ISSN (Print) 1225-9918
ISSN (Online) 2287-3406
Journal of Life Science 2017 Vol. 27. No. 11. 1290~1298 DOI : https://doi.org/10.5352/JLS.2017.27.11.1290

A PCR Denaturing Gradient Gel Electrophoresis (DGGE) Analysis of Intestinal Microbiota in Gastric Cancer Patients Taking Anticancer Agents

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Received July 31, 2017 /Revised September 13, 2017 /Accepted September 27, 2017

Intestinal microbiota is an important factor in the development of immune defense mechanisms in the human body. Treatments with anticancer agents, such as 5-Fluorouracil, Cisplatin, and Oxaliplatin, significantly change the temporal stability and environment of intestinal bacterial flora. The anticancer treatment chemotherapy often depresses the immune system and induces side effects, such as diarrhea. This study investigated the effects anticancer agents have on the intestinal microbial ecosystems of patients with gastric cancer. An exploration of the diversity and temporal stability of the dominant bacteria was undertaken using a DGGE with the *16S* rDNA gene. Researchers collected stool samples from patients zero, two and eight weeks after the patients started chemotherapy. After the treatment with anticancer agents, the bacteria strains *Sphingomonas paucimobilis*, *Lactobacillus gasseri*, *Parabacteroides distasonis* and *Enterobacter sp.* increased. This study focused on the survival of the beneficial microorganisms *Bifidobacterium* and *Lactobacillus* in the intestines of cancer patients. The administration of antigastric cancer agents significantly decreased *Lactobacillus* and *Bifidobacterium* populations and only moderately affected the main bacterial groups in the patients' intestinal ecosystems. The results showed the versatility of a cultivation independent - PCR DGGE analysis regarding the visual monitoring of ecological diversity and anticancer agent-induced changes in patients' complex intestinal microbial ecosystems.

Key words : Anticancer agent, diversity, intestinal microbiota, PCR-DGGE

Introduction

Gastric cancer is the leading cancer type in Korea. Treatment for cancer may include surgery, chemotherapy and radiation therapy. Gastric cancer patients often have to undergo major abdominal surgery along with chemotherapy. Combination chemotherapy [1] using 5-fluorouracil, carmustine, semustine, and doxorubicin, and more recently cisplatin, and platinum, have been used for the treatment of advanced cancer. These drugs are highly effective antibacterial agents [5, 12], of which some can be administered orally, thereby having major pharmacoeconomic advantages over other antibiotics [4]. Yet these agents have been shown to induce substantial short- and long-term side effects, including diarrhea and constipation [20]. Evidence demonstrates that chemotherapy-induced diarrhea is associated with an imbalance in the intestinal bacteria ecosystem [22].

The distal intestinal tract of humans harbors a complex bacterial community termed the microbiota, the composition of which is represented in the feces. Methods in molecular microbiology have become a valid supplement to traditional techniques [14]. Molecular methods that identify bacteria, especially those that can sequence the genes encoding for ribosomal 16S rDNA, have become very important tools in studying bacterial communities in patients' fecal samples [14]. Culture-independent methods are now often used because they are believed to overcome problems associated with selective cultivation and isolation of bacteria in cultured samples. The principal reason to use culture-independent techniques is the lack of knowledge about the real conditions under which most bacteria grow in their natural habitat; it is also difficult to develop media for cultivation that accurately resemble these conditions[13].

In recent years, the intestinal microflora has been simulated by developing and marketing preparations of living microbial cells that, when consumed, are believed to influence the composition of the bacteria in the intestine and ben-

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efit the well being of the consumer [16]. Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit to the host. *Lactobacillus* and *Bifidobacterium* are commonly used as probiotics. Several studies reported the protective effect of probiotic consumption on patients receiving anticancer agents [11].

Microbiological investigations of the compositions of terrestrial and microbial communities have shown the versatility of denaturing gradient gel electrophoresis (DGGE) combined with PCR as a molecular analytical method. In this technique, DNA is extracted from patients' fecal samples and a variable sequence region of the 16S rDNA gene is amplified by PCR [19]. In the last decade, molecular fingerprinting techniques such as DGGE, terminal restriction fragment length polymorphisms or single strand conformation polymorphisms became important and frequently used tools in microbial ecology.

In DGGE, DNA fragments of the same length but with different base-pair sequences (G+C contents) can be separated. Separation in DGGE is based on the electrophoretic mobility of a partially melted DNA molecule in polyacrylamide/bis gels. Sequence variants of particular fragments will therefore stop migrating at different positions in the denaturing gradient and hence can be separated effectively by DGGE. This technique has been successfully applied to identifying sequence variations in a number of genes from several different organisms [19].

The current study was designed to investigate the effect of chemotherapy on the intestinal ecosystem of gastric cancer patients. For this purpose, bacterial population fingerprinting of feces was determined using PCR followed by DGGE analysis of the 16S rDNA gene with universal and species-specific primers. Microbial changes detected in the microbiota profiles were further characterized.

Materials and Methods

Subject and sample collection

The subjects of this study were 3 patients who were previously diagnosed with gastric cancer in the Gastroenterology Clinic at Pusan National University Hospital in Korea. The patients were given 5-fluorouracil, cisplatin, and oxaliplatin chemotherapy for 8 weeks.

The ward staff collected stool samples from the patients on sampling days – 0, 2 and 8 weeks after the start of chemotherapy. Fecal specimens were collected in sterile tubes, immediately delivered to the laboratory, and frozen at -70°C where they were stored until analysis.

Prepared of genomic DNA for denaturing gradient gel electrophoresis (DGGE)

Total DNA was extracted from fecal samples by using the QIAamp® DNA Stool Mini Kit (QIAgen, Canada) according to the manufacturer's instructions with some modifications [10]. Briefly, fecal samples were lysed in ASL buffer (QIAgen), which is specially developed to remove inhibitory substances from stool samples, centrifuged, and then the supernatant was transferred to a clean 2 ml tube to remove cell debris. The InhibitEx tablet, provided by the manufacturer, was suspended in each sample. The role of the tablet is to remove the DNA-damaging substances and polymerase chain reaction (PCR) inhibitors. DNA was precipitated with ethanol and applied to a column provided in the kit and dissolved in AE buffer (10mM Tris-HCl, 0.5mM EDTA, pH 9.0). Finally, genomic DNA samples were stored at -20°C.

Polymerase chain reaction (PCR) of 16s rDNA with universal primers

PCR amplification of the 16S rDNA genes was carried out with the universal primers Bact-11F and Bact-1492R (Table 1). The PCR program consisted of the following steps: initial denaturing at 95°C for 10 min, followed by 30 cycles of denaturing at 95°C for 1 min, primer annealing at 55°C for 1 min, extension at 72°C for 1 min, and final extension for 10 min at 72°C. Amplification products were purified using a PCR products purification kit (QIAgen).

The V1-V3 region of the 16S rDNA gene of bacteria in the intestinal contents was amplified with primers Bact-11F (GC) and Bact-536R (Table 1). Touchdown PCR [7] was performed by using 20 cycles consisting of denaturation at 95°C for 1 min, primer annealing at 65°C for 1 min (temperature was decreased by 0.5°C every cycle until the touchdown temperature of 55°C was reached) and primer extension at 72°C for 1 min. Fifteen additional cycles were carried out at an annealing temperature of 55°C. For the last extension step, tubes were incubated for 10 min at 72°C.

PCR of 16s rDNA with species-specific primers

The bifidobacterial population was investigated by nested PCR [3]. The first PCR applied primers Bif-26F and Bif-3R (Table 1) described by Kaufmann *et al.* [8] and amplified a

Table 1. List of primers

Primer	Sequence (5′→3′)
Bact-011F	AGA GTT TGA TCC TGG CTC AG
Bact-1492R	ACG CGT ACC TTG TTA CGA CTT
Bact-011F(GC)	(GC-clamp) AGA GTT TGA TCC TGG CGC AG
Bact-0536R	GW[1]A TTA CCG CGG CK[2]G CTG
Bif-26F	GAT TCT GGC TCA CGA TGA ACG
Bif-3R	CGG GTG CTN[3] CCC CAC TTT CAT G
Bif-0164F(GC)	(GC-clamp) GTG GTA ATG CCG GAT G
Bif-0662R	CCA CCG TTA CAC CGG GAA
Lac-0159F(GC)	(GC-clamp) AAA CAG R[4]TG CTA ATA CCG
Lac-0677R	CAC CGC TAC ACA TGG AG
GC clamp	CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG

*[1]W is A or T, [2]K is G or T, [3]N is A, G, T and C, [4]R is A or G

1417 bp fragment of the *Bifidobacterium*′ s 16S rDNA gene. The following PCR program was used: initial denaturation at 94°C for 5 min, three cycles of denaturation at 94°C for 4 sec, annealing at 57°C for 2 min, extension at 72°C for 1 min, 30 cycles of denaturation at 94°C for 20 sec, annealing at 57°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 5 min. A second PCR was performed using the amplicons of the first PCR as template DNA. PCR amplification of the V1-V3 regions of bacterial 16s rDNA genes was carried out using the specific bacterial primers set Bif-0164F (GC) and Bif-0662R. The forward primer contained a GC clamp (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG-3′) to facilitate separation of the amplicons in a DGGE gel.

PCR amplification of the *Lactobacillus* species 16S rDNA genes was carried out with the universal primers Bact-11F and Bact-1492R (Table 1). The amplification program was the same as previously described. And the lactobacilli population was *Lactobacillus* species-specific primers were Lac-159F (GC) and Lac-677R (Table 1). The amplification program was 92°C for 2 min followed by 30 cycles of 95°C for 30 sec, 30 sec at the 55°C annealing temperature, and 72°C for 30 sec. A cycle of 72°C for 5 min concluded the program.

Moreover, to check for specificity, the sequences of the selected PCR primers were compared to the sequences available in the BLAST database search program (www.ncbi.nlm.nih.gov/BLAST).

Denaturing gradient gel electrophoresis (DGGE) analysis

DGGE was performed with a D-Code system (Bio-Rad, Hercules, CA) utilizing 16-cm × 16-cm × 1-mm gels. Eight percent polyacrylamide gels were prepared and run with 0.5X TAE buffer, (20 mM Tris base, 10 mM acetic acid, 0.5 mM EDTA, pH 8.0) diluted from 50X TAE buffer. The gels contained 30 to 50% gradient of urea and formamide increasing in the direction of electrophoresis. A denaturing solution containing 40% formamide and 7 M urea was used. The electrophoresis was conducted with a constant voltage of 70 V at 65°C for about 16 hr 30 min. After electrophoresis, the gels were stained with ethidium bromide (10 μg/ml in DW) solution for 30 min, observed by UV illumination. DGGE fingerprints obtained from the 16S rDNA banding patterns were digitized by using Image J program (http://rsbweb.nih.gov/ij),

Sequencing and sequence analysis

To confirm the identification of the intestinal microbiota established by DGGE and species-specific primers, we amplified and sequenced the variable region of the 16S rDNA gene of each fragment and conducted a ribosomal database project (RDP) search (www.rdp.cme.edu/). Amplification of the variable region was accomplished with primers (lacking the GC clamp) and the same thermal cycler (BioRad) program as described above for DGGE. DNA sequencing was performed by the Genotech staff (Genotech co., Daejeon, Korea).

Statistical analyses

PCR-DGGE profiles were compared using XLStat program (http://xlstat.com). The analysis included the number, position, and intensity of PCR-DGGE bands (PCR-amplified 16S rDNA fragments) in the gel.

Results

Diversity of bacterial species represented in the denaturing gradient gel electrophoresis (DGGE) patterns

Fecal samples were analyzed by polymerase chain reaction (PCR)-DGGE to characterize and compare bacterial survival in the large intestines of patients administrating anticancer agents. Briefly, the V1-V3 region of the 16S rDNA genes (positions 11 to 536 in the *Escherichia coli* gene) of bacteria in the fecal samples was amplified by universal primers Bact-11F (GC) and Bact-536R. PCR reaction mixtures and the amplification program were the same as previously described (Material and Methods).

Analysis of PCR products by DGGE resulted in a fingerprint that represents the diversity of the rDNA nucleotide sequences due to the different bacterial types. The mobility of these amplicons after DGGE was compared to those obtained from the 16S rDNA of the same feces sample at different time points, as shown in Fig. 1. The bands in the profiles represented most of the dominant microbial populations in the community, and their appearance and disappearance reflected approximate temporal changes in the microbial community composition. Using a statistical analysis of each lanes using XLstat program, we observed the change of intestinal microbiota ecosystem, after dosing the anticancer agents just after 2 weeks.

To amplify the products, 33 bands were excised from the gels and purified with a QIAEX gel extraction kit (QIAgen). Some of the bands did not amplify with PCR to prepare amplicons for sequencing. The sequences were verified with

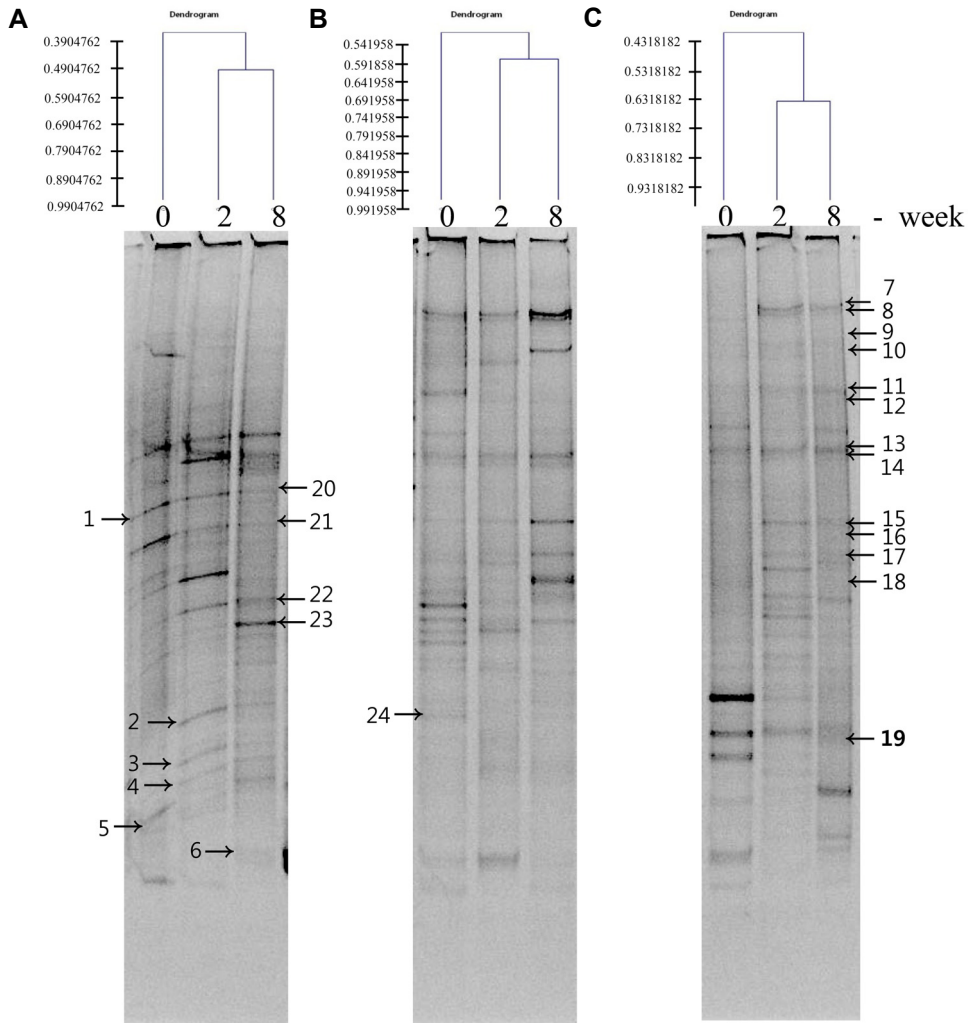

Fig. 1. PCR-DGGE profiles representing the bacterial diversity in fecal samples. DGGE of PCR fragments on 30% to 50% denaturing gradient gel. Each lane reveals the microbial ecosystem at 0, 2 and 8 weeks Numbered bands were identified with 86.8% similarity to 100.0% through ribosomal database project (RDP) search (www.rdp.cme.edu/).

Table 2. List of DGGE band profiles

Band	Closest relative	% identity	GenBank Accession No.	Temporal change
1	*Pseudomonadales aeruginosa*	98.7	EU 194237	Up
2	*Escherichia coli*	95.8	FJ 789760	Up
3	Uncultured *gamma Proteobacteria*	98.6	EU 447540	Up
7	*Sphingomonas paucimobilis*	98.0	U 37337	Up
8	*Lactobacillus gasseri*	96.3	CP 000413	Up
9	*Parabacteroides distasonis*	97.2	AB 238923	Up
10	Uncultured *bacterium*	100.0	EF 509276	Up
11	Uncultured *bacterium*	99.1	FJ 371651	Up
12	*Vibrio sp.*	97.1	AY 864627	Up
16	*Enterobacter sp.*	98.5	EU 301807	Up
17	Uncultured *Faecalibacterium sp.*	95.0	AJ 494671	Up
20	*Helicobacter apodemus*	99.6	AY 009129	Up
21	*Citrobacter sp.*	100.0	AF 451253	Up
18	Uncultured *bacterium*	94.6	EU 642893	Down
19	Uncultured *Escherichia sp.*	94.6	EU 642893	Down
4	*Citrobacter sp.*	97.8	AB 064498	NC
5	*Stenotrophomonas maltophilia*	86.8	AB 180661	NC
6	Uncultured *Firmicutes bacterium*	96.7	FJ 651692	NC
13	*Faecalibacterium prausnitzii*	99.6	AJ 413954	NC
14	Uncultured *bacterium*	97.3	FJ 36332	NC
15	*Morganella morganii*	97.1	FJ 418576	NC
22	Uncultured *Firmicutes bacterium*	97.1	FJ 650829	NC
23	Uncultured *Bacteroidales bacterium*	100.0	EF 710426	NC
24	Uncultured *alpha proteobacterium*	96.6	EU 811018	NC

*NC : not changed

an RDP database, from which we identified 24 nucleotide sequences (Table 2). The DGGE profiles obtained from the three patients were the change of the 16S rDNA sequences present in the feces during the treatment of chemotherapy. Most of the predominant bands (for example the bands indicating *F. prausnitzii, M. morganii,* uncultured *Firmicutes* sp. and uncultured *Bacteroidales* sp.) were present in all feces. Some DGGE bands, however, suggested that the presence of several intestinal microflora was dependent on the individual.

Temporal stability of the DGGE pattern over time after treatment with anticancer agents

Figure 1 showed the DGGE patterns of the patients undergoing anticancer therapy for 8 weeks. The band patterns were complicated in all cases. At the week follow-up stool examination, the DGGE profile remained unstable. DGGE bands' profiles were generated for the samples the temporal changes were graphed in Fig. 2. Most microbiota detected in this experiment was intestinal microflora. Characterization of the microflora appears in Table 3. Among the bands, bands 6, 13, 14, 15 and band 23 remained stable over 8 weeks in all lanes. These bands had a high sequence homology to uncultured *Firmicutes* bacterium (band 6), *Faecalibacterium prausnitzii* (band 13), uncultured bacterium (band 14), *Morganella morganii* (band 15) and uncultured *Bacteroidales* bacterium (band 23).

Pseudomonas aeruginosa (band 1) was a dominant band at

Table 3. Identification of DGGE bands using *Lactobacillus*-specific primers

Closest relative	% identity	GenBank Accession No.	Temporal change
Lactobacillus pantheris	90.9	AB257867	Up
Lactobacillus ruminis	97.9	AY851760	Up
Uncultured *Lactobacillus sp.*	78.2	GQ911089	Up
Uncultured *bacterium*	85.3	FJ808988	Down
Lactobacillus sp.	93.1	GQ911109	Down
Uncultured *bacterium*	83.3	DQ647971	Down

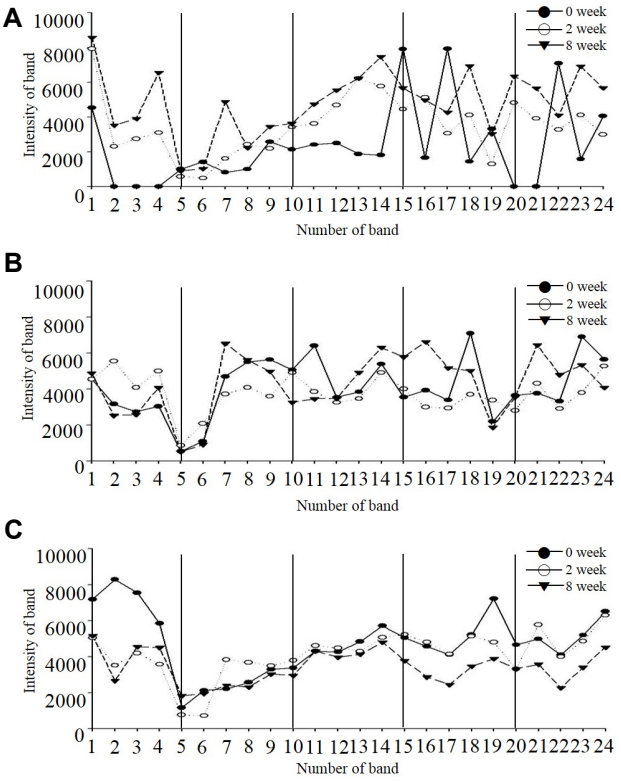

Fig. 2. Temporal stability of the microflora in intestine. Bands of DGGE patterns were digitized by using Image J program (http://rsbweb.nih.gov/ij). Symbol '●' means intensity of band at 0 week during chemotherapy treatment in gastric cancer patients. Symbol '○' means intensity of band at 2 week during chemotherapy treatment in gastric cancer patients. Symbol '▼' means intensity of band at 8 week during chemotherapy treatment in gastric cancer patients.

the middle of the profile. *Escherichia coli* (band 2), uncultured *gamma Proteobacteria* (band 3), uncultured *bacteria* (band 10), *Vibrio* sp. (band 12), uncultured *Faecalibacterium* sp. (band 17), *Helicobacter apodemus* (band 20) *and Citrobacter* sp. (band 21) bands appeared as results of chemotherapy treatment in a time-dependent manner. Bands 4, 5, 22 and 24 were related to *Citrobacter* sp., *Stenotrophomonas maltophilia,* uncultured *Firmicutes* bacterium and uncultured *alpha Proteobacterium,* respectively.

Remarkably, the bands of uncultured bacterium (band 18) and uncultured *Escherichia* sp. (band 19) significantly decreased during the treatment with anticancer agents. However, the bands related to *Sphingomonas paucimobilis* (band 7), *Lactobacillus gasseri* (band 8), *Parabacteroides distasonis* (band 9), uncultured bacterium (band 11) and *Enterobacter* sp. (band 16) were increased.

Lactobacillus gasseri, Pseudomonas aeruginosa and Escherichia coli are normal flora found in human intestines. It has been reported that a side effect of chemotherapy is diarrhea, which is related to *Faecalibacterium prausnitzii* and *Morganella morganii*. Increases in that microflora with chemotherapy were indeed observed in the stool samples.

Diversity of *Lactobacillus* and *Bifidobacterium* in fecal samples using DGGE

Bacteria of the genus *Lactobacillus* and *Bifidobacteria* genus are widely used as probiotics because they interact with both host and intestinal microbiota [17], which were an abundant group; it is important to analyze the role they play to balance the gut ecosystem.

The *Lactobacillus* genus forms a biofilm on the intestinal epithelium [21]. We observed PCR-DGGE profiles of the *Lactobacillus* sp. found in fecal samples of two patients. The *Lacobacillus* specific primer set of Lac-159F (GC) and Lac-677R resulted in amplicons for fecal samples, and DGGE analysis of the PCR-amplified 16S rDNA fragments revealed identical profiles for both samples (data not shown). Some DGGE bands were not specifically identified, indicating *Lactobacillus*-like species, but obtained from amplicons using *Lacobacillus* specific primers. These results might be because the species were below the detection limit or absent. Six bands were identified as *Lactobacillus pantheris, Lactobacillus ruminis,* and uncultured *Lactobacillus sp.* through the sequence database (Table 3).

Bifidobacterial species-specific primers were used to amplify a fragment of the 16S rDNA gene (data not shown). These amplicons served as template DNA for the V1-V3 primer combination V1F and V3R during a second PCR step. The high mobility of the PCR products is due to the G+C content of *bifidobacterium*. DGGE of rDNA gene amplicons revealed that there were four bands identified as *Bifidobacterium pseudolongum* subsp. and *Bifidobacterium dentium, Asterionellopsis glacialis and* uncultured *Bifidobacterium* sp. (Table 4). Two of the bands were from a uncultured bacterium.

Epidemiological and experimental studies showed that ingestion of certain lactic cultures, such as *Lactobacillus* and *Bifidobacterium*, or their fermented dairy products, reduce the risk of certain types of cancer and tumor growth [18]. It has been reported that in Japan, the incidence of colon cancer was lowest when the colonic populations of *Bifidobacteria* were highest and diarrhea-inducing *Clostridium perfringens* was lowest [9]. Therefore, if the population of *Lactobacillus* and *Bifidobacterium* is enriched in cancer patient's intestine

Table 4. Identification of DGGE bands using *Bifidobacterim*-specific primers

Closest relative	% identity	GenBank Accession No.	Temporal change
Uncultured bacterium	87.9	DQ 648000	Up
Asterionellopsis glacialis	97.6	FJ 002234	Up
Bifidobacterium pseudocatenulatum	97.2	AB 507148	Down
Uncultured bacterium	90.6	EF 51093	Down
Bifidobacterium dentium	84.5	AB 507095	NC
Bifidobacterium dentium	87.2	GQ 179694	NC

*NC : not changed

with some administration, the efficacy will be increased for ongoing cancer diseases.

Discussion

The present study using molecular profiling of bacterial 16S rDNA genes, we found that dominant species of human fecal microbiota were markedly modulated within 0 to 8 weeks of treatment with anticancer agents. We demonstrated that PCR-DGGE can be used to monitor the successive microbiota populations in the intestinal tracts of gastric cancer patients undergoing chemotherapy. Bands in the DGGE profiles were identified by analyzing part of the 16S rDNA sequence and comparing the sequences through the use of RDP databases [23]. The levels of similarity ranged from 86.8% to 100.0%. It was possible that the levels of similarity can change somewhat when the complete sequences are compared, even though finding the same closest relatives and the same levels of similarity between partial and complete sequences.

Colonization describes a bacterial population in the gastrointestinal tract that remains stable in size, with no need for periodic reintroduction of bacteria by oral doses or other means. This implies that colonizing bacteria multiply in a given intestinal niche at a rate that equals or exceeds their rate of washout or elimination from that site. Normal colonization of the human intestine is a complex ecosystem [15]. *Bifidobacteria* and *Escherichia coli* are predominant bacteria, followed by *Streptococcus, Lactobacillus, Bacteroides* and Gram-positive bacteria, all in similar quantities. In the patients who participated in the present study, the identities of many of the dominant bands in the DGGE profiles were consistent with some of these patterns of succession.

Anticancer agents, such as cisplatin, oxaliplatin and 5-flurouracil, are very commonly used to treat cancer without taking into consideration their impact on gut microbiota. Most studies of the relationship between normal microbiota and chemicals have been done in patients undergoing decontamination procedures for intestinal surgery. The studies have reported a decrease in anaerobes and aerobes and an increase in yeast and *E. coli*. One of the most common side effects that arise during chemotherapy is an overgrowth of *Clostridium* sp.. This bacterium induces diarrhea and pseudomembranous colitis, conditions associated with *Clostridium* sp. that have outbreaks with a high rate of mortality in hospitalized patients. However, that microflora was not detected in our patients' feces.

Despite several advances in the treatment of gastric cancer, the therapeutic outcome of the malignancy has posed a significant challenge to modern medicine. In previous studies, ingestion of cultures of lactic acid-producing bacteria, such as *Lactobacillus* and *Bifidobacterium*, already present in human intestine, inhibited tumor incidence and diarrhea-induced imbalances of microbiota in the intestinal ecosystem [2, 6].

DGGE of PCR-amplified rDNA gene amplicons is a useful technique for monitoring dynamic changes in mixed bacterial populations over time. All of the rDNA gene sequences from bacterial species in a mixed culture were first amplified using conserved bacterial primers that bracket a hypervariable region of the rDNA gene, producing amplicons of the same sizes, but with different sequences that are specific for a given species. DGGE allows separation of these amplicons, producing a 'molecular fingerprint' of the bacterial species.

In conclusion, we demonstrated that PCR-DGGE is a powerful tool for monitoring the effects of chemotherapy administration in gastric cancer patients' gut ecosystems. The recent development and application of genus-specific primers for intestinal microbes in combination with DGGE expand the potential of this technique for detection of specific populations in some ecosystems.

Acknowledgement

This work was supported by a 2-year Research Grant of Pusan National University.

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초록 : PCR-DGGE를 통해 분석한 항암치료에 따른 장내 미생물 변화

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인체의 장내에 존재하는 장내 미생물은 서로 공생 또는 길항 관계를 유지하며 우리 몸의 면역 방어 기전에 중요한 요소로 작용한다. 본 연구는 항암제가 위암 환자의 장내 미생물 생태계에 미치는 영향을 조사 하였다. 항암치료를 받는 환자의 분변에서 genomic DNA를 추출하고, 16S rDNA 유전자에 대한 denaturing gradient gel electrophoresis (DGGE)를 수행하였다. 분석된 균주는 개체간의 차이가 있었으나, 대부분 사람의 장내에 살고 있는 normal flora로 동정되었다. 모든 분변에 존재하는 5 개 밴드의 서열 분석 결과에 의하면 *Faecalibacterium prausnitzii*, *Morganella morganii* 및 Uncultured bacterium sp.가 나타났고, 항암제 처리 후 *Sphingomonas paucimobilis, Lactobacillus gasseri, Parabacteroides distasonis* 및 *Enterobacter* sp.가 증가하였다. 이 연구에서 probiotic으로 알려진 *Bifidobacterium*과 *Lactobacillus* 를 특이적 PCR primer를 이용하여 동정한 결과, 항암제 투여로 인해 *Bifidobacterium* 과 *Lactobacillus*의 개체군이 현저하게 줄어들어 diarrhea와 같은 부작용의 원인을 예상하게 하며, 장내 생태계의 주요 박테리아 집단에도 중요한 영향을 미치는 것을 알 수 있었다. 이러한 결과는 항암제 투여와 같이 시간의 흐름에 따른 균총의 변화를 시각적으로 모니터링하기 위하여 PCR-DGGE 분석법이 유용하다는 것을 나타낸다.