PCR Analysis: Detection Of Bacteriophage Contamination In Biotechnology Used To Produce Recombinant drugs

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Abstract:
Contamination is a major drawback of the world in biotechnology field and it has cost of losing important biological products like recombinant drugs or valuable research. The causative agents are different chemicals, invertebrates, bacteriophages, bacteria, fungi, parasites, viral species and even other cell lines. In this study, E. coli BL-21 DE3 (DE3 has \( \lambda \) - bacteriophage genome) which is cloned with therapeutic drug were studied during 2 years (2008-2010) to detect their contaminations and the causative organisms. Samples were examined for bacteriophage contamination using conventional molecular biology techniques. Using polymerase chain reaction (PCR) technology, primers were specifically designed for lambda phage DE3 genome in E. coli BL-21 (DE3) and bacteriophage was detected in positive control. No bacteriophage was detected in any of the negative control samples. PCR results were confirmed by virus isolation experiments performed with PCR-positive and negative samples. This indicates \( \lambda \)DE3 from E. coli BL-21 (DE3) is expressed and this led to cell lysis. The results obtained in this study furnish evidence suggesting that the employed assay system constitutes an effective tool for the detection of contamination in culture systems. To sum up, PCR represents a sensitive, specific, accurate, inexpensive, and quick bacteriophage detection assay that is suitable for the routine screening of cultures.

Key words: E. coli BL-21 (DE3); PCR; Bacteriophage;

1. Introduction:
Molecular methods have been developed for direct detection of phage components. They include immunochemical detection of bacteriophages with antibodies raised against specific structural protein components of the virion (Schouler et al., 1992; Moineau et al., 1993b; Chibani Azaiez et al., 1998; Ledeboer et di., 2002), and molecular DNA detection techniques (e.g. DNA hybridization, P CR) with DNA primers and probes that are specific for nucleic acid sequences of a given phage, or group of phages (Moillieall et al.. 1992; Labrie and Mohican, 2000; Binetti et al., 2005; Dupont a.. 2005). These techniques are generally well suited for rapid detection, can be quite sensitive (e.g. PCR), and can be adapted to provide quantitative data e.g. Q-PCR, ELISA (H. Dvorakova, L. valicek, M. Reichelova, 2005).

Moreover, a number of commonly used strains of Escherichia coli, one of the most frequently employed host bacteria for overexpression of recombinant genes, contain lambdoid prophages that often bear some regulatory genetic elements useful in the control of the expression of cloned genes. However, under certain conditions, prophage induction occurs and may have similar effects on a bacterial culture as phage infection has.

BL21(DE3) E. coli are ideal for use with bacteriophage T7 promoter based expression systems .BL21(DE3) carry the lambda DE3 lysogen. Recombinant proteins that are non-toxic to E. coli are generally expressed at higher levels in BL21(DE3) cells than in BL21(DE3)pLysS or BL21(DE3)pLysE. However, the basal expression levels of heterologous genes are significantly high by using BL21(DE3) E. coli . Although \( \lambda \)DE3 is normally dormant, the induction can occur as the result of expressing proteins that damage the E. coli chromosome, either directly or indirectly. This may lead to cell lysis.

In this study, Samples were examined for bacteriophage contamination using conventional molecularbiology techniques. Using polymerase chain reaction (PCR) technology, primers were specifically designed for lambda phage DE3 genome in E. coli BL-21 (DE3) and bacteriophage was detected in positive control. PCR
analysis suggested that PCR is effective tool for the
detection of contamination in fermentation culture systems.

**Materials**

1. **JM 109 E. coli**
2. **BL21(DE3) E. coli**
3. PBS (phosphate-buffered saline) : 140 mM NaCl, 27 mM KCl, 7.2 mM Na2HPO4, 4.7 mM KH2PO4, pH 7.2. Autoclave 20 min at 121°C to sterilize the solution.
4. 50X TAE (Tris–acetic acid–EDTA): 2 M Tris base, 5.71% glacial acetic acid (v/v), 100 mM EDTA. Adjust to pH of approx 8.5.
5. DNA extraction and purification system (e.g., phenol–
chloroform extraction and ethanol precipitation, or
DNA extraction kits).
7. Taq DNA polymerase (Qiagen,). 6X Loading buffer:
0.09% (w/v) bromophenol blue, 0.09% (w/v) xylene
cyanol
8. FF, 60% glycerol (v/v), 60 mM EDTA.
9. Primers:
   Primer set:
   Forward primer:
   5’- gctggatgctatacggtggt -3’
   Reverse Primer :
   5’- atggttacgccgttcttgac -3’
   The expected size of amplicon is: 513 bp
10. Primer stock solutions: 100 µM in dH2O, stored frozen
at –20°C. Working solutions: mix of forward primers at
5 µM each (Myco-5’) and mix of reverse primers at 5
µM each (Myco-3’) in distilled water (dH2O), aliquoted
in small amounts (i.e., 25 to 50-µL aliquots), and stored
frozen at –20°C.
11. Internal control DNA
12. Positive control DNA:
13. Deoxy-nucleotide triphosphate mixture (dNTP mix):
mixture contains 5 mM each of deoxyadenosine
triphosphate (dATP), deoxycytidine triphosphate
(dCTP), deoxyguanosine triphosphate (dGTP), and
deoxythymidine triphosphate (dTTP) (Peqlab,
Erlangen, Germany) in H2O and stored as 50-µL
aliquots at –20°C.
14. 1.3% Agarose–TAE gel

**Method**

1. The sample was filtered through 0.22-micron filter.
2. **E.coli** [JM-109 and BL-21 (DE3) RP] bacterial
cultures were revived from the glycerol bank.
3. Single isolated colony of JM-109 was grown [Figure
1] in LB medium supplemented with magnesium ions
(Mg++) and maltose.
4. Similarly an isolated colony of BL-21 (DE3) RP was
grown in LB medium [Figure 1].
5. The inoculated cultures were grown to log phase at
37°C.
6. The JM-109 culture grown in LB medium
supplemented with Mg++ and maltose was split in to
two aliquots.
7. To one aliquot the filtered phage-attacked broth was
added whereas the other aliquot was used as a negative
control.
8. The BL-21 (DE3) RP was used as a positive control.
9. After infection the cultures were incubated for 4 hours.
10. The cells were washed with the Tris buffer and
genomic DNA was extracted by phenol: chloroform
method.
11. The genomic DNA samples were resolved and
quantified using UVP gel documentation system
[Figure 2].
12. PCR was set up with the above DNA samples
including positive and negative controls using one sets
of primers.
Primer set:
   Forward primer:
   5’- gctggatgctatacggtggt -3’
   Reverse Primer :
   5’- atggttacgccgttcttgac -3’
   The expected size of amplicon is: 513 bp
13. PCR conditions were: 30 cycles of Denaturation at
94°C for 45 sec; Annealing at 62°C for 45 sec;
Polymerization at 72°C for 45 sec. Extension at 72°C
for 10 min
14. Agarose gel electrophoresis of the PCR products was
carried out and documented in [Figure 3].

**Results and Discussion**

Figure 1: The two **E.coli** cultures BL-21 (DE3) RP and JM-109
were streaked on agar.
Figure 2: Agarose gel of Genomic DNA: Shows the gel of the genomic DNA extracted by phenol: Chlor.

Lane 1: JM-109 Mg++ (negative Control)
Lane 2: JM-109 Mg++ (Virus infected)
Lane 3: BL-21 (DE3) RP (positive control)
Lane 4: 1 Kb DNA ladder

The PCR products were analyzed on 1.5% agarose gel. The primer set showed the expected size band in the positive control [BL-21 (DE3) RP], in the experimental samples. Whereas no band was seen in the negative control (JM-109) (Figure 3).

Figure 3: PCR using two sets of gene specific primer for T7 phage

Lane 1: Negative control (JM-109) DNA
Lane 2: JM-109 Mg++ (Virus infected) DNA
Lane 3: Positive control BL-21 (DE3) DNA
Lane 4: PCR Negative control
Lane 5: 1 Kb Marker

1-5 with primer set-- (expected size ~513 bp),

The PCR results prove the presence of DE3 phage in the Phage attacked fermentation and culture sample.

Conclusion

The PCR results prove the presence of DE3 phage in the Phage attacked fermentation and culture sample.

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