P0421 Identification of Chlamydia Species by Electrophoretic Separation of omp2 Gene PCR Products in the Presence of Bisbenzimide-PEG

V. DEMKIN[1], A. ZIMIN[1], M. EDELSTEIN[2], I. EDELSTEIN[2], M. SUVOROV[2]

[1]Laboratory for Molecular Diagnostics, Institute for Molecular Genetics, Moscow

[2]Department of Clinical Pharmacology and Antimicrobial Chemotherapy, Smolensk State Medical Academy, Smolensk

P.O.Box 5, Smolensk, 214019, Russia; ☎: +7 (0812) 55 34 01, fax: 55 06 24; Email: me@cliph.keytown.com

ABSTRACT

Objectives: To develop a simple and rapid molecular method for detection of organisms belonging to the genus Chlamydia and their subsequent identification to the species level.

Methods: The omp2 gene of *Chlamydia* spp. encoding the 60Kda cysteine-rich outer membrane protein was selected as a target for amplification by PCR. The following primers (5'-CAAACTCATCAGACGAG(C/T)AGT-3' and 5'-CCTTCTTTAAGAGGTTTTACCC-3') which anneal to the two conserved regions of the gene were used to amplify 582bp DNA fragment of *C.psittaci* CP-16, 579bp fragment of *C.pneumoniae* K7 and 552bp fragment of *C.trachomatis* L7. The PCR products reveal significant genetic variability (40-54.5%) between different species of Chlamydia while retain sequence conservation at the subspecies level. This observation permitted a sequence specific separation of amplification products by electrophoresis in the agarose gel containing 1U/ml of DNA ligand bisbenzimide-PEG (H.A.Yellow).

Results: Due to the different number of bisbenzimide binding sites, which consist of at least 4 consecutive A/T bases, an expected and clearly different mobility of the amplicons derived from three species was observed. The migration rate of the amplicons was directly proportional to the number of bisbenzimide binding sites: *C.trachomatis* -24, *C.pneumoniae* - 25, *C.psittaci* - 27.

Conclusions: We have demonstrated that a simple agarose gel electrophoresis in the presence of bisbenzimide-PEG makes possible a rapid identification of 3 reference strains of Chlamydia. Analysis of the currently available sequences of chlamydial omp2 gene allows us to suggest that our method may be successfully applied for identification of different isolates of Chlamydia to the species level.

INTRODUCTION AND PURPOSE

There are 4 species of intracellular bacteria that recognised within the genus Chlamydia. Three of them: *C.trachomatis* (TR), *C.psittaci* (PS) and *C.pneumoniae* (PN) may cause a wide range of human diseases. Although some of diseases are associated with a particular species or strains, all three species may be responsible for respiratory infections. This fact provokes the interest in developing efficient diagnostic procedures for detection and differentiation of Chlamydia species.

Several research groups have applied a two-step procedure for the successive detection of the organisms belonging to this genus and their subsequent identification to the species level, by the amplification of a common genus-specific DNA sequence followed by a digestion with restriction enzymes (RFLP) or by a nested-PCR.

Recently a simple method has been described allowing a separation of PCR-amplified DNA-fragments which are identical in length but differ in base composition. This method utilises a typical electrophoresis procedure in an agarose gel containing bisbenzimide-PEG (H.A.Yellow; Hanse Analytik GmbH, Germany). The bisbenzimide binds to the specific DNA motifs which consist of at least 4 consecutive A/T bases and being loaded with the long PEG chains leads to selective retardation and separation of the DNA sequences which differ in the number of bisbenzimide binding sites (M.Müller, 1997).

The purpose of our study was to evaluate the applicability of this method for identification of three chlamydial species (TR, PS and PN).

METHODS

Bacterial strains. Partially purified and heat-inactivated elementary bodies (EB) of *C.pneumoniae* K7, *C.psittaci* CP-16 and *C.trachomatis* L2 were kindly donated by Prof. Pekka Saikku (National Public Health Institute, Finland).

DNA purification and amplification by PCR. Prior to PCR, suspensions of EB were digested with proteinase K, boiled and diluted to introduce approximately 10 EB into each PCR reaction. The omp2 gene of *Chlamydia* spp. encoding the 60Kda cysteine-rich outer membrane protein was selected as a target for amplification by PCR. The following primers (5'-CAAACTCATCAGACGAG(C/T)AGT-3' and 5'-CCTTCTTTAAGAGGTTTTACCC-3') previously described by M.W.Watson et al. (1991) were used to amplify 582bp DNA fragment of *C.psittaci*, 579bp fragment of *C.pneumoniae* and 552bp fragment of *C.trachomatis*. The PCR reaction mixture contained 50mM KCl, 10mM Tris-HCl (pH 8.4), 2.5mM $MgCl_2$, 200μM each dNTP, 0.25μM each primer and 1U Taq polymerase (IMG RAS, Moscow) in 30μl final reaction volume. Reaction mix was then overlaid with 10μl mineral oil and subjected to initial denaturation at 94°C for 3 min followed by 35 cycles of 20 sec denaturation at 94°C, 20 sec annealing at 45°C and 30 sec elongation at 72°C. The final elongation step was extended to 5 min. Amplification was performed with a DNA Thermal Cycler 480 (Perkin Elmer, USA).

Electrophoretic separation of PCR products. Amplification products were separated with the Wide Mini-Sub Cell GT electrophoresis system (BioRad, USA) in an agarose gel containing 3% (w/v) AmpliSize agarose (BioRad, USA) in 0.5x TBE buffer with U=100V for 3h. The H.A.Yellow was added to the agarose solution at a temperature of 50°C to a final concentration of 1U/ml as recommended by manufacturer. After electrophoresis the gels were stained with ethidium bromide and documented using the PhotoDoc-IT Link Gel Documentation System (UVP, USA).

RESULTS

All chlamydial strains yielded a single DNA fragment of 552-582bp after amplification by PCR. The resultant DNA fragments were slightly larger in the case of PS and PN because of the several nucleotide deletions in the 5'-end of omp2 gene sequence from TR. This difference in the length of PCR products may be detected simply by electrophoretic separation in an agarose gel without a DNA-ligand, however PS and PN may not be differentiated by this way (Fig.1A).

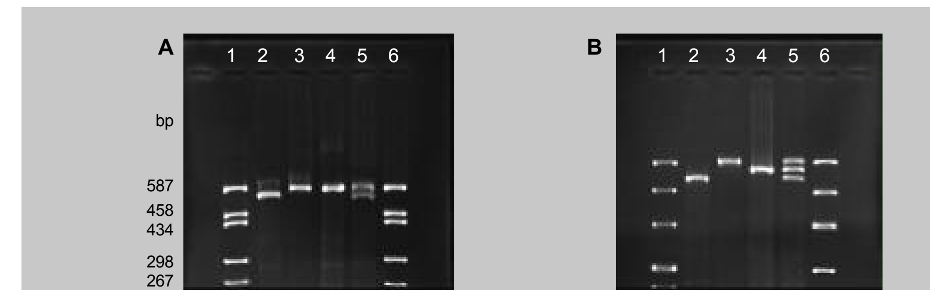

Figure 1. (**A**) Analysis of PCR products derived from three chlamydial species by conventional agarose gel electrophoresis. Lanes 1, 6: DNA size standard pUC18 Hae III; Lane 2: 552bp PCR fragment from TR; Lane 3: 582bp PCR fragment from PS; Lane 4: 579bp PCR fragment from PN; Lane 5: equimolar mixture of fragments from TR, PS and PN.
(**B**) PCR and size standards as in (A) but electrophoresed in an agarose gel containing 1U/ml H.A.Yellow. Buffer and conditions were otherwise identical.

Figure 1B illustrates that all three fragments corresponding to the different species of Chlamydia were successfully resolved by bisbenzimide-PEG-agarose gel electrophoresis. Notably, the fragments from PS and PN which could not be separated by conventional electrophoresis have clearly different mobility in the presence of H.A.Yellow. This observation is in good concordance with the expected number of bisbenzimide binding sites contained in the amplified sequences of PS (27 sites) and PN (25 sites).

By extending the time of electrophoresis to 4h, even better separation of the fragments was achieved (data not shown). However, because the bisbenzimide-PEG slowly migrates in the opposite direction to the DNA, the use of a long gel and consequently larger amount of the DNA ligand was necessary to prevent “smearing” of bends.

The reproducibility of bisbenzimide-PEG-agarose gel electrophoresis was tested in several consecutive experiments. Each time a consistent and reproducible separation of the respective PCR products was observed.

CONCLUSION

Our study has demonstrated that the PCR with a single pair of primers followed by a simple agarose gel electrophoresis in the presence of bisbenzimide-PEG makes possible a rapid identification of 3 reference chlamydial strains.

Additional experiments with a larger number of strains are needed to determine an influence of intraspecies genetic variability of the amplified region of omp2 gene on the electrophoretic mobility of PCR products and applicability of this technique for the practical identification of Chlamydia species.

ACKNOWLEDGEMENT: We are very grateful to Prof. Pekka Saikku (National Public Health Institute, Oulu, Finland) for a very kind gift of the reference chlamydial strains.

We would like to thank Hanse Analytik GmbH for generously providing the H.A.Yellow and for additional information accompanying this product.