Conference Paper

Optimization of Polymerase Chain Reaction to Overcome Contamination of Deionized Water and Plumbing Premises By *Pseudomonas* spp. in Molecular Biology Laboratory

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Abstract

The purpose of the current study is to introduce specific optimization steps to overcome non-specific binding of primers to contaminating DNA. The applied modifications provide applicable solutions especially if large number of primer aliquots were contaminated and the cost to replace them is high. Several steps were taken to achieve complete mitigation of non-specific binding: reducing the concentration of both forward and reverse primers, reduction in the total number of PCR cycles from 35 to 25, increasing the annealing temperatures, doing filter sterilizations (0.2 µm Thermo Scientific polyethersulfone membrane) for the deionized water (DI) used in PCR and in certain cases reducing the extension time. The optimization steps carried in this work were successful in eliminating non-specific binding of primers to contaminating DNA found in primer aliquots.

Keywords: Plumbing Premises, Water Contamination, PCR, *Pseudomonas*, Deionized Water

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Received: 18 September 2018
Accepted: 10 October 2018
Published: 15 October 2018

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the Sustainability and Resilience Conference Committee.

1. Introduction

Contamination of piping and pluming premises with gram negative bacteria is well documented [1–3]. Among other gram negative bacteria, *Pseudomonas* species inhabit most of the water systems in both industrial and research laboratories [4]. Reports on enumeration of such species became basic routine in certain countries [4]. In University of Bahrain there is continues monitoring for changes in numbers of *Pseudomonas* in water systems. Premises at university use "deionization units" to provide required deionized water (DI) for laboratory procedures. Tested water samples showed high titers of isolated *Pseudomonas* spp. In both tap and deionized waters.

Deionized water used in PCR is usually sterilized by autoclaving for 15 minutes at 121°C and 15 psi [5]. After autoclaving, heat-stable DNA from dead cells would contaminate subsequent processes such as PCR mixtures and primer aliquots. As a consequence of this step, nonspecific binding of primers due to contaminating DNA would result in false amplifications. The purpose of the current study is to introduce specific optimization steps to overcome nonspecific binding of primers to contaminating DNA coming from contaminated primer aliquots. The applied modifications provide applicable solutions especially if large number of primer aliquots were contaminated and the cost to replace them is high. An estimated cost for single primer would range between $10-35. Thus for research projects that use a large number of primers, it would cost a lot to replace all primer aliquots contaminated with DNA.

2. Protocol

2.1. Culture media for isolation of *Pseudomonas* spp.

Conventional isolation techniques (standard most probable number techniques) for isolation and enumeration of *Pseudomonas* bacteria were used [6]. *Pseudomonas* were cultivated on asparagine agar media for isolation and enumeration [6]. Other Conventional methods applied to test for presence of coliforms and other possible pathogenic species were carried out too [7].

2.2. PCR conditions

The amplifications were carried using the KAPA2G Fast PCR kit under the following conditions (total reaction volume 25 µl): 0.5 units of KAPA2G Fast DNA polymerase, 0.2mM of dNTPs, 0.5µM forward and reverse primers. The PCR conditions were as following (TECHINE GENIUS thermocycler): initial denaturation at 95°C for 2 minutes, 35 cycles of 10 seconds at 95°C, 10 seconds at 62°C, and 1 second at 72°C. Final extension was for 10 minutes at 72°C.

2.3. Gel electrophoresis

1% agarose was prepared for most amplification runs in this study. The gels were stained with ethidium bromide (0.5µg/ml) for 30 minutes and de-stained before visualization. DNA ladder used is GeneRuler from Thermo Fisher Scientific.

3. Representative Results

The optimization process started with the reduction in the concentrations of both forward and reverse primers (Figure 1-panel B). In the original protocol, the final concentration of both primers was 0.5 µM [8] and was reduced to 0.1 µM. The intensity of the false bands was reduced but not eliminated (Figure 1-panel B). Then the following steps were performed, reduction in total number of PCR cycles from 35 to 25, increasing the annealing temperatures (upon the Tm of primers), doing filter sterilizations (0.2 µm Thermo Scientific polyethersulfone membrane) for the deionized water (DI) used in PCR and in certain cases reducing the extension time (depends on product size). After applying these optimization steps, no non-specific binding was noticed (Figure 1-panel D).

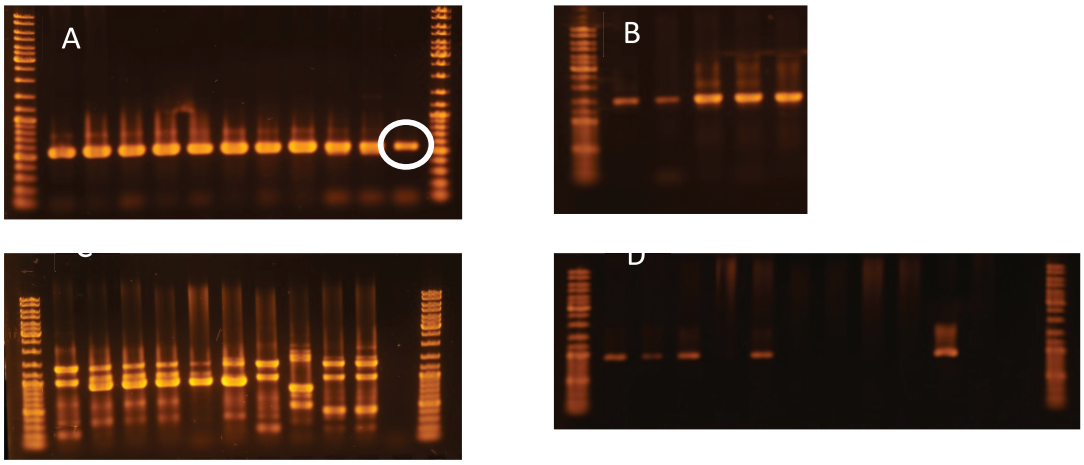

Figure 1: Panel A. Amplification of PCR products using autoclaved deionized water. The white circle shows the non-specific PCR product in the negative control. Panel B. A non-specific PCR amplicon produced in a negative control sample. In this part, different concentrations of primers were used (from left to right the concentrations of primers were: 0.1, 0.2, 0.3, 0.4 and 0.5 µM). Panel C. After the use of filter sterilized deionized waters (0.2 µm Thermo Scientific polyethersulfone membrane) no false amplifications were noticed in the negative control (last column). Panel D. The same amplification in panel C but after applying the optimization steps (e.g., reduction in total number of PCR cycles from 35 to 25, increasing the annealing temperatures). DNA ladder used is GeneRuler from Thermo Fisher Scientific.

4. Discussion

Molecular diagnosis laboratories in hospitals and universities use deionization units to provide water for identification and detection of pathogens and reporting of human diseases [9]. However, as reported before these piping systems are considered the source of contamination for molecular diagnostics protocols as different bacterial populations inhabit these premises [9]. The current study shows the importance of using (0.2 µm) filters to sterilize (DI) water. The study used 0.2 µm filters instead of 0.45

µm to avoid possible contamination of certain species of bacteria such as *Mycoplasma*, known to pass through the 0.45 µm filters [10].

The study investigated the presence of *Pseudomonas* spp in water premises of University of Bahrain. However, other bacterial species were noted too (data not shown). No pathogenic or coliforms were detected with conventional procedures in samples studied.

The optimization process started with the reduction in the concentrations of both forward and reverse primers 0.1µM. According to previous studies primer concentrations in PCR reactions are the most likely cause of non-reproducible amplifications and that most of these are dependent on primers' sequences [11]. However, further optimization steps were required to mitigate the effects of contamination. According to [8] the primers are not completely used up during the amplification. However, due to the accumulated products at the end of the amplification process the primers would straggle to find their target sequences. Therefore, to avoid high primer concentrations which might increase unspecific priming I reduced the number of PCR cycles to 25 instead of 35 (Figure 1-panels D). Reducing the number of PCR cycles has been found to enhance the amplification process and reduces the smear effects as can be seen in Figure 1-panels C and D [12]. The elimination of the non-specific binding was achievable after applying all optimization steps mentioned above and in certain cases reducing the extension time.

The optimization steps observed exclude the need for other methods previously applied to reduce reagents' contamination: UV and gamma radiations, DNase, restriction digests, plus other chemical treatments [13–20]. All of these methods come with drawbacks such as reduction in enzyme activity due to radiation or DNase treatments, or an increase in the amount of contaminating DNA if restriction enzymes were used [13–20].

The current investigation pinpoint to the importance for more controls and tests when working with culture independent methods as contaminants from different sources might intervene with the obtained results and subsequent analysis.

5. Conclusion

In the current study several steps were applied to reduce/eliminate the effects of contaminating DNA coming from deionized water. The optimization steps were successful to eradicate the effects of the contaminants; however, the optimization steps might not suite different amplification protocols such as multiplex PCR. Moreover, the

optimization steps will not optimize for contaminants of the same species tested if the primer aliquots were the source of contamination.

Disclosures

I have nothing to disclose.

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