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Rapid Detection of *Borrelia burgdorferi* Strains by Nested Polymerase Chain Reaction

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Abstract: This study was carried out to evaluate the nested PCR for specific detection of different strains of *B. burgdorferi*. Five strains of *B. burgdorferi* including ACA-1, B-31, 2B45, 3B45, 7B49 obtained from different countries were used in this study. The strains of *B. hermsii*, *Escherichia. coli* and *T. pallidum* were also included as control strains. Two pairs of nested PCR primers were used to amplify the gene encoding the Osp-A protein of *B. burgdorfer* under standard PCR condition. In a two stage procedure, nested PCR yielded a positive reaction for five tested strains of *B. burgdorferi*. None the strains including *B. hermsii*, *E. coli* and *T. pallidum* showed positive reaction when used as control strains in PCR. In conclusion, nested PCR showed acceptable specificity for rapid detection of *B. burgdorferi*.

Key words: Lyme borreliosis, *Borrelia burgdorferi*, rapid detection, PCR

INTRODUCTION

Lyme borreliosis is caused by *Borrelia burgdorferi*. This bacterium is maintained in nature in an enzootic cycle and is transmitted by Ixodes ticks (Li *et al*., 2007; Steere *et al*., 1983; Barbour and Fish, 1993; Srivastava and De-Desilva, 2008). The vertebrate hosts are infected through the tick's salivary glands (Anguita *et al*., 2003). Lyme is a multi-system infection with early and late manifestations involving the skin, heart, nervous system and joints. This disease is now recognized as the most common thick-borne infection in Europe, North America and other parts of the world with a temperate climate (Nardelli *et al*., 2008; Bacon *et al*., 2003).

Laboratory diagnosis of this disease is complicated. The most of the related clinical symptoms are not unique to lyme disease. Moreover, the culture of *B. burgdorferi* takes a long time to grow and the accuracy of serological tests is doubtful in patients, strong and specific immunologic responses do not always accompany infection and spirochetes generally cannot be directly detected or isolated from infected individuals (Wallet *et al*., 2008; Craft *et al*., 1984; Dattwyler *et al*., 1988; Magnarelli *et al*., 1987; Rosa *et al*., 1991).

At present, serology based tools remains the main diagnostic methods for laboratory diagnosis of this disease. Some molecular methods such as Polymerase Chain Reaction (PCR) based techniques have been recently applied for diagnosis of *B. burgdorferi* but, until now, a reliable, easy-to-perform and sensitive method has not been described (Santino *et al*., 2008).

Molecular approach has provided powerful tools for diagnosis, epidemiological surveillance and tracking of key genes among the microbial pathogens (Ranjbar *et al*., 2007a; Ghasemi *et al*., 2008). PCR as a powerful technique can be useful especially for the detection of pathogens whose *in vitro* cultivation is difficult (Persing *et al*., 1990). Recently some novel PCR-based methods has been developed for detection *B. burgdorferi* infections (Joss *et al*., 2008).

Among PCR-based techniques, nested PCR has been evaluated as the most sensitive method for the demonstration of *Borrelia* sp. in clinical samples such as erythema migrans skin lesions (Cerar *et al*., 2008).

The main aim of current study was to evaluate the nested PCR for specific detection of different strains of *B. burgdorferi*.

MATERIALS AND METHODS

Five strains of *B. burgdorferi* including ACA-1 (obtained from Sweden), B-31 (obtained from USA), 2B45, 3B45, 7B49 (obtained from Germany) were used in this study. The strains of *B. hermsii* (obtained from USA), *Escherichia. coli* (obtained from Southampton, UK) and *T. pallidum* (obtained from Birmingham, UK) were

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also included as control strains. *B. burgdorferi* strains were cultured in BSK-II medium and grown for 4-14 days at 33°C. After harvesting, cells (10^4) were lysed by heating at 100°C in 200 mM NH_4OH and after removal of excess ammonia; a portion (25 µL) of the remaining solution was added directly to the PCR reagents. Two pairs of nested PCR primers were used to the gene encoding the Osp-A protein of *B. burgdorferi*. Primer pair 1, OspA-Nl (5'- GAGCTTAAAGGAACTTCTGATAA-3'), OspA-Cl (5'-GTATTGTTGTACTGTAATTGT-3') and primer pair 2, OspA-N2 (5'-ATGGATCTGGAGTACTTGAA-3'), OspAC2 (5'-CTTAAAGTAACAGTTCCTTCT-3') are correspond to nucleotide 334-356, 874-894, 362-381 and 693-713, respectively, of the OspA gene (Guy and Stanek, 1991).

First step of PCR amplification was done by following concentrations and cycling program: dNTPs 1.25 mM, 1x PCR buffer, 20 µL from each of primers OSPA-N1 and OSPA-C1 and 1.25 U Taq DNA polymerase. PCR amplification was done in 35 cycles including: denaturation in 94°C for 1 min, 37°C for 2 min in order to anneal the primers, 72°C for 3 min for extension. Each sample was then transferred to a second PCR reaction mix in which the first primers were replaced with second primers OspA-N2 and OspA-C2 and amplified for a further 25 cycles. Electrophoresis of PCR products was done by submarine agarose gel electrophoresis and finally the gel was stained by ethidium bromide.

RESULTS AND DISCUSSION

As shown in Fig. 1, in a two stage procedure, nested PCR yielded a positive reaction for five tested strains of *B. burgdorferi* including ACA-1, B-31, 2B45, 3B45 and 7B49. A single strong band of approximately 352 bp was produced all *B. burgdorferi* strains tested. None the strains including *B. hermsii*, *E. coli* and *T. pallidum* showed positive reaction when used as control strains in PCR.

Different approaches such as microscope-based assays, detection of specific proteins or nucleic acids and culture have been used for diagnosis of *B. burgdorferi* in the clinical laboratory (Aguero-Rosenfeld *et al*., 2005).

The different molecular methods could provide sensitive interpreting keys suitable for microbiological studies (Ranjbar *et al*., 2007b; Ranjbar *et al*., 2008a, b). Molecular tools, particularly amplification of specific markers using the PCR have revolutionized detection and identification of pathogenic organisms such as this organism.

PCR-based methods have been used for confirmation of the clinical diagnosis of suspected lyme borreliosis,

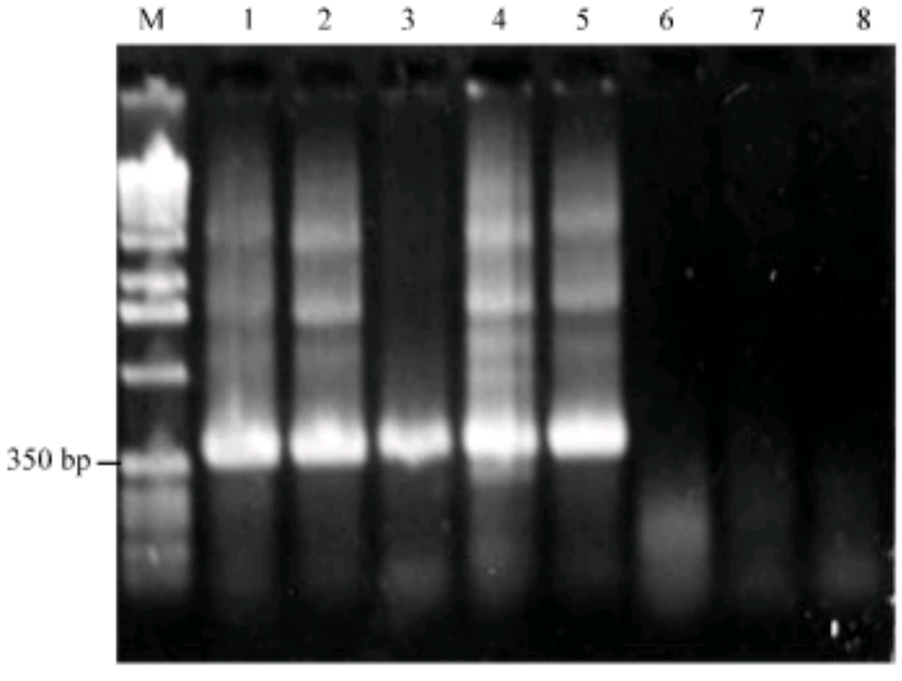

Fig. 1: Specific nested PCR amplification of Osp operon of *B. burgdorferi* strains. Lane M: Molecular weight marker, lane 1-5: Five strains of *B. burgdorferi* including ACA-1, B-31, 2B45, 3B45, 7B49, respectively, lane 6-8: Three control strains including *B. hermsii*, *E. coli*, *T. pallidum*, respectively

molecular identification of species and/or molecular typing of spirochetes and detection of coinfection of *B. burgdorferi* and other tick-borne pathogens (Aguero-Rosenfeld *et al*., 2005).

In current study, a nested PCR targeting the gene encoding the outer surface protein (Osp)-A was evaluated for rapid detection of *B. burgdorferi*. Outer surface proteins are one of the specific markers of this organism (Robertson *et al*., 2000). Thirty one and 34 kDa outer surface proteins are designated Osp-A and Osp-B (Heikkila *et al*., 2002; Howe *et al*., 1985). Osp-A is produced as borreliae enter the tick vector and remains a major surface antigen during midgut colonization (Battisti *et al*., 2008). The nucleotide sequence of the Osp-A and Osp-B structural genes has been determined by Magnarelli *et al*. (2002) and Bergstrom *et al*. (1989). These genes are good targets for diagnosis purpose in this bacterium.

Guy and Stanek (1991) described direct detection of *B. burgdorferi* in the serum samples recovered from patients with Lyme disease. They used two pairs of nested PCR primers complementary to Osp-A gene of *B. burgdorferi* and detected two positive cases among five patients with erythema migrans. They concluded that their method could form the basis of a useful routine laboratory test in those cases of early lyme disease where conventional serological testing commonly yields equivocal or false negative results (Guy and Stenek, 1991). Some researcher around the world have been earlier used the PCR to direct detection of *B. burgdorferi* in its vectors and clinical samples (Lebechdouble-Dagger *et al*., 1998;

Nocton *et al*., 1994). Persing *et al*. (1990) used PCR to amplify DNA sequences of *B. burgdorferi* in its tick vector. Their target gene for PCR amplification was the Osp-A of strain B31. They showed that this gene could be used to identify the most North American isolates. (Persing *et al*., 1990). More recently Cerar *et al*. (2008) described a nested PCR targeting the rrf-rrl region and found the technique was the most sensitive method for the demonstration of *Borrelia* sp. in erythema migrans skin lesions when compared by culture and PCR targeting the flagellin gene.

In this study, we used nested PCR with primers applied by Guy and Stanek (1991) to evaluate the technique for rapid diagnosis of different strains of *B. burgdorferi* obtained from different countries including USA, Sweden and Germany. Nested PCR could produce a single strong band approximately 352 bp for all strains tested but no bands were seen with *B. hermsii*, *E. coli* and *T. pallidum* demonstrating the specificity of the reaction for *B. burgdorferi*. Current study showed the amplification of *B. burgdorferi*-specific sequences using the nested PCR can be considered as a specific detection of this bacterium and it is hoped that the technique can be useful for direct detection of organism in the vector or clinical samples.

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