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The evidence of contaminant bacterial DNA in several commercial Taq polymerases

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Abstract

Bacteria identification through PCR with 16S rDNA gene universal primers was widely used in clinical, environmental and applied microbiology. In practice, it is a considerable challenge to achieve high sensitivity and a clear negative control at the same time, due to contamination of commercial Taq polymerases with trace of bacterial DNA. In this paper, besides the methods proposed so far to avoid amplification in negative controls, we propose using of 16S rDNA gene universal primer pair (27F and 1407R), which unlike other primer pairs result with a clean control. Additionally, even if the negative control checked by agarose gel is clean, it is still possible presence of detectable PCR product, and we have identified the source of DNA contamination in six commercial Taq polymerases. We tested seven commercial Taq polymerases for presence of trace contaminating DNA and only one Taq polymerase was bacterial DNA free. These data can be useful to eliminate the false positive products when mentioned polymerases are used.

Keywords: *polymerase contamination, PCR, 16S rDNA gene*

Introduction

Bacteria are colonizing every niche of biosphere and very adapted microorganism to extreme life conditions. It is not easy to have a sterile space with no bacteria. They are enzyme resource for biotechnological research and pathogen for animal and human infections and food poisoning. Taq polymerase contaminations with bacterial DNA during its preparation was reported before (J.K. KOPONEN & al. [1], S. TONDEUR & al. [2], R. KONCAN & al. [3], H. MÜHL & al. [4]). The PCR using 16S rRNA gene for bacterial identification is a sensitive method and has a broad range of applications in screening of pathogen in clinical samples, characterization of environmental bacterial community, food contamination, etc. Different strategies for removal of DNA contamination from commercial Taq polymerases were proposed including purification using extraction kit reagents (T. MOHAMMADI & al. [5]), UV radiation (C.E. CORLESS & al. [6]), treatment with restriction enzymes (T. MOHAMMADI & al. [7], T.-H. WANG & al. [8]) or ethidium azide (A. RUECKERT and H.W. MORGAN [9]). Digestion of contaminating DNA with DNase I, was investigated, but the removal of DNase is much more complicated (F. MARTEL & al. [10], A. HEININGER & al. [11]). The simplest method proposed to avoid DNA amplification in negative controls was the dilution method of Taq polymerase (R. SPANGLER & al. [12]). All these strategies have their disadvantages: the decreasing protein concentration, inhibition or lower PCR amplification in subsequent reactions. A new strategy for production of Taq

polymerases were proposed consisting in expression of recombinant polymerases in eukaryotic host (H. NIIMI & al. [13]), but the contamination of commercial Taq polymerases are not from the host, but from manipulation of proteins during purification.

The aim of this study was the identification of bacterial DNA contamination in commercial Taq polymerases by 16S rDNA amplification and improvement of PCR conditions in order to avoid amplification in PCR negative controls.

Materials and methods

PCR amplification of 16S rDNA

The PCR amplifications were done using seven different Taq polymerases: A) DreamTaq Green DNA Polymerase (Thermo Scientific); B) GoTaq Flexi DNA Polymerase (Promega); C) MangoTaq DNA Polymerase (BIOLINE); D) MyTaq DNA Polymerase (BIOLINE); E) BioMix Red (BIOLINE); F) VELOCITY DNA Polymerase (BIOLINE); G) Phusion High-Fidelty DNA Polymerase (Thermo Scientific). Two different samples for each Taq polymerase were tested for the presence of bacterial DNA contamination.

All PCR reactions were done in final volume of 50 µl with 1 µM forward and reverse primers and no added DNA template. For each PCR reaction was respected all manufacturer standard recommendations. The PCR amplification was performed at 95°C for 5 min, 35 cycles, with each cycle consisting in 95°C for 30 sec, 54-68°C for 30 sec and 72°C for 30-90 sec, and a final extension at 72°C for 10 min. All manipulations were done in sterile conditions using sterile plastics and filter tips. Universal 16S rDNA primers are presented in Table 1.

Table 1. Universal 16S rDNA primers used for PCR

Name	Primer sequence 5'→3'	Reference
27F	AGA GTT TGA TCM TGG CTC AG	(D. LANE [14])
518R	ATT ACC GCG GCT GCT GG	(G. MUYZER & el al. [15])
533F	GTG CCA GCA GCC GCG GTA A	(W.G. WEISBURG & el al. [16])
1381R	CGG TGT GTA CAA GRC CYG RGA	(B.P. HODKINSON and F. LUTZONI. [17])
1407R	GAC GGG CGG TGW GTR CA	(O. NERCESSIAN & el al. [18])
1492R	GGT TAC CTT GTT ACG ACT T	(D. LANE [14])

16S rDNA cloning and sequence analysis

16S rDNA pool amplified by PCR was purified from agarose gel and cloned using CloneJET^{TM} PCR Cloning Kit (Thermo Scientific) according to manufacturer's recommendations. The ligation mixture was used for transformation of *Escherichia coli* cells strain XL1 blue (C.T. CHUNG and R.H. MILLER [19). About 20 colonies were picked and cultured in 5 ml of LB media for plasmid DNA extraction. Sequencing was done via a custom sequencing service (Macrogen Company, Amsterdam). The plasmid DNA was sequenced with vector sequencing primer (pJET1.2R).

The 16S rDNA gene sequences were manually checked using the free analyzing software BioEdit Version 7.0.9 (Tom Hall, Ibis Biosciences). The sequences were compared to the NCBI (National Centre for Biotechnology Information) database (http://www.ncbi.nlm.nih.gov/) using BLASTN (Basic Local Alignment Search Tool) to retrieve similar sequences.

Results

In order to analyze an effective and easy method to avoid DNA amplification in negative PCR controls for bacteria detection using 16S rDNA, a series of PCR reaction were done using different primer sets. PCR amplifications with no template were done using five

sets of 16S universal bacterial primers and Taq polymerases A. Seven different annealing temperatures were tested ranging from 54 to 68°C. The positive control was done using *Escherichia coli* genomic DNA. PCR products were observed at all aligning temperatures (Fig. 1). No PCR products in negative PCR were detected only in the case of one primer set 27F and 1407R. Higher amounts of DNA were detected in the case of smaller fragments using primer set 338F and 518R.

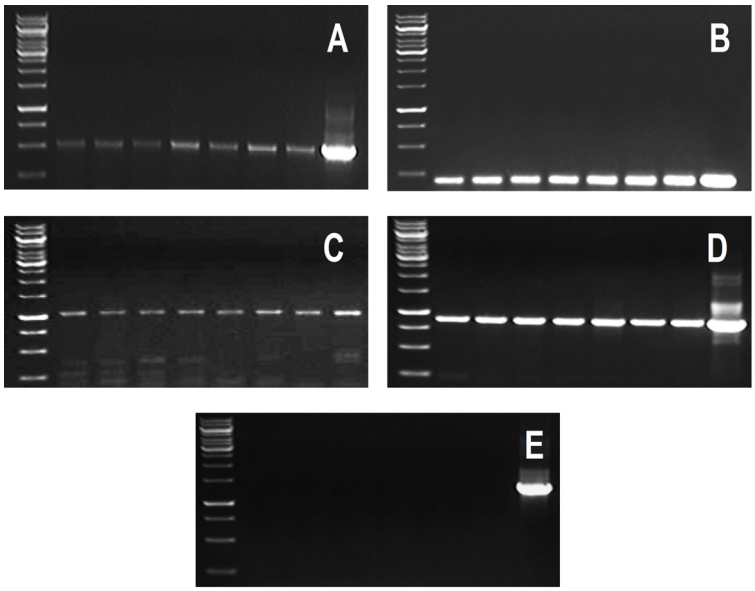

Fig. 1. Amplification in negative controls with Taq polymerase A using 16S universal primers: A - 27F 518R; B - 338F 518R; C - 338F 1381R; D - 533F 1381R; E - 27F 1407R. 15 µl of PCR products were loaded on the agarose gel (1.5 %). 1 – Gene Ruler 1 kb DNA Ladder (Thermo Scientific), 2-8 – different annealing temperatures ranging from 54 to 68°C, 9 – positive control with gDNA from *E. coli*.

PCR amplification with primers 27F and 518R and seven different Taq polymerases were done in order to identify the source of bacterial DNA contamination. No visible PCR products were detected only in reaction with Taq polymerase G. PCR products from amplifications with six different Taq polymerases (A-F) were cloned into pJET1.2 vector. From each library 20 clones were analyzed and sequenced. The sequence analysis revealed a presence of different bacterial DNA (Table 2) in each Taq polymerase.

Table 2. Diversity of bacterial contaminating DNA in tested Taq polymerases.

Taq Polymerase	Strain	Accesion number	Identity %
A	*Geobacillus pallidus* strain DSM 3670	NR 026515	99
	Caldalkalibacillus uzonensis strain JW/WZ-	NR 043653	94
	Halomonas shengliensis strain SL014B-85	NR 044099	98
	Halomonas campaniensis strain : 5AG	NR 042157	98
	Halomonas axialensis strain Althf1	NR 027219	98
	Nesterenkonia flava strain CAAS 251	NR 044353	100
B	*Caulobacter vibrioides* strain CB51	NR 037099	96
	Arthrobacter russicus strain A1-3	NR 024783	88
	Pelomonas puraquae strain : CCUG 52769	NR 042615	99
	Petrobacter succinatimandens strain 4BON	NR 025725	98
	Geobacillus pallidus strain DSM 3670	NR 026515	99
	Arthrobacter ramosus strain DSM 20546	NR 026193	98

C	*Pelomonas puraquae* strain : CCUG 52769	NR 042615	99
	Pedomicrobium australicum strain IFAM	NR 026337	96
	Byssovorax cruenta strain : By c2 = DSM 14553	NR 042341	91
	Propionibacterium acnes	NR 040847	98
	Porphyrobacter donghaensis strain SW-132	NR 025816	99
	Methylobacterium jeotgali strain S2R03-9	NR 043878	99
	Micrococcus luteus strain DSM 20030	NR 037113	99
	Caulobacter vibrioides strain CB51	NR 037099	96
D	*Solibacillus silvestris* strain HR3-23	NR 028865	94
	Caulobacter vibrioides strain CB51	NR 037099	96
	Solibacillus silvestris strain HR3-23	NR 028865	99
	Gemella haemolysans strain ATCC 10379	NR 025903	99
	Bacillus thuringiensis strain IAM 12077	NR 043403	95
	Streptococcus mitis strain NS51	NR 028664	99
	Solibacillus silvestris strain HR3-23	NR 028865	95
	Bacillus thuringiensis strain IAM 12077	NR 043403	99
	Xanthobacter agilis strain SA35	NR 026306	99
E	*Caulobacter vibrioides* strain CB51	NR 037099	96
	Methylobacterium organophilum strain ATCC	NR 041027	95
	Propionibacterium acnes	NR 040847	100
	Citrobacter braakii strain 167	NR 028687	99
	Solibacillus silvestris strain HR3-23	NR 028865	99
	Micrococcus luteus strain DSM 20030	NR 037113	100
F	*Blastobacter denitrificans* strain IFAM 1005;	NR 041827	99
	Caulobacter vibrioides strain CB51	NR 037099	96
	Methylobacterium jeotgali strain S2R03-9	NR 043878	100

A) DreamTaq Green DNA Polymerase (Thermo Scientific); B) GoTaq Flexi DNA Polymerase (Promega); C) MangoTaq DNA Polymerase (BIOLINE); D) MyTaq DNA Polymerase (BIOLINE); E) BioMix Red (BIOLINE); F) VELOCITY DNA Polymerase (BIOLINE).

Discussion

Bacterial DNA detection by PCR is a powerful technique for clinical, applied and environmental microbiology. Using of 16S universal primers targeting conserved regions of rDNA enables the detection of lower quantity of template DNA. Bacterial DNA contamination in commercially available Taq polymerases was estimated at 10-1000 genome copies per enzyme Unit (R. SPANGLER & al. [12]). The number of 16S gene copies is higher due to their multiple copy number per bacterial genome. In the present study we identified the presence of contaminant DNA in six of the seven tested Taq DNA polymerases.

Sources of contamination during handling can be DNA or even bacterial cells. The 16S sequence analysis revealed the presence of various bacterial strains. These strains could be of human (*Propionibacterium acnes, Gemella haemolysans, Streptococcus mitis, Micrococcus luteus*), air (*Methylobacterium jeotgali, Micrococcus luteus* (J.M. KOOKEN & al. [20])) or water (*Porphyrobacter donghaensis*) origin. The 18 % of the airborne bacteria are associated with human skin microbiome (J. QIAN & al. [21]). The presence of some thermophilic bacterial strains like *Caldalkalibacillus uzonensis, Petrobacter succinatimandens* can indicate its use for overexpression of recombinant proteins, information that is not provided by the producing companies. An other important source of bacterial contamination is represented by ventilation systems, contributing to the spread of bacteria if inadequate filters are used (E. MIASKIEWICZ-PESKA and M. LEBKOWSKA [22]).

Regardless of the source of bacterial DNA contamination, investigators need DNA-free Taq polymerases. In order to avoid bacterial DNA amplification in negative control samples the following strategies are recommended: choice of primers pair, increase the amount of DNA template and decrease of the amount of Taq polymerase used (R. SPANGLER & al. [12]).

We hope our findings will help researchers to avoid bacterial DNA amplification in negative controls on one hand, and to identify false positives in 16S PCR products, on the other hand.

Conclusion

The pair of universal primers 27F and 1407R is the best choice in avoiding the amplification in negative controls. The sources of bacterial DNA contamination in commercial Taq polymerases are most from the laboratory environment during the protein purification.

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The authors declare that they have no conflict of interest.

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