Research in Microbiology 165 (2014) 813–825

www.elsevier.com/locate/resmic

Original article

Evolution of the RNase P RNA structural domain in *Leptospira* spp.

Vigneshwaran Ravishankar [a], Ahmed Ahmed [b], Ulaganathan Sivagnanam [a], Krishnaraja Muthuraman [a], Anbarasu Karthikaichamy [a], Herald A. Wilson [a], Ajay Devendran [a], Rudy A. Hartskeerl [b], Stephen M.L. Raj [a,*]

[a] *Department of Biotechnology, Mepco Schlenk Engineering College, Sivakasi 626005, Tamilnadu, India*

[b] *WHO/FAO/OIE, National Collaborating Centre for Reference and Research on Leptospirosis, KIT Biomedical Research, Meibergdreef 39, 1105 AZ Amsterdam, The Netherlands*

Received 9 September 2014; accepted 15 October 2014
Available online 23 October 2014

Abstract

We have employed the RNase P RNA (RPR) gene, which is present as single copy in chromosome I of *Leptospira* spp. to investigate the phylogeny of structural domains present in the RNA subunit of the tRNA processing enzyme, RNase P. RPR gene sequences of 150 strains derived from NCBI database along with sequences determined from 8 reference strains were examined to fathom strain specific structural differences present in leptospiral RPR. Sequence variations in the RPR gene impacted on the configuration of loops, stems and bulges found in the RPR highlighting species and strain specific structural motifs. In vitro transcribed leptospiral RPR ribozymes are demonstrated to process pre-tRNA into mature tRNA in consonance with the positioning of *Leptospira* in the taxonomic domain of bacteria. RPR sequence datasets used to construct a phylogenetic tree exemplified the segregation of strains into their respective lineages with a (re)speciation of strain SH 9 to *Leptospira borgpetersenii*, strains Fiocruz LV 3954 and Fiocruz LV 4135 to *Leptospira santarosai*, strain CBC 613 to *Leptospira kirschneri* and strain HAI 1536 to *Leptospira noguchii*. Furthermore, it allowed characterization of an isolate P2653, presumptively characterized as either serovar Hebdomadis, Kremastos or Longnan to *Leptospira weilii*, serovar Longnan.

Keywords: *Leptospira*; RNase P RNA; Phylogeny; Ribozyme

1. Introduction

Differentiation of microorganisms based on nucleotide sequences of RNase P RNA (RPR) was demonstrated in many bacterial genera [1–4]. RPR genes of various species have shown highly conserved stretches interspersed with varying sequences unique to each organism. RPR is a subunit of an essential tRNA processing enzyme, ribonuclease P (RNase P). The 5′ leader sequence of precursor tRNA (pre-tRNA) is catalytically cleaved by RNase P to produce mature tRNA [5,6]. Generally, RNase P holoenzyme is a ribonucleoprotein complex comprising the RPR subunit, and one or more protein cofactor(s) [7–10]. In all eukaryotic organisms, both RNA and protein subunit(s) are required for catalytic activity at physiologic concentration of Mg^{2+} whereas in bacteria and archaea, RPR alone can cleave pre-tRNA at elevated Mg^{2+} concentration [11,12].

RPR sequences of many species representing the domains of life have been catalogued in the RNase P database where secondary structures of RNA subunits were constructed and made available for public use. Phylogenetic comparative analyses and biochemical studies performed on various bacterial

* Corresponding author.
E-mail addresses: rvigneswaran9490@yahoo.com (V. Ravishankar), a.ahmed@kit.nl (A. Ahmed), ulags.iitm@gmail.com (U. Sivagnanam), krish_raja0@yahoo.co.in (K. Muthuraman), anbu4biotech@gmail.com (A. Karthikaichamy), heraldwilson89@gmail.com (H.A. Wilson), dajayneo@gmail.com (A. Devendran), r.hartskeerl@kit.nl (R.A. Hartskeerl), stephenraj@mepcoeng.ac.in, rajste1965@yahoo.com (S.M.L. Raj).

http://dx.doi.org/10.1016/j.resmic.2014.10.007
0923-2508/

RPRs [13–16] have helped understanding how tertiary interaction between different domains facilitated the formation of higher order structures. Analysis of the crystal and solution structures of bacterial RPR revealed the presence of two distinct structural components called the catalytic and the pre-tRNA binding substrate specific domains [17–22]. All RPR structures known so far have a common core but vary in the presence of peripheral structural elements playing role in tertiary interactions [23] to offer stability in the overall conformation [14].

Variations observed in the helices of RPR secondary structures provided useful phylogenetic information among bacterial species and strains. Our study focused on understanding the sequence and structural variations that necessitate evolution of RPR in distinct strains of *Leptospira*. The genus *Leptospira* is highly heterogenous comprising pathogenic (*Leptospira interrogans*, *Leptospira santarosai*, *Leptospira borgpetersenii*, *Leptospira alstonii*, *Leptospira noguchii*, *Leptospira weilii*, *Leptospira kirschneri*, *Leptospira alexanderi* and *Leptospira kmetyi*), saprophytic (*Leptospira biflexa*, *Leptospira meyeri*, *Leptospira vanthielii*, *Leptospira terpstrae*, *Leptospira yanagawae*, *Leptospira idonii* and *Leptospira wolbachii*) and intermediate species (*Leptospira inadai*, *Leptospira licerasiae*, *Leptospira broomii*, *Leptospira fainei* and *Leptospira wolffii*) [24,25]. To date, about 300 serovars of *Leptospira* spp. have been identified and the genomic sequences of 158 strains are available in the public database. The RPR gene sequences determined in this study were used along with sequences obtained from the database to construct secondary structures of different leptospiral RPRs and compared to evaluate phylogeny based on sequence variations contributing to unique conformations of distinct RPRs.

2. Materials and methods

2.1. Bacterial strains and culture condition

Eight *Leptospira* strains were from the collection of WHO/FAO/OIE and National Leptospirosis Reference Centre in Amsterdam, The Netherlands. Leptospires were propagated at 30 °C in EMJH liquid media as described elsewhere [26] with modifications suggested by Johnson and Harris, 1967 [27]. Strain P2653 is an isolate from a human patient in The Netherlands and has been serologically typed as serogroup Hebdomadis, either serovar Hebdomadis, Kremastos or Longnan.

2.2. Genomic DNA extraction

Genomic DNAs were extracted from 17 reference strains of *Leptospira* using QIAamp DNA extraction kit (QIAGEN GmbH, Germany) followed by RNase treatment using RNase Cocktail enzyme mix (Ambion, TX, USA). Seventeen reference strains consist of eight strains for which the RPR gene sequences were determined in this study and RPR gene sequences of another nine strains were obtained from the database (Table 1), cloned and in vitro transcribed. Purified DNA was eluted with 0.1 × TE buffer, pH 8.0 in accordance with manufacturer's instructions. The quantity of genomic DNA was estimated by spectrophotometry using the Nanodrop spectrophotometer (Nanodrop, DE, USA).

2.3. Cloning and sequencing of RPR gene

RPR genes were amplified from nine strains of *Leptospira* using Taq polymerase (NEB, MA, USA). RPR specific P4 sense and P4 antisense primers (Table 2) were deduced 280–300 bp apart from sequences present in the universally conserved P4 region of the gene (Fig. 1), thus comprising 85–90% of the RPR gene sequences. Degeneracy was introduced at three consecutive nucleotide positions of the antisense primer where R and K denote A/G and G/T, respectively. Ten picomoles each of the above primers were used in PCR mixture with 5 ng of leptospiral genomic DNA, 200 μM dNTPs and 2.5 U of Taq polymerase (NEB, MA, USA) in a total volume of 20 μl reaction mix. Amplification was performed on DNA Engine (MJ Research, CA, USA) using 95 °C for 5 min for initial denaturation followed by 95 °C for 30 s, 48 °C or 50 °C for 30 s and 72 °C for 1 min of 40 cycles. Final extension was performed at 72 °C for 30 min before terminating the reaction at 4 °C. PCR products were analyzed by agarose gel electrophoresis according to standard procedures. An aliquot of the fresh PCR product was incubated at 65 °C for 15 min, then brought to room temperature, ligated into vector pCR2.1 and subsequently transformed into TOPO cells (Invitrogen, CA, USA). Reaction conditions for ligation and transformation were followed as prescribed by the manufacturer. Transformants were picked up from the agar plate and inoculated into LB broth containing ampicillin (50 μg/ml) and incubated overnight at 37 °C. Plasmid DNA was extracted from the cultures using the plasmid mini kit (QIAGEN GmbH, Germany) and the extracted plasmids were digested with the restriction enzyme *Eco*RI (NEB, MA, USA). For each serovar, at least three clones were subjected to sequencing (SciGenom, Cochin, India) using M13 reverse primer. Sequences with 100% sequence identities between all three RPR clones were included for further analysis. Sequences were deposited in Genbank. Accession numbers of the sequences are listed in Table 1. The single copy nature of RPR gene was supported by an in silico analysis of genomic sequences of 150 *Leptospira* strains available in the NCBI database (Table 1). The *secY* gene sequences for 158 leptospiral strains included in this study were also retrieved from the NCBI database (Table 1) and employed to evaluate the phylogenetic utility of RPR gene.

Full length RPR gene was amplified from genomic DNAs of nine *Leptospira* strains (Table 1) using species specific primers as listed in Table 2. Primers were designed based on RPR gene sequences available in the database. T7 promoter sequence and appropriate restriction recognition sequences were included in the primers for in vitro transcription and cloning. PCR reaction was performed as described before.