammam-Lif, Tunisia. E-mail : abdohassen@yahoo.fr
** Institute for International Cooperation, Japan International Cooperation Agency (JICA), 10-5, Ichigaya Honmura cho, Shinjuku-ku, Tokyo 162-8433, Japan. E-mail : Yoshida.Mitsuo.2@jica.go.jp

Abstract

Microbial characteristics of contaminated lagoon bottom sediment collected from the Bizerte Lagoon, Mediterranean coast of northern Tunisia is investigated using polymerase chain reaction (PCR) technique. Preliminary results are summarized in Table 1 and Figure 1. The rDNA-PCR technique can be used in strains identification (16S-ARDRA) and phylogenetic relationship investigations.

Keywords : PCR, DNA, Microbial species, Contaminated sediment

The PCR can amplify specific sequences of DNA and has been developed as a recent specific and sensitive technique that can be used for direct detection of microorganisms in aquatic, food or dairy products, and terrestrial environments. PCR can also be used to detect microorganism environments that are difficult to culture in vitro and to determine the fate of particular organisms or genetically engineered microorganisms. This technique can also be used to study complex natural environments from which only a small percentage of the microflora can be isolated in vitro such as enteric viruses. In fact, enteric virus transmission due to the consumption of feacally-contaminated potable water, foods and shellfish is a significant public health concern. While the fecal coliform groups of bacteria are accepted as the criterion for the sanitary quality universally of potable water, foods and shellfish, the scientific consensus is that these indicators do not reflect the occurrence of enteric viruses in the marine environment. In the absence of indicator systems, investigators have attempted the effective detection of viruses from marine sediments, shellfish and their harvesting direct waters. These techniques have relied on cumbersome methods of virus extraction and subsequent detection based on virus infectivity for susceptible, live laboratory hosts. These virus concentration and detection steps are expensive, time-consuming, technically difficult, and lacking in sensitivity. They are further limited by the need for large sample sizes to detect the low virus levels that still pose a health risk and the lack of susceptible hosts for the more epidemiologically important viruses, such as hepatitis A virus (HAV) and the Norwalk- type viruses. Reverse transcriptase (RT) PCR is a rapid, economical, sensitive, and specific approach, which has been used recently for the amplification of nucleic acid sequences for the HAV, rotaviruses, and Norwalk enteroviruses virus in clinical fecal specimens for which expected viral contamination levels are high. However, there is a need to develop methodology designed specifically to detect the lower levels of viral contamination typically expected in environmental samples.

In order to develop procedures to detect intact enteric viruses from soil/sediment samples, in the first step of our research project, we have chosen contaminated sediments collected from the Bizerte lagoon in Tunisia as a pilot sample for identifying dominant microbial species and analyzing the phylogenetic relationship between these strains.

We have used the ribosomal operon (a classic molecular marker) commonly used to summarize genetic relationships and to identify strains rapidly. Moreover, ribosomal operon analysis can differentiate between the genomic lineages of a single species, as in the case of

This collaborative research was made as a part of the technical cooperation programme between INRST and JICA (2000-2002).

Pediococcus acidilactici strains that produce, or not, pediocin AcH/PA1 and in bacteriocinogenic baccilli respect to sensitive strains. Of all the different regions of the ribosomal operon, the 16S rDNA and the internal transcribed spacers (ITS) between 16S and 23S rDNA are frequently used as molecular markers to identify microbial species and analyze the phylogenetic relationship between strains. To extract DNA 1.5 ml of an overnight culture in LB broth was pelletted and the cells washed three times with physiological saline. Total DNA was extracted from the washed cells by SDS-proteinase K treatment. Amplification of the 16S rDNA and the ITS was performed using the already described primers (Table 1). PCR was performed with a *BIOMETRTA T-Personal* automated thermocycler with 0.2-ml thin walled PCR tubes. Reactions were carried out in 50 ul-volumes containing 5 ul of 10 X PCR buffer (supplied with *Taq* DNA polymerase, Appligene), 2.0 mM $MgCl_2$, 50 pM of each oligonucleotide primer (Table 1), 0.2 mM each deoxynucleotide triphosphate (dATP, dCTP, dTTP and dGTP), 1 U of *Taq* DNA polymerase (Appligene) and 50 ng of extracted DNA. Amplification of the 16S rDNA and the ITS was attempted with 30 cycles at 95°C for 30s, 55°C for 45s and 72°C for 45s, followed by a final extension step at 72°C for 7 mn.

Table 1. Sequences and positions of the used primers.

Primer	Target Sequence	Position	Sequence 5'---3' [a]	Expected size
S-D-Bact-0008-a-S-20	ADNr 16S	8-28	AgAgTTTgATCCTggCTCAg	1486
S-D-Bact-1495-a-A-20	ADNr 16S	1474-1494	CTACggCTACCTTgTTACgA	
S-D-Bact-1494-a-S-20	ITS 16S-23S	1494ADNr 16S	gTCgTAACAAggTAgCCgTA	Pattern
S-D-Bact-0035-a-A-15	ITS 16S-23S	35 ADNr 23S	CAAggCATCCACCgT	

For each strain the PCR-products were analyzed individually by electrophoresis. The ITS-PCR and the 16S amplifications patterns were electrophoresed on standard 2% agarose gels in 1× Tris-Acetate-EDTA buffer (50X is 2 M Tris base, 57.1 ml Acetic Acid, 0.05 M EDTA-Na_2, pH 7.9) and stained for 30 min in a 0.5 mg/l solution of ethidium bromide.

Preliminary results were displayed in Figure 1. Example of ITS-PCR fingerprinting patterns of Bacillus strains resolved by 2% agarose gel electrophoresis, showing the migration of some amplified products. Lanes M, 50 bp ladder. Lanes 1-9, *B. cereus* strains 256, 282, 582, 846, 7700, 7702, Cepanzo, 4229, Davis TE702. The 100 bp and 500 bp bands of the ladder are indicated.

The rDNA-PCR technique can be used in strains identification (16S-ARDRA) and phylogenetic relationship investigations. Subsequent analysis can be performed such as sequencing in order to confirm identification.

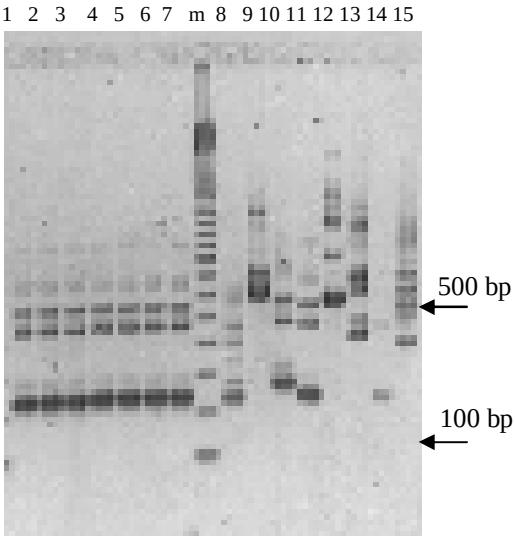

Figure 1. ITS-PCR patterns