Direct comparison of single-strand conformation polymorphism (SSCP) and denaturing gradient gel electrophoresis (DGGE) to characterize a microbial community on the basis of 16S rRNA gene fragments

Tomoyuki Hori \textsuperscript{a}, Shin Haruta \textsuperscript{a,*}, Yoshiyuki Ueno \textsuperscript{b}, Masaharu Ishii \textsuperscript{a}, Yasuo Igarashi \textsuperscript{a}

\textsuperscript{a} Department of Biotechnology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan

\textsuperscript{b} Kajima Technical Research Institute, Tobitakyu 2-19-1, Chofu-shi, Tokyo 182-0036, Japan

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Abstract

Characterization of microbial communities using single-strand conformation polymorphism (SSCP) was compared with that using denaturing gradient gel electrophoresis (DGGE). This comparison was based on the V3–4 region (Escherichia coli positions: 341–806) of 16S rRNA gene of bacterial or archaeal communities obtained from a methanogenic bioreactor. Significant differences in the bacterial banding profiles were observed while attempting to detect the diversity of the community and its succession during the reactor operation. The SSCP produced a number of sharp bands and differentiated the bacterial community structures to which the DGGE gave an identical pattern. On the other hand, the SSCP and DGGE provided similar succession patterns for archaeal community.

Keywords: Single-strand conformation polymorphism; Denaturing gradient gel electrophoresis; 16S rRNA gene fragment

It is widely recognized that microbial diversity is underestimated when culture-dependent approaches are used. In the last two decades, a number of culture-independent methods have been used for investigating characteristics of the microbial communities, e.g., physiological examination such as community-level physiological profiling (CLPP), chemical biomarker examination such as phospholipid fatty acid analysis (PLFA), and nucleic acid examination such as terminal restriction fragment polymorphism (T-RFLP), denaturing gradient gel electrophoresis (DGGE), and single-strand conformation polymorphism (SSCP) (Kowalchuk et al., 2004). Recently, SSCP has been applied to various environmental samples (Junca and Pieper, 2004; Lee et al., 1996; Wenderoth and Abraham, 2005). SSCP is one of the PCR-based fingerprinting techniques and has advantages in identifying members of the microbial community because the DNA bands can be recovered from the gel and sequenced. The determination of the community members can be also performed in DGGE. SSCP separates PCR amplicons having the same length but different nucleotide sequences on the basis of the conformation of single-stranded DNA (Orita et al., 1989; Schwieger and Tebbe, 1998), while the separation
of PCR amplicons by DGGE depends on the denaturation of double-stranded DNA in the gel containing DNA denaturants (Muyzer et al., 1993). DGGE requires the use of long PCR primer with region of high GC content (GC clamp) and is affected by heteroduplex of PCR amplicons; these possibly produce pseudobands on the DGGE profile (Ferris and Ward, 1997; Lee et al., 1996). It has been thought that SSCP profile is not affected by the unusual PCR using the long primer and/or the heteroduplex. However, there has been no proper comparison between the efficacies of these methods in the examination of microbial communities. In this study, we investigated whether SSCP and DGGE provided the same information regarding the diversity and succession of a microbial community in a methanogenic bioreactor.

In order to compare these fingerprinting techniques, we applied the PCR amplicons of V3–4 region of 16S rRNA gene (rDNA) to both SSCP and DGGE. In addition, comparative analysis with DGGE based on V3, which has been reported to produce the most informative amplicons for DGGE (Yu and Morrison, 2004), was also performed.

V3–4 region of 16S rDNA was selected to evaluate the fingerprinting techniques; the length of this region is approximately 450 bp, longer than the V3 region (approximately 170 bp). The PCR primers which have been reported (Casamayor et al., 2002; Hansen et al., 1998; Takai and Horikoshi, 2000; Watanabe et al., 2001) were modified to widen the availability on the basis of the nucleotide database updated in 2004 using ARB program (http://www.arb-home.de/) (Table 1). The availability of the primers was confirmed using Probe_Match tool of the ribosomal database project II (http://rdp.cme.msu.edu/). The primer set B342If/ U806Ir was used to amplify the V3–4 region of bacterial 16S rDNA. The archaeal V3–4 fragment was amplified by nested PCR. The first and second primer sets were U341If/A1399r and A348If/U806Ir, respectively. PCR amplification was performed under the thermal cycle conditions as follows; a total of 25 cycles, each including 60 s at 93 °C, 60 s at 50 °C, and 70 s at 72 °C; followed by a final extension step of 5 min at 72 °C, except for the first PCR cycle for archaeal DNA that employed 35 cycles. SSCP analysis was carried out according to the procedure described previously (Schmalenberger and Tebbe, 2003). Conversion of PCR amplicons to single-stranded DNA was performed by enzymatic digestion with lambda exonuclease (New England BioLabs, Beverly, MA). We used a gel matrix (MDE®, FMC Bioproducts, Rockland, ME) at a dilution of 0.675-fold and prepared a gel of 16 cm length and width, 1 mm thickness in 0.5 × TBE (45 mM Tris–HCl, 45 mM boric acid, 1 mM EDTA, pH 8.0). Electrophoresis was performed at 20 °C at 400 V for 20 h in a running buffer of 0.5 × TBE. DGGE analysis was performed as reported previously (Haruta et al., 2002). The polyacrylamide and denaturing gradient for V3–4 region were optimized as follows: a 6–9% polyacrylamide and a 30–55% denaturant. The gel dimensions and the electrophoresis apparatus (DCode system, Bio-Rad Laboratories, Hercules, CA) used for SSCP and DGGE were the same in this study.

Successive changes in bacterial and archaeal communities have been observed during the start-up period of anaerobic bioreactors (Leclerc et al., 2001; Liu et al., 2002), and these successions are thought to be appropriate for the comparison of the fingerprinting techniques. Anaerobic digestion sludge obtained from a thermophilic methanogenic bioreactor that had been fed with garbage slurry was cultivated in a 1.4 l stirred tank reactor at 55 °C. A previously described medium that contained glucose (1%, w/v) as a sole carbon and energy source (Ueno et al., 2001) was continuously supplied to the reactor at a dilution rate of 0.1 day−1.

Fig. 1 shows the change in the reactor performances. The pH gradually decreased from 8.0 to 7.1 during the first 14 days of operation, and was then maintained at 7.1 by automatic titration with NaOH. The methane gas production rate randomly fluctuated within the range of 1 L day−1.
Acetate degradation was observed before day 6. Propionate concentration gradually decreased and declined to less than 1.1 mmol l$^{-1}$ on day 16. The culture samples, which were taken every three days, were subjected to DNA extraction (Zhu et al., 1993). The V3–4 region of 16S rDNA from bacterial or archaeal communities was analyzed by SSCP or DGGE (Fig. 2A, B, D, and E). The bacterial SSCP profile (SSCP/V3–4, Fig. 2A) produced 16 sharp bands that could be easily differentiated. In contrast, the bacterial DGGE profile (DGGE/V3–4, Fig. 2B) produced fewer smear bands (8 bands). The bacterial SSCP profile of V3–4 showed a high resolution, e.g., the band indicated by arrowhead in Fig. 2A that was observed in the period from day 12 to day 18; however, such temporary appearance of the band was not detectable on DGGE/V3–4 (Fig. 2B). On the other hand, the DGGE profile utilizing V3 (DGGE/V3, Fig. 2C) produced clear bands and detected appearance of particular band (indicated by arrowhead in Fig. 2C) as well as SSCP/V3–4. Moreover, the community succession was characterized by the simultaneous changes of several bands on the profile. Thus, in order to compare the succession pattern of these profiles, we applied principle component analysis (PCA) using the software JMP version 5.1 (SAS Institute, Tokyo, Japan) (Fig. 3). The bacterial SSCP and DGGE fingerprints were converted to binary data on the basis of the migration and presence of bands using the software Quantity One version 3.1 (Toyobo, Tokyo, Japan) and the data were applied to PCA. The PCA plots demonstrated the clear differences between SSCP/V3–4 and DGGE/V3–4. The bacterial communities studied were classified into 8 points on the PCA plot of SSCP/V3–4 (Fig. 3A), while they were classified into 4 points on the PCA plot of DGGE/V3–4 (Fig. 3B). The bacterial succession revealed by SSCP/V3–4 possibly reflects the successive changes in the substrate during the start-up period. PCA of DGGE/V3 indicated a similar succession as that of SSCP/V3–4. However, no difference was observed in DGGE/V3 on days 12 and 15 (Fig. 3C); although there was a difference in propionate concentration and methane gas production rate on these days. Only three bands detected on the archaeal SSCP/V3–4 during the operation (Fig. 2D). The archaeal DGGE/V3–4 also produced three dominant bands (Fig. 2E). In contrast with the multiple patterns of the bacterial community succession (Fig. 3), both archaeal fingerprints showed a similar succession along with the duration of operation, i.e., gradual disappearance of bands b and f at the late stage, delayed appearance of bands c and e, and continuous existence of bands a and d. The succession of the archaeal community was more pronounced than that of the bacterial community. Archaeal DGGE/V3–4 was comparable to that of SSCP/V3–4, possibly because of the simple structure of the archaeal community, which is composed of few divergent species.

The results of this study demonstrated that, on the basis of V3–4 region of 16S rDNA, SSCP was superior in detecting the dynamics of this microbial community to the DGGE (Fig. 2A,B and Fig. 3A,B). A longer DNA fragment has known to induce a lower resolution of the DNA fingerprint (Yu and Morrison, 2004). However, SSCP/V3–4 was capable of detecting the subtle difference of the community succession which DGGE/V3 could not recognize (Fig. 3A,C). It is also noteworthy that SSCP/V3–4 revealed the comparable or higher resolution to DGGE/V3.
Fig. 2. SSCP and DGGE profile of bacterial and archaeal communities within the methanogenic bioreactor. (A) Bacterial SSCP profile of V3–4 region; (B) Bacterial DGGE profile of V3–4 region; (C) Bacterial DGGE profile of V3 region; (D) Archaeal SSCP profile of V3–4 region; (E) Archaeal DGGE profile of V3–4 region. The culture samples were taken every 3 days from day 0 to day 30 and the extracted DNA were applied to PCR-SSCP and -DGGE. Arrowheads indicate the bands which specifically occurred in the period from day 12 to day 18 on the bacterial banding profiles. Arrows in (D) and (E) indicate the dominant bands designated a to f on the archaeal banding profiles. Archaeal SSCP and DGGE profiles showed a similar succession pattern, i.e., gradual disappearance of bands b and f at the late stage, delayed appearance of bands c and e, and continuous existence of bands a and d.

Fig. 3. Principal component analysis of the bacterial SSCP and DGGE profiles. (A) SSCP of V3–4 region; (B) DGGE of V3–4 region; (C) DGGE of V3 region. The successive time points (days) are connected by arrows.
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References


