Lab 3
Identification of Bacteria in an Environmental Sample

Introduction

Polymerase chain reaction (PCR) is a standard method for DNA identification of organisms. One of the key parameters in PCR process is the primer. A number of studies have been done on the selection of PCR primer (Youvraj Sohni, et al., 2008), (K.N. Ballantyne, et al., 2008), and it was believed that a primer producing larger fragment size has a better effect for UV analysis, because of the high visibility and resolution of the fragment (Morag GLEN, et al., 2001), also it has a better capability for DNA discrimination (Rui Jorge Nobre, et al., 2008). In this study, a PCR process was performed on several DNA samples collected from single colonies cultured from bioreactor water, using two different primer pairs targeting 16S rRNA (341F/518R producing 200bp and 341F/907R producing 550bp), in order to compare the effect of different fragment size on PCR process. Gel electrophoresis and UV analysis were conducted after PCR. Nanodrop and DNA sequencing analysis were also performed.

Method

In this study, 5 single colonies were taken from 5 different streak plates with bacteria from bioreactors on them. These colonies were put into 10 ml tubes of nutrient broth separately and inoculated in an incubator shaker at 30C overnight. After that, DNA extraction was performed on each liquid culture we got, using DNA extraction kit (Qiagen DNA Stool Mini Kit Catalog #51504).

In PCR operation, two different primers were used on DNA samples: primer pair 341F/518R and primer pair 341F/907R. And distilled water was used as negative control. The mastermix component used in PCR was shown as below, followed by operating parameters:

Master Mix Component	Volume (μL)
10x PCR Buffer	35
dNTP mix	7
Primer F	3.5
Primer R	3.5
HotStarTaq DNA Polymerase	3.5
Distilled Water (sterile)	290.5
Template DNA	7

Operating Parameters:

1. Denature 15min 94□
2. Denature 1min 94□
3. Anneal 1min 55□
4. Extension 1min 72□
5. Repeat steps 2 through 4 for 29 times
6. Extension 10min 72□

After the PCR process, samples were stored at 4□ for 24 hours.

Before gel electrophoresis, the PCR products were purified by Qiagen QIAquick PCR Purification Kit Catalog #28104. The gel electrophoresis was operated on a 1% agarose gel with two rows of wells, and MBI Fermentas GeneRuler DNA ladder Catalog #SM0333 was used as DNA ladder. All the samples were operated gel electrophoresis under 90mA and 100V for 50min. After that, a staining process was operated on the gel using 10,000xSYBR Gold Stain.

The image analysis of the gel was performed on an UV gel imager to measure the DNA concentration determine that if PCR products contain the correct materials. Nanodrop was also used to measure DNA concentration. Then, a DNA sequencing analysis was operated on each PCR product (an aliquot of the original one) at the KU DNA sequencing facility, using Chromas as the sequencing analyze program. And the DNA sequencing information of the colonies was reported.

Result

The DNA concentration values of PCR products were collected from Nanodrop analysis (Table 1).

Table 1. DNA concentration (ng/μL)

Primer\Group	GC	G1	JJJ	MNG
341F/518R	6.0±4.5	22.7±5.8	8.2±3.9	27.7±8.6
341F/907R	16.0±4.1	2.9±1.0	3.9±1.3	7.5±3.3

The results of group G1, JJJ and MNG have the same pattern that the PCR product using 341F/518R has a higher DNA concentration than using 341F/907R. However, group GC has an opposite result.

For the DNA identification, the final result was gained by inputting the result of DNA sequencing analysis into the website of "http://www.ncbi.nlm.nih.gov/blast/" (Table2).

Table 2. DNA identification

Sample/Group	GC	G1	JJJ	MNG
341F/518R_1	Comamonas sp			Aeromonas sp
341F/518R_2	Comamonas sp		Citrobacter sp	Comamonas sp
341F/518R_3		Aeromonas sp	Comamonas sp	Comamonas sp
341F/518R_4	Comamonas sp	Aeromonas sp	Comamonas sp	Aeromonas sp
341F/518R_5	Citrobacter sp	Aeromonas sp	Citrobacter sp	Staphylococcus sp
341F/907R_1	Comamonas sp			Aeromonas sp
341F/907R_2	Comamonas sp			Comamonas sp
341F/907R_3	Comamonas sp		Comamonas sp	
341F/907R_4			Comamonas sp	
341F/907R_5	Citrobacter sp		Citrobacter sp	

*Blank boxes mean that the DNA sequencing result was not good.

The result showed a perfect coherence of two primer pairs, and a total number of all four groups' samples species: four. The most common species in the samples was Comamonas sp.

Discussion

Firstly, Nanodrop analysis is based on UV spectroscopy (Huw Kidwell, al.,

2008), thus the PCR product with larger fragment size should have a higher value because of its high visibility and resolution. However, most of groups showed that 341F/518R PCR product had a higher DNA concentration than 341F/907R, which was contrary to my hypothesis. The explanation for this result should be that primer 341F/518R had a higher reproducibility than 341F/907R (Torsten W. Remmerbach, et al., 2004), and as a result, it made more fragments than 341F/907R in PCR process, which leaded to a higher DNA concentration of PCR product.

On the other hand, for the DNA identification result, there were four kinds of species were detected: Comamonas sp, Citrobacter sp, Aeromonas sp and Staphylococcus sp. The number of detected species was much smaller than the total number of the species in bioreactor water. The main reason for this significant difference is the way that DNA extract was collected. In this study, DNA extract was collected from nutrient agar plates, on which bacteria in bioreactor water was cultured for several days. As a result, many of the species exist in the bioreactor water will not show up or hard to identified on the plate, because of the effect of competition and predation (Subir Kumar Nandy, et al., 2007). And most of the space on the plate will be occupied by one or several species. In this case, they were Comamonas sp, Citrobacter sp and Aeromonas sp. If DNA extract was collected directly from the bioreactor water, there will be more species be detected.

Finally, these two kinds of primers had exactly same result of species identification, which showed that they have same effect on general DNA identification. This result is not contrary to the statement that larger fragment size has a better DNA identification capability (Rui Jorge Nobre, et al., 2008), because that identification in this study was general, if a more detailed identification is needed, larger size fragment will show more accuracy than smaller one. However, the result also showed that primer 341F/518R had more available result than 341F/907R, which revealed that primer producing shorter fragment has a higher rate to get valid result than primer producing longer fragment. As a result, in general DNA identification, primer producing relatively shorter fragment is better.

Conclusion

In this study, two kinds of primers were used to run a PCR process, which followed by DNA sequencing analysis. Several comparisons were made between their results.

- Primer 341F/518R has a higher reproducibility than 341F/907R in PCR process.
- Primer 341F/518R and 341F/907R have the same effect on general DNA identification, but 341F/518R has a higher success rate to get valid result than 341F/907R.
- The species detected in the study were Comamonas sp, Citrobacter sp, Aeromonas sp and Staphylococcus sp. The total species number in the bioreactor water will be much larger than this.

References

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Questions

1. The PCR mastermix contains several kinds of material and they have different

function.

PCR buffer: keeps the master mix at the proper pH so the PCR reaction will take place

dNTP mix: provide both the energy and nucleosides for the synthesis of DNA

Primers: Short pieces of DNA (20-30 bases) that bind to the DNA template allowing Taq DNA polymerase enzyme to initiate incorporation of the deoxynucleotides

HotStarTaq DNA Polymerase: A heat stable enzyme that adds the deoxynucleotides to the DNA template

Template DNA: The DNA which will be amplified by the PCR reaction

2. The annealing temperature depends on the length and composition of primers, thus if a PCR program use a different primer from ours, the annealing temperature will also be changed.
3. A. The distilled water used in PCR probably was contaminated by bacteria, which will make all the PCR results become invalid.

   B. It is possible that all the samples and ladder were not added into the gel wells. It is also possible that the electrophoresis buffer was not good. Another possible reason is that the electrophoresis time was too long.

   C. Probably the ladder was not good, or ladder was not added into the gel well.
4. PCR amplification from natural microbial communities using a single set of 16S rRNA primers typically generates a single gel band containing amplified DNA fragments of a single size. However, the band typically contains many highly related but not identical genes. As a result, an additional step is needed to resolve the different forms of the gene before sequencing can proceed. DGGE and cloning are two ways to resolve the different forms of gene.
5. Take DNA extracts from soil samples, and use PCR, DGGE and sequencing analysis to determine that if there is the corresponding DNA sequencing for producing ribulose bisphosphate carboxylase in the samples. If there is, the organisms capable of autotrophic growth using the Calvin cycle are exist in soil sample.
6. Bryant et al. have discovered a new thermophilic phototroph from a poorly characterized bacterial phylum with no previously known photosynthetic members, using Metagenomic data from the phototrophic microbial mats of alkaline siliceous hot springs in Yellowstone National Park. The new organism is called Candidatus Chloracidobacterium thermophilum. (Bryant et al, 2007)