OPTIMUM CONDITIONS FOR DGGE OF 16S rDNA FROM SUT TILAPIA INTESTINAL TRACT MICRO FLORA†

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Abstract

Denaturing Gradient Gel Electrophoresis (DGGE) is a molecular fingerprinting method that separates similar size (bp), but different sequence of PCR products using acrylamide gel that contain denaturing gradient of urea and formamide. GC clamp is added at the 5′ end of one of the primer to prevent the PCR amplicon from completely denature. Different bacteria have different DNA sequences that will denature at different denaturant concentrations. Different DGGE conditions are suitable for different bacterial communities. This research aimed to optimize the conditions for determining bacterial community of fish intestinal tract. The percent of polyacrylamide gel (8.0% and 10.0%), denaturant concentrations (45 - 55%, 30 - 60%, and 30 - 70%), running times (5 h and 12 h) and voltages used (120 V and 200 V) were varied. The results indicated that, 8.0% polyacrylamide gel, 30 - 60% denaturant, 12 h running time and 120 V was the optimum condition for separation of different species of fish intestinal tract micro flora.

Keywords: GC-clamp, 16S rDNA-DGGE, DGGE condition

Introduction

Denaturing Gradient Gel Electrophoresis (DGGE) is a molecular fingerprinting method that separates similar size (bp), but different sequences of PCR products using acrylamide gel that contains denaturing gradient of urea and formamide. Many researchers have used this technique to identify microbial communities such as using 8% polyacrylamide gel, 30 - 70% denaturant at 200 V, 5 h to identify bacterial communities in soil samples (Yang *et al.*, 2001). Another group used 6.5% polyacrylamide gel, 45 - 55% denaturant at 200 V, 5 h to identify bacterial diversity in purified water (Kawai *et al.*, 2002). Eight percent polyacrylamide gel, 30 - 60% denaturant at 120 V, 12 h was used to identify bacterial community in fish (Le Nguyen *et al.*, 2008) and 10% polyacrylamide gel, 30 - 60% denaturant at 130 V, 5 h was used to identify Microbial Diversity in a Malaysian Crude Oil (Liew and Jong, 2008). Several conditions were followed but no good result was obtained. Therefore, the screening conditions of bacterial community in SUT tilapia intestinal tract were optimized.

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Materials and Methods

Bacterial DNA samples were extracted from intestinal tract of five tilapias individually followed the protocol of Le Nguyen *et al.*, 2008. Portion of 16S rDNA was amplified using the extracted DNA as template with primers GC338f (5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACG GGGGGACTCCTACGGGAGGCAGCAG-3′) and 518r (5′-ATTACCGCGGCTGCTGG-3′) (Muyzer *et al.*, 1993; ∅vreas *et al.*, 1997; Ampe *et al.*, 1999; Moeseneder *et al.*, 1999). Each mixture (final volume 25 µl) contained template DNA, 0.4 µM primers, 0.2 mM dNTPs, 2 mM $MgCl_2$, 1x of $MgCl_2$ free buffer and *Taq* polymerase (homemade @ SUT). First stage; 1 cycle at 94°C for 5 min, second stage; 35 cycles at 94°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec and the last stage; 1 cycle at 72°C for 7 min. Firstly, PCR products (3 µl) were analyzed by conventional electrophoresis on 2% (w/v) agarose gel with 1x TAE buffer and quantified by using a standard (DNA mass ladder 100 bp, Promega, USA).

The PCR products were then analyzed on DGGE using Bio-Rad the DCode^{TM} universal mutation detection system (Bio-Rad Laboratories, USA). PCR products were loaded onto several gel conditions that vary percent of polyacrylamide gel (8% and 10% (w/v)), percent of denaturing gradient (ranging from 45% to 55%, 30% to 60% and 30% to 70%), voltage (120 V and 200 V) and running time (5 h and 12 h) in 1x TAE buffer. All electrophoresis experiments were performed at 60°C. The gels were stained for 1 min with ethidium bromide and rinsed for 15 min in water and then photographed on an UV transilluminator.

Result and Discussion

The DNA samples extracted from intestinal tract of 5 tilapias were used as template to amplify bacterial 16S rDNA using GC clamp primer. The results showed that the PCR products were about 220 bp on agarose gel (Figure 1). Then, the rest of the samples were loaded on several conditions of DGGE. When compare the percent of polyacrylamide gel, in Figure 2 the 8% polyacrylamide gel showed better results than the 10% polyacrylamide gel indicating that the 220 bp GC clamp PCR products were better separated in this condition. This result is in agreement with the results of Le Nguyen *et al.*,

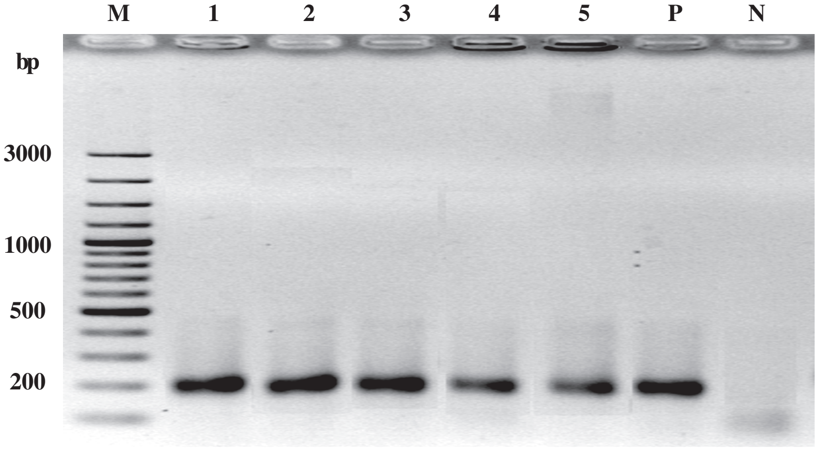

Figure 1. PCR products on 2% agarose gel (M = 100 bp marker, lane 1-5 = DNA samples from five tilapia intestinal tracts no.1-5, respectively, P = positive control (*E. coli*) and N = negative control)

2008 that also use 8% polyacrylamide gel to separate bacterial community from fish. However, this result contradict the results of Liew and Jong, 2008 that use 10% polyacrylamide gel to identify microbial community in crude oil. This may indicated that identifying bacterial community from fish is better in 8% polyacrylamide gel. Kawai *et al.*, 2002 used 6.5% polyacrylamide gel to separate bacterial community from water, so we also wanted to try these conditions, however, the gel we prepared were not able to polymerize in all percent denaturant (45 - 55%, 30 - 60% and 30 - 70%) tested. Even though increased amount of APS and TEMED were used, the gel still did not polymerize. Therefore the 6.5% gel was not tested.

In Figure 3 the running time of 12 h showed better results than 5 h run time when 120 V was used. In these conditions, 5 h is not enough to separate the PCR products obtain from fish intestinal micro flora. However, in the lane P (Figure 3(a)) using *E. coli* as a positive control,

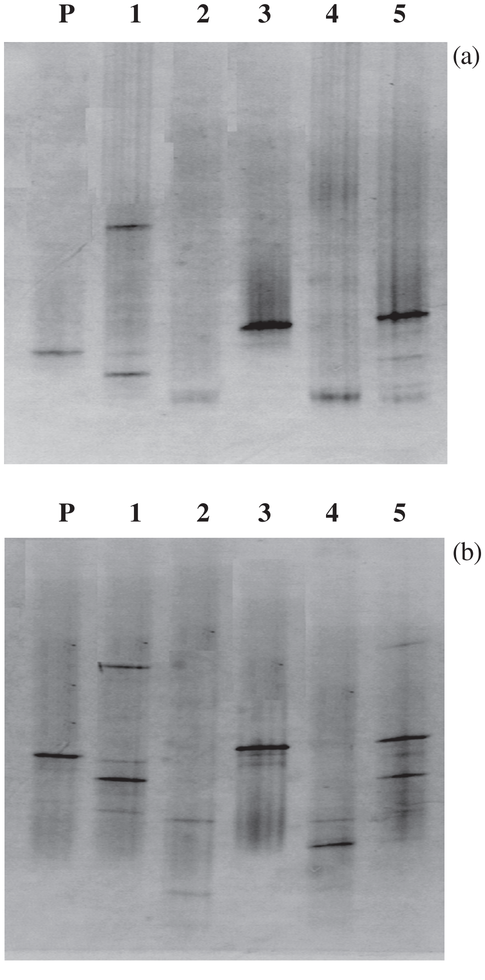

Figure 2. DGGE conditions: 30 - 60% denaturant, 120 V, 12 h and A = 10% polyacrylamide gel and B = 8% polyacrylamide gel. Lane 1-5 = DNA samples from five tilapia intestinal tracts no.1-5, respectively, P = positive control (*E. coli*)

we can see that the running time of 5 h (Figure 3(a) lane P) and 12 h (Figure 3(b) lane P) does not show much different. In Figure 4, when higher percent of polyacrylamide is used (10%) with narrower percent denaturant (44 - 55%) 5 h running time with either 200 V or 120 V (Figure 4(a) and 4(b), respectively) the PCR products from fish intestinal micro flora cannot be separated, but the PCR products from *E. coli* can somewhat be seen clearly (Figure 4(a) and 4(b) lane P). These results indicated that 10% polyacrylamide, 45 - 55% denaturant and 5 h running time are not good conditions to separate PCR products from fish intestinal micro flora.

The comparison of percent denaturant in Figure 5(a) and 5(b) showed that 30 - 60% denaturant (Figure 5(b)) gave better separation of the PCR products than the 45 - 55%

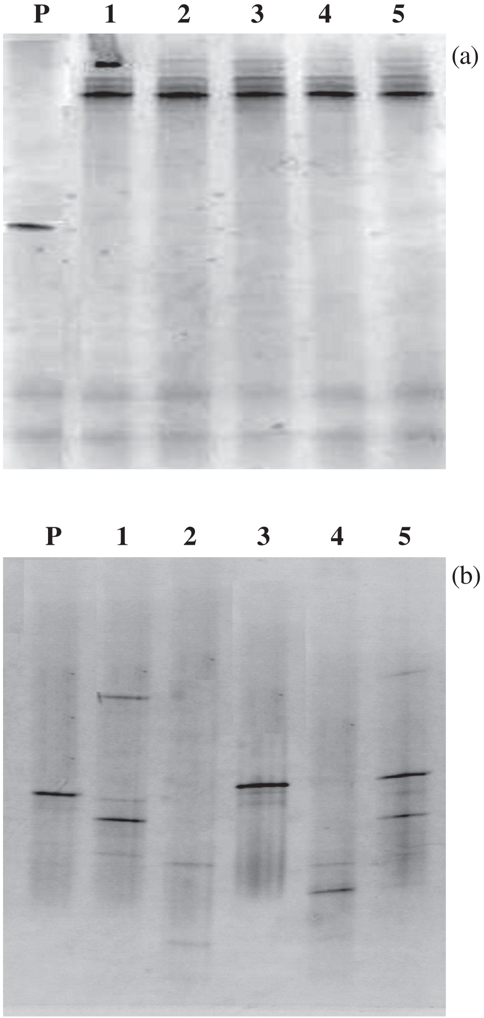

Figure 3. **DGGE conditions: 8% polyacrylamide gel, 30 - 60% denaturant, 120 V and A = 5 h and B = 12 h. Lane 1-5 = DNA samples from five tilapia intestinal tracts no. 1-5, respectively, P = positive control (*E. coli*)**

denaturant (Figure 5(a)). The 30 - 70% denaturant was also tried, however, at this condition the separation was not better than using 30 - 60% denaturant (data not shown).

From these experiments, the optimum conditions for analysis of bacterial community in tilapia intestinal samples indicated that the condition of 8% polyacrylamide gel, 30 - 60% denaturant, 120 V and 12 h is the best condition for these samples.

Conclusions

Many researchers use different condition of DGGE for bacterial community analysis. Therefore, to obtain good results, optimization of condition used is important and should be considered. All parameter including the size of

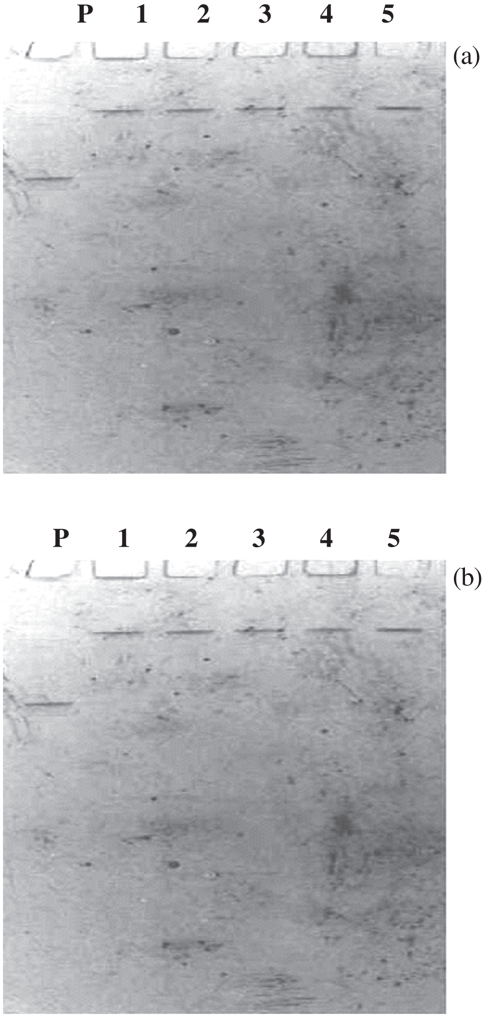

Figure 4. DGGE conditions: 10% polyacrylamide gel, 45 - 55% denaturant, 5 h and A = 200 V and B = 120 V. Lane 1-5 = DNA samples from five tilapia intestinal tracts no. 1-5, respectively, P = positive control (*E. coli*)

PCR products, percent denaturant, percent polyacrylamide, running time and voltage used need to be optimized to obtain good sharp results. The best condition for tilapia intestinal Micro flora analysis was as mention above.

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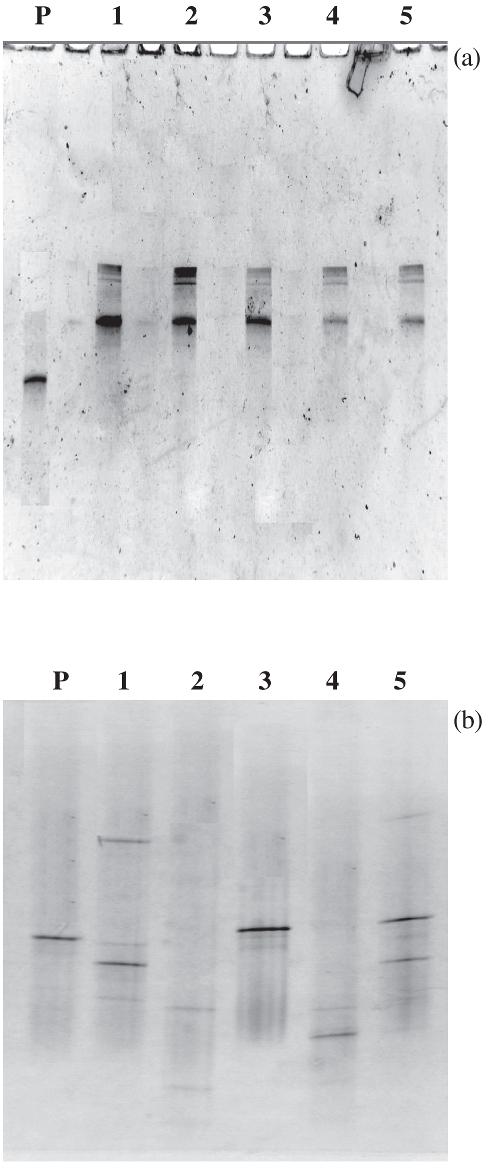

Figure 5. DGGE conditions: 8% polyacrylamide gel, 120 V, 12 h and A = 45 - 55% and B = 30 - 60% denaturant. Lane 1-5 = DNA samples from five tilapia intestinal tracts no. 1-5, respectively, P = positive control (*E. coli*)

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