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Rapid identification and quantitation for oral bacteria based on short-end capillary electrophoresis

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ABSTRACT

High-speed capillary electrophoresis (HSCE) is a promising technology applied in ultra-rapid and high-performance analysis of biomolecules (such as nucleic acids, protein). In present study, the short-end capillary electrophoresis coupled with one novel space domain internal standard method (SDIS) was employed for the rapid and simultaneous analysis of specific genes from three oral bacteria (*Porphyromonas gingivalis* (P.g), *Treponema denticola* (T.d) and *Tannerela forsythia* (T.f)). The reliability, reproducibility and accuracy properties of above mentioned SDIS method were investigated in detail. The results showed the target gene fragments of P.g, T.d and T.f could be precisely, fast identified and quantitated within 95 s via present short-end CE system. The analyte concentration and the ratio of space domain signals (between target sample and internal standard sample) featured a well linear relationship calculated via SDIS method. And the correlation coefficients R^2 and detection limits for P.g, T.d, T.f genes were 0.9855, 0.9896, 0.9969 and 0.077, 0.114 and 0.098 ng/μl, respectively.

1. Introduction

To date, recent evidences have suggested that hundreds of bacteria species (around 700) exist in human oral cavity [1]. Some of them may result in oral disease when the abnormal population happens. For instance, *Streptococcus mutans* (S.m) and *Lactobacillus beijerinck* (L.b) have been considered as the major pathogens related to caries [2,3]; *Porphyromonas gingivalis* (P.g), *Treponema denticola* (T.d) and *Tannerela forsythia* (T.f) play key roles in periodontitis happening [4,5]. Moreover, some oral bacteria could also induce some other serious illnesses, such as gastropathy, diabetes and cardiovascular disease, which have been demonstrated by previous work [6–8]. Recently, more researchers have suggested that above pathogenic bacteria, which are closely associated with oral diseases, could be adopted as effective indicators for clinical examination and diagnosis.

Unfortunately, until now how to realize facile, efficient and low-cost analysis for oral bacteria is still a formidable challenge in clinical research. The traditional methods (such as culture, morphology and immunology technologies) are still in prevalence [9–12]. However, the clinical application of above technologies is strictly limited due to their intrinsic shortages of long-time, complicated operation, and poor-accuracy. In addition, most bacteria in oral cavity can't be cultivated and duplicated because of the very strict requests in culture method [13,14]. In recent decades, the real-time polymerase chain reaction (PCR) and gene probe technologies, based on the specific and conserved genes originated from microorganism, have been widely studied due to the merits of noninvasive monitoring and extreme accuracy [15]. Whereas, the methods mentioned above are not only complicated but also costly.

Since nucleic acid molecules were successfully separated via capillary electrophoresis (CE) in 1988, CE has been applied extensively in gene analysis because of its excellent advantages including fast-separation, high-resolution, and less sample consumption [16,17]. Nowadays, CE is often coupled with PCR, which also could be called post-PCR route, for the target gene analysis. In 1991, the concept of high-speed CE (HSCE) was firstly presented by Jorgenson's pioneer work [18]. The purpose of HSCE is achieving ultra-rapid separation and high performance within a very short time (< 3 min) via using short-pathway or high-voltage. It mainly

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Table 1
Primers' sequences of target genes for PCR.

Bacteria	Primers' sequences	
	Forward	Reverse
P.g	5′-TGTAGATGACTGATGGTGAAAACC-3′	5′-ACGTCATCCCCACCTTCCTC-3′
T.d	5′-AAGGCGGTAGAGCCGCTCA-3′	5′-AGCCGCTGTCGAAAAGCCCA-3′
T.f	5′-GCGTATGTAACCTGCCCGCA-3′	5′-TGCTTCAGTGTCAGTTATACCT-3′

includes two strategies: microfluidic chip electrophoresis and short-end CE [19,20]. It is worth mentioning that the fabrication and manipulation of a microfluidic chip are more complicated compared with the mature fused silica capillary. Therefore, short-end CE features more attractive in simplicity and convenience for routine biomolecule detection.

Precisely quantitative determination is an important point of concerns for bacteria analysis in clinical field. However, it is still a tricky technical difficulty for CE in spite of the remarkable performances in biomolecule identification. In previous quantitative CE reports, internal standard (IS) method is the typical analysis approach, which determines the analyte content based on the ratio of electrophoretic peak area of target sample (TS) and that of IS sample [21]. Electrophoretic peaks come from the variation of fluorescent signal relying on time. So, the IS method based on electrophoretic peak area is defined as time domain IS (TDIS) for convenient elaboration. However, the exact relationship between time domain signal and actual content of sample was not carefully studied. Thus the precision of quantitative analysis based on TDIS is doubtful.

In present work, to overcome the shortages of existed TDIS method, one novel and simple-to-use space domain IS method (SDIS) was provided and coupled with short-end CE technology for the rapid analysis of three target genes from oral pathogens P.g, T.d and T.f. The relationship between actual space domain signal and detected time domain signal of electrophoretic sample band was deeply investigated. Furthermore, the reliability, reproducibility and accuracy properties of proposed SDIS method were studied in detail. To our knowledge, there is rare similar work has been reported to date. Such work may present an effective analysis method for clinical diagnosis of human oral diseases.

2. Experimental

2.1. Short-end CE detection system

In this work, the short-end CE system, mainly based on an inverted fluorescent microscope (DSY 5000X, Aopu, Chongqing, China), was self-built in our lab. And a high-voltage power supply (Model HSR-25P (A), MATSUSADA, Osaka, Japan) was employed to drive the electrophoresis. The excitation light from the mercury lamp was filtered to be 460–495 nm, which matched the maximum excitation wavelength of dye-nucleonic acid conjugate, via an optical filter (U-MWIB3, Olympus, Tokyo, Japan). The emitted fluorescence signal was collected by a 60 × objective and detected via a photomultiplier tube (PMT) (H8429-101, Hamamatsu Photonics, Japan). The applied voltage adjustment and raw data collection were digital processed by NI USB-6212 DAQ card and LabVIEW software, which were all purchased from National Instrument (NI) (Austin, TX, USA). The uncoated fused silica capillary with inner diameter of 50 μm was bought from Polymicro Technologies (Phoenix, AZ, USA). The effective length (L_e) and total length (L_t) of our experimental short capillary were designed as 2.5 and 4 cm, respectively. The whole system was enclosed in a dark box at room temperature (~25 °C).

2.2. Chemicals and reagents

100 bp DNA ladder (including 11 fragments ranging from 100 to 1500 bp), 10 × Tris-borate-EDTA (TBE) buffer and SpeedSTAR HS DNA polymerase were purchased from Takara (Dalian, China). 10 × TBE buffer was diluted to 0.5 × and kept at room temperature. Hydroxyethylcellulose (HEC, Mw = 1300 K) powder was provided by Polysciences (Warrington, PA, USA), and prepared to be 1.5% HEC solution with 0.5 × TBE. 50 and 400 bp standard DNA samples, SYBR Green I dye were gained from Thermo Fisher Scientific (Waltham, MA, USA). SYBR Green I (10^4 ×) was diluted to be 100 × with 0.5 × TBE and stored under light-blocking at 4 °C, the final using concentration was 5 × in present work. The PCR templates of P.g, T.d, T.f were extracted by Wizard Genomic DNA purification (Promega, Madison, WI, USA). Primers of target genes for PCR were synthesized by Sangon Biotech (Shanghai, China), their detailed information was shown in Table 1.

2.3. PCR for target DNA fragments

For each bacterium, the PCR reaction solution was prepared with 3 μl 10 × Fast Buffer I, 2.4 μl dNTP mixture (2.5 mM), 2 μM primers, and 0.15 μl SpeedSTAR HS DNA polymerase. The final 30 μl reaction solution was obtained after adding sterile water into above reagents mixture. In present work, the PCR process was carried out by Takara TP350 thermal cycler (Dalian, China). Firstly, the PCR thermal-cycling start with an initial denaturation step at 95 °C for 2 min. Then the program, (95 °C, 10 s) for denaturation and (64 °C, 30 s) for annealing and extension, was executed 30 cycles sequentially. According to the designed primers shown above, the sizes of final amplified products were 197, 311 and 641 bp for P.g, T.d and T.f, respectively.

3. Results and discussion

3.1. Space domain IS method (SDIS)

The content of a component in the sample is usually presented in terms of mass or mole. In this paper, mass and mass concentration were preferred to measure the content and concentration of DNA, in accordance with the purchased DNA samples adopted in our experiments. It is well known that there is a linear relation between fluorescent signal and content of fluorescent substance. In the case of fluorescence detection of DNA capillary electrophoresis, SYBR Green I dye molecules uniformly intercalated into dsDNA minor groove [22,23]. Thus, the content of DNA fragments containing in a DNA band is linearly reflected by the practical amount (labeled as *PA*) of spatially distributed fluorescent signal.

Assuming Q is the content of DNA in a certain band. The band was divided into numbers of small parts (q_1, q_2, ……, q_n) along with the axis of capillary. Correspondingly, the spatially distributed fluorescent signals of small parts are (I_1, I_2, ……, I_n). Then, the value of *PA* could be calculated as