Journal of Microbiological Methods 89 (2012) 71–75

Contents lists available at SciVerse ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Rapid identification of oral isolates of *Aggregatibacter actinomycetemcomitans* obtained from humans and primates by an ultrafast super convection based polymerase chain reaction

M. Karched, D. Furgang, N. Sawalha, D.H. Fine *

Department of Oral Biology, New Jersey Dental School, University of Medicine & Dentistry of New Jersey, Newark, New Jersey, USA

ARTICLE INFO

Article history:
Received 23 November 2011
Received in revised form 23 January 2012
Accepted 26 January 2012
Available online 2 February 2012

Keywords:
A. actinomycetemcomitans
Localized aggressive periodontitis
rapid PCR
Super convection

ABSTRACT

Aggregatibacter actinomycetemcomitans is a Gram negative oral bacterium associated with localized aggressive periodontitis (LAP). Detection of *A. actinomycetemcomitans* in clinical samples is routinely done by PCR. Our aim was to develop a rapid and reliable PCR method that can be used as a chair-side tool to detect *A. actinomycetemcomitans* in clinical samples. Sensitivity and specificity assessment was performed on buccal and plaque samples obtained from 40 adolescents enrolled in an ongoing LAP study by comparing 20 *A. actinomycetemcomitans*-positive subjects and 20 who were negative. In a second study, *A. actinomycetemcomitans* presence was tested in oral samples from eighty-six primates that included rhesus monkeys, chimpanzees, marmosets, tamarins and baboons. All samples were processed for detection of *A. actinomycetemcomitans* by means of culture, conventional PCR (cPCR) and rapid PCR (rPCR) using a Super Convection based AmpXpress thermal cycler (AlphaHelix, Sweden). For human samples, culture, cPCR and rPCR showed perfect agreement. Using this method *A. actinomycetemcomitans* was detected in 27 of 32 rhesus monkeys, 4 of 8 chimpanzees and 1 of 34 marmosets. Rapidity of AmpXpress thermal cycler, combined with Ready-To-Go PCR beads (GE Life sciences), a quick DNA extraction kit (Epicentre Biotechnologies, Madison, Wisconsin, USA) and a bufferless fast agarose gel system, made it possible to obtain results on *A. actinomycetemcomitans* detection within 35 min. We conclude that AmpXpress fast PCR can be conveniently used as a chair-side tool for rapid detection of *A. actinomycetemcomitans* in clinical samples.

1. Introduction

Aggregatibacter actinomycetemcomitans is a Gram negative coccobacillus implicated in localized aggressive periodontitis (Zambon, 1985; Christersson, 1993). *A. actinomycetemcomitans* colonizes the oral cavities of humans (Slots, 1976; Socransky and Haffajee, 1992) and non-human primates (Eke et al., 1993) and belongs to the HACEK group of organisms believed to be associated with a number of systemic diseases including infective endocarditis (Das et al., 1997; Ellner et al., 1979; Paturel et al., 2004).

In addition to conventional culture based methods used to identify *A. actinomycetemcomitans*, PCR is now a well-established and widely used technique (Flemmig et al., 1995; Tran and Rudney, 1999). A large number of reports exist in the literature describing PCR based identification of *A. actinomycetemcomitans* (Flemmig et al., 1995; Goncharoff et al., 1993; Kim et al., 2005). While several studies have aimed at identifying only *A. actinomycetemcomitans* in the specimen, others have identified additional oral bacteria apart from *A. actinomycetemcomitans* e.g., using multiplex PCR (Tran and Rudney, 1999). Furthermore, 16S rDNA has been used as the target in PCR in many studies, but other genes such as *lktA* (Flemmig et al., 1995; Goncharoff et al., 1993; Tonjum and Haas, 1993) have also been used to identify *A. actinomycetemcomitans*. PCR based detection of bacteria in clinical specimens is sensitive and specific (Ficarra and Eversole, 1992; Olive, 1989). Conventional PCR, however, is time-consuming more often than not. This is mainly due to poor heat transfer on conventional PCR machines, resulting in longer time required to complete the reaction. In a clinical study setting during field screening of patients, generally samples are collected and brought to the laboratory where samples are processed and PCR performed for *A. actinomycetemcomitans* identification. The overall time for conventional PCR can vary from 2 to 4 h to overnight (Kramer and Coen, 2001). It is advantageous when conducting a screening examination to identify subjects who harbor *A. actinomycetemcomitans* at chair side within a short time period so that they can be informed that they are carriers of this potentially pathogenic organism. Extended time periods required for conventional PCR are inconvenient and can result in loss of subject interest and participation in ongoing studies. Therefore, rapid attainment of data at chair side during screening examinations could provide a great advantage and should improve

* Corresponding author at: 185 S Orange Ave., MSB C636, UMDNJ, Newark, New Jersey, USA. Tel.: +1 973 972 7053; fax: +1 973 972 0045.
E-mail address: finedh@umdnj.edu (D.H. Fine).

0167-7012/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.mimet.2012.01.016

recruitment of subjects. In our approach to develop a rapid PCR method for the detection of *A. actinomycetemcomitans*, we utilized samples from *A. actinomycetemcomitans*-positive and negative subjects who were involved in a longitudinal study of the relationship of *A. actinomycetemcomitans* to the initiation of localized aggressive periodontitis. In addition, oral samples from several primate species were used to compare culture to conventional PCR and to a new Super Convection rapid PCR technique. In this report we demonstrate that the new ultrafast PCR technique can be conveniently used as a chair-side tool for rapid *A. actinomycetemcomitans* detection.

2. Materials and methods

2.1. Bacterial culture

Buccal and plaque samples were suspended in *A. actinomycetemcomitans* Growth Medium (AAGM) broth [trypticase soy broth with 0.8% glucose (8 g/l), 0.6% yeast extract (6 g/l) and 0.4% sodium bicarbonate (4 g/l), 75 μg/ml bacitracin and 5 μg/ml vancomycin] and brought to the laboratory for processing. In some cases, plaque and buccal samples were collected using cytology brushes, and then the brushes were stabbed in half-strength AAGM agar in small glass vials before being sent to our laboratory. Once samples reached the laboratory, serial 10-fold dilutions were made and spread on AAGM plates. After 3 days of incubation at 37 °C and 10% CO_2, *A. actinomycetemcomitans* colonies were preliminarily identified by colony morphology and catalase positivity. Presumptive *A. actinomycetemcomitans* colonies were subcultured from each sample. Human *A. actinomycetemcomitans* isolate IDH781 and *Aggregatibacter aphrophilus* ATCC® 33389™, a phylogenetic relative of *A. actinomycetemcomitans*, were also grown on AAGM as above.

2.2. Purification of DNA

DNA from the buccal/plaque samples from humans and primates, and genomic DNA from *A. actinomycetemcomitans* isolates was purified using DNeasy® blood and tissue kit from Qiagen (QIAGEN Sciences, Germantown, Maryland, USA). Briefly, the samples were treated with a lysis buffer and proteinase-K overnight at 56 °C, followed by extraction and purification of DNA using Qiaquick spin columns (Qiagen). DNA from buccal samples from subjects with or without LAP were extracted using QuickExtract™ DNA extraction kit from Epicentre Biotechnologies (Madison, Wisconsin, USA). The swab samples were suspended first in a quick DNA extract solution and heated at 65 °C for 6 min and then the tubes were transferred to 98 °C and incubated for 2 min. The extracted DNA was stored at −20 °C.

2.3. Human sampling and analysis

Buccal samples from 40 subjects (29 females and 11 males, mean age = 14 year) enrolled in an ongoing LAP study (20 *A. actinomycetemcomitans*-positive subjects and 20 *A. actinomycetemcomitans*-negative) were used for detecting *A. actinomycetemcomitans* by both cPCR and rPCR. All volunteers gave consent, using a form that was reviewed and approved by the Institutional Review Board (IRB) of the University of Medicine & Dentistry of New Jersey (UMDNJ).

2.4. Primate sampling

Monkey samples were collected from the North East Regional Primate Research Center (NEPRC) at Harvard University, Southwest National Primate Research Center (SNPRC), Yerkes Regional Primate Research Center at Emory University and Laboratory Animal Services facility at Rutgers University. All monkeys (Table 2) had an intact dentition and were housed in separate cages. Prior to sampling all primates were anesthetized using ketamine hydrochloride (15 mg/kg) and a supplement of isoflurane. Buccal mucosa of the monkeys was sampled with sterile wooden tongue depressors. Plaque samples were collected using autoclaved dental scalers. The samples were suspended in AAGM broth and processed for bacterial culture as described above. Sample collection from primates was approved by the Institutional Animal Care and Use Committees of the UMDNJ, Harvard University and Rutgers University.

2.5. Super convection rapid PCR

Table 1 shows primers and amplicon sizes. For all PCR reactions, Ready-To-Go beads (GE HealthCare Biosciences, Buckinghamshire, UK) were used. When each bead was reconstituted to 25 μl final volume, the concentration of each dNTP was 200 μM in 10 mM Tris–HCl (pH 9), 50 mM KCl and 1.5 mM $MgCl_2$. Primers were used at a concentration of 0.5 μM and the amount of template DNA was 50–100 ng per reaction. PCR reactions were performed on the rapid PCR machine AmpXpress (Alpha Helix Molecular Diagnostics AB, Sweden). Rapid PCR is facilitated by a centrifugation based convection technology used in the instrument (Martensson et al., 2006). A typical thermal profile consisted of an initial denaturation of 94 °C for 1 min followed by 30 cycles of 94 °C for 0 s, 55 °C for 6 s and 72 °C for 7 s. No final elongation was required.

2.6. Conventional PCR

A Techne TC-412 PCR machine (Techne Inc. Burlington, NJ, USA) was used. All PCR reagents were the same as described above for rapid PCR. Except for variable annealing temperatures for different primer pairs, the temperature profile was as follows: Initial denaturation 94 °C for 10 min followed by 30 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min.

2.7. Agarose gel electrophoresis

PCR products were visualized by electrophoresis through a 2% agarose gel after adding the dye EZ-Vision™ Three (Amresco, Ohio, USA) to the entire PCR product, i.e., 25 μl. In rPCR experiments, a rapid and bufferless agarose gel system that completes in 6 min at 250 V (Febe bufferless agarose gel; Biokeystone Co, California, USA) was used. The gels were then exposed to UV light on a trans illuminator and pictures were taken by the attached Kodak DC290 camera.

3. Results

3.1. Validation of the efficacy of rPCR

In order to test the reliability of the Super Convection rPCR, we performed PCR for *lktA* of *A. actinomycetemcomitans* strains in parallel both on the AmpXpress machine as well as the conventional PCR machine. Fig. 1A shows that all *A. actinomycetemcomitans* strains tested produced an expected 262-bp band of similar intensity from both PCR machines. Sensitivity of the super convection rPCR was also compared with that of the conventional PCR. Genomic DNA from a serial 10-fold dilutions of *A. actinomycetemcomitans* IDH781 was used in the same PCR reaction as above using *A. actinomycetemcomitans*-specific *lktA* primers. It was possible to amplify the fragment at a bacterial concentration as low as 10^3/ml using rPCR, similar to cPCR (Fig. 1B). Comparison of rPCR with cPCR in terms of time requirements is schematically shown in Fig. 2. The time requirement for each step of either PCR method was established after running at least 3 experiments. Regardless of using a quick DNA extraction method and a fast agarose gel electrophoresis system, the cPCR takes approximately 2 h, while rPCR requires as little as 35 min to obtain results (Fig. 2).