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Molecular Characterization of *Blastocrithidia culicis* L17 Ribosomal Protein

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Summary. *Blastocrithidia culicis* is a protozoan of the family Trypanosomatidae. It is a parasite of insects, but the presence of bacterium-like endosymbionts in its cytoplasm led some investigators to study this protozoan. This trypanosomatid does not infect humans and although it is phylogenetically distant from *Trypanosoma cruzi*, it presents many morphological characteristics, which are similar. In previous studies our group showed the presence of a L27 ribosomal protein in *T. cruzi* (named TcrL27) using a RT-PCR, which also resulted in the cloning, sequencing and expression of an unexpected ribosomal protein, L17, in *Blastocrithidia culicis* (BcL17). In this paper, Western blot analysis demonstrated that the anti-BcL17 antibody recognizes the presence of the same ribosomal protein either in *Blastochritidia culicis* and *T. cruzi* nuclear extracts. Besides, two similar bands (40 and 47 kDa) appeared also in *T. cruzi* isolated ribosomal proteins and *B. culicis* nuclear extract corroborating with the findings showed in the phylogenetic reconstruction. With respect to their localization within the ribosome, both the L17 and L27 ribosomal proteins appear to belong to the peptidyl-transferase site, and are therefore part of the key step in protein synthesis. Both ribosomal proteins bind spiramycin derivatives, being therefore compounds of the macrolides connection sites in the ribosome. These findings would open a possibility to better evaluate this issue.

Key words: *Blastocrithidia culicis*, L17 ribosomal protein, recombinant ribosomal protein, trypanosomatids.

INTRODUCTION

Trypanosomatid ribosomal proteins, such as histones and heat shock proteins, have an elevated sequence identity and preserved cellular function on an evolutionary timescale (González *et al*. 2004). The order Kinetoplastida is divided into two sub-orders: Trypanosomatina and Bodonina; the sub-order Bodonina is divided in two families: Bodonidae and Cryptobiidae and the sub-order Trypanosomatina includes only one family: Trypanosomatidae. In this family the genera *Trypanosoma*, *Leishmania* and *Blastocrithidia* are included. These genders were defined as monophyletics (a group that involves an ancestral species and all its descendants) (Lukes *et al*. 1997, Wright *et al*. 1999, Stevens and Gibson 1999). However, recently Maslov *et*

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al. (2001) defined the gender *Blastocrithidia* as polyphyletic (a group derived from two or more ancestral species) suggesting that the morphological classification of the Kinetoplastida order may not be general to the family Trypanosomatidae. The genus *Blastocrithidia* is classified as monogenetic while the *Trypanosoma* and *Leishmania* are digenetic (Neves *et al*. 2000). More than 30 species belonging to the genus *Blastocrithidia* have been described, which infect dipters (mosquitoes and flies) and hemipters. The epimastigote forms of *Blastocrithidia* sp can be cultivated *in vitro* and the cells measure between 10 and 50 μm in length, with flagella of about 5 to 12 μm. This trypanosomatid does not infect humans and although it is phylogenetically distant from *Trypanosoma cruzi*, presents many morphological, biochemical and immunological characteristics, which are similar; in *B. culicis* culture some trypomastigotes can occur ranging from 2-7 % (Souza 1994, d'Ávila-Levy *et al.* 2005). Due to these morphological similarities among trypanosomatids, the gender *Blastocrithidia* provides to be an excellent model system for the investigation of *Trypanosoma cruzi* and Chagas' disease.

In previous studies we showed the presence of a L27 ribosomal protein in *T. cruzi* (Perone *et al*. 2003) using RT-PCR; this strategy resulted in the cloning sequencing and expression of an unexpected protein, which codes for another ribosomal protein, L17, in *Blastocrithidia culicis* (BcL17). Both the L17 and L27 ribosomal proteins appear to belong to the peptidyl-transferase site within the ribosome, and are therefore part of the key step in protein synthesis (Monro *et al*. 1969). However, studies in *Escherichia coli* have shown that the location of the L17 protein in the ribosome is still controversial (Bischof *et al*. 1995). Both ribosomal proteins (L17 and L27) bind spiramycin derivatives (Bischof *et al*. 1995), being therefore, compounds of the macrolides connection sites in the ribosome (Vazquez 1979). Spiramycin macrolide derivatives act in the initial stages of peptide formation and compete with aminoacyl-tRNAs for the A site (Dinos *et al*. 1993). This effect is comparable with the typical inhibition of the peptidyl-transferase provoked by chloramfenicol (Vazquez 1979).

We report herein the cloning, sequencing, structural analysis and gene expression of the L17 ribosomal protein from *Blastocrithidia culicis* (BcL17). The results show details of the amino acid sequence of the protein, features of its physical chemistry and its similarity with homologues from other trypanosomatids. The expressed recombinant protein was used to produce antibody that reacted with the native protein in parasite extracts.

MATERIALS AND METHODS

***Blastocrithidia culicis* parasites.** *Blastocrithidia* epimastigotes (ATCC14806) were cultured *in vitro* at 28ºC in LIT medium (Fernandes and Castellani 1966) supplemented with 10% fetal bovine serum, until reaching a total cell count of approximately 10^8 forms.

Genomic DNA, total RNA and nuclear extract preparation. The genomic DNA and total RNA were prepared using DNAzol® and Trizol® reagents (Invitrogen), respectively. 400 μl of nuclear extract were prepared using protease inhibitors (50 mM PMSF, leupeptin 2 μg/ml, 0,5M DTT) following the protocol described by Kovacs *et al*. (1993). The proteins were precipitated with 100 % cold acetone.

cDNA preparation, amplification and sequencing. RT-PCR was carried out using the 3'RACE kit (Life Technologies) for cDNA synthesis in the case of the trypanosomatid. The PCR reaction was performed using the oligonucleotide (5'-GCTGTACTATATTGTGGCTTC-3') designed from the nucleotide sequence of the 5'-UTR gene region that codes for the L27 ribosomal protein in *Trypanosoma cruzi*. The PCR product was sized on agarose electrophoresis and purified from the gel using the Qiaquick gel extraction kit (Qiagen). The PCR product was cloned using the *TOPO TA* Cloning for sequencing kit (pCR®4-TOPO® vector - Invitrogen), transformed in *E. coli* competent cells and sequenced on an ABI PRISM 377 DNA Sequencer (Perkin Elmer), using Big Dye Terminator (Applied Biosystem). Sequence-comparison analysis of the protein BcL17 was carried out using the program GENE RUNNER version 3.0 (Hastings Software, Inc.).

Sequence analysis. The BLASTn and BLASTp tools were used for the identification of sequences of interest (www.ncbi.nlm.nih.gov/blast). The BcL17 sequence was analyzed for functional motifs or domains using the PROSITE (http://myhits.isb-sib.ch/cgi-bin/motif_scan) and Conserved Domain (www.ncbi.nlm.nih.gov/Structure/) databases. Secondary structure prediction was accomplished using the PSIPRED Protein Structure Prediction Server (http://bioinf.cs.ucl.ac.uk/psipred/) and the isoelectric point predicted using GENE RUNNER version 3.0 (Hastings Software, Inc.). The BcL17 sequence was aligned with other sequences of ribosomal proteins obtained from the NCBI database (www.ncbi.nlm.nih.gov) using the BioEdit Sequence Alignment Editor (Hall 1999).

Phylogenetic analysis. For the phylogenetic studies, the BcL17 sequence was aligned with ribosomal proteins of the other organisms using BioEdit version 7.0.0 (Hall 1999) and the phylogenetic tree was built using MEGA (Molecular Evolutionary Genetics Analysis) version 2.1 (Kumar *et al*. 2001) with the distance methodology (distance-p) and neighbor-joining algorithm. Bootstrap values of 1,000 replications were used.

Southern blot analysis. Ten μg of *Blastocrithidia culicis* genomic DNA were fully digested with *Hind*III (0.6 U/ml - Amersham Biosciences) and *Pst*I (0.4 U/ml - Life Technologies), which does not

cut inside the ORF, electrophoresed on 0.8% agarose gel and transferred to a nylon membrane (Amersham Biosciences) using 10X SSC (0.3 M sodium citrate, pH 7.0 and 3 M sodium chloride). Labeling and hybridization were performed using the ECL Nucleic Acids Labelling and Detection System kit (Amersham Biosciences) following the manufacturer's instructions. The membrane was exposed to the film for 30 min at room temperature.

Expression of His-tagged 20 kDa protein and immunization of rabbits. To clone the 20 kDa cDNA into the pET28a vector (Novagen), *Nde*I and *Xho*I restriction sites were added via PCR amplification to the N and C termini, respectively, of the 20 kDa EST. The PCR fragment was cloned into the pET expression vector thus encoding the full-length 20 kDa protein fused to a N-terminal His tag. Correct orientation and the presence of an ORF were verified by DNA sequencing. The recombinant protein was expressed in *Escherichia coli* BL21 cells for 3 h at 37°C after induction with 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside). The protein was found both on inclusion bodies (insoluble) and supernatant (soluble fraction); this latter was separated by preparative 15 % SDS-PAGE, and the interest band (approximately 17 kDa) was excised the gel, cut with sterile scissor, mixed with 1 ml of saline solution and stored -20°C. The concentration of the protein was estimated using the standard molecular weight according with the manufacturer (Bio-Rad). The excised band (approximately 200 µg of recombinant protein) was homogenized in 1X PBS (Phosphate Buffer Saline) plus complete Freund adjuvant (GibcoBRL) and then inoculated in rabbits (70-75 µg of protein/inoculation). Two boosters with incomplete Freund adjuvant were done after 15 days each one. Pre-immune serum (non-absorbed with *E. coli* total protein lysate) was collected before rabbit immunization. The immune serum was previously absorbed with *E. coli* total protein lysate and the polyclonal antibodies were tested by Western blot against the BcL17 recombinant protein to titration.

Trypanosoma cruzi **nuclear extracts and ribosomal proteins.** *T. cruzi* epimastigotes of two different strains (Bol and Y) were cultured *in vitro* at 28°C in LIT medium (Fernandes and Castellani 1966) supplemented with 10 % fetal bovine serum for 7-8 days and the parasites were used to prepare 400 µl of nuclear extracts using protease inhibitors (50 mM PMSF, leupeptin 2 µg/ml, 0.5 M DTT) following protocol described by Kovacs *et al.* (1993). The proteins were precipitated with 100 % cold acetone and resuspended in 40 µl SDS-PAGE loading buffer (20 µl of samples were loaded per slot). *T. cruzi* (Tulahuen strain) ribosomal fraction proteins were kindly provided by Dr. Mariano Levin lab (INGEBI-CONICET, Buenos Aires, Argentina).

Western blot analysis. Protein fractions (recombinant BcL17 and *T. cruzi* ribosomal proteins) and total and nuclear parasite extracts were separated on 15 % SDS-PAGE gel and transferred to nitrocellulose using Hoefer SemiPhor (Pharmacia) and transfer buffer (25 mM Tris, 192 mM glicine, 20 % methanol). The membrane was blocked using 3 % low fat milk (1h at room temperature), washed 3 times with PBS-Tween 20 and incubated with anti-BcL17-antibody diluted in 2 % low fat milk/PBS for 18 hours at 4°C under agitation. After washings (PBS-Tween 20), the blot was incubated 1 hour with protein-A-horseradish peroxidase (Sigma, 1:5,000 in 2 % low fat milk/PBS) at room temperature and revealed using DAB (0.6 g in 5 ml of 0.05 M Tris-HCl, pH 7.6, and 1 µl/ml H_2O_2) for 10 min. Scanner captured the bands.

RESULTS

The cDNA insert of the BcL17 clone contains 534 nucleotides and includes a single open reading frame together with the 3' flanking region. The open reading frame of 420 nucleotides codes for a 139 amino acid protein, with an estimated molecular mass of 15,044 Da. The nucleotide sequence (GenBank accession number AAY78546) and the deduced amino acid sequence are shown in Fig. 1. The initiation codon is located at position one and the stop codon (TAA) at position 420. The BcL17 protein shows an excess of basic residues (9.22 % arginine and 9.22 % lysine) over acidic ones and a theoretical isoeletric point (pI) of 10.11. These residues confer a high level of positive charge to the protein, a common feature of ribosomal and hystone proteins, which are involved in interactions with nucleic acids in eukaryotic organisms (Aslund *et al.* 1994).

BLAST analysis of the *Blastocrithidia culicis* L17 protein with other L17 sequences of different trypanosomatids demonstrated 96% identity with the homologue from *Leishmania infantum* and 82% with that from *Trypanosoma cruzi.* The identity detected with other L17 sequences belonging to different non-trypanosomatid organisms was lower than expected: 76 % with *Pan troglodytes* (XP_511444) and *Arabidopsis thaliana* (AAB70426), 70 % with *Homo sapiens* (AAH62716) and 67 % with *Drosophila melanogaster* (NP_523813).

The protein displays highly conserved regions with respect to other members of the protein family as well as zones with remarkable differences. The L17 signature sequence is located between amino acid positions 78 and 104, which is similar to that of trypanosomatids but differs from that shown by proteins from the other species considered. There are two myristoylation sites at position 18-GLPVGA and 123-GSTIAG and three protein kinase C (PKC) phosphorylation sites at positions 40, 63 and 86, respectively (Fig. 1).

The macrolide binding sequence (Bischof *et al.* 1995) is present between Cys^{11} and Lys^{37} and is highly conserved, displaying high levels of sequence identity (close to 90 %) not only with those from other Trypanosomatidae, but also with *Arabidopsis thaliana, Drosophila* and humans. In the case of the L17 ribosomal protein from *Blastocrithidia culicis*, the possible lysine for ligation to the macrolide seems to be present at position 35. Between positions 73 and 88 (KKVLNAVIIRQRKSWR) a nuclear location sequence

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         10        20        30        40        50        60
....|....|....|....|....|....|....|....|....|....|....|....|
ATGGGTAAGGAACAGATCAACGTTAAGGGTTGCCGCTTCCGCGTGTCCGTCGGTCTCCCC
 M  G  K  E  Q  I  N  V  K  G  C  R  F  R  V  S  V  G  L  P

         70        80        90       100       110       120
....|....|....|....|....|....|....|....|....|....|....|....|
GTCGGCGCCGTCGTGAACTGCGCGGATAACACCGGTGCCAAGAACCTGTACGTGATTTCC
 V  G  A  V  V  N  C  A  D  N  T  G  A  K  N  L  Y  V  I  S

        130       140       150       160       170       180
....|....|....|....|....|....|....|....|....|....|....|....|
GTCAAGGGTTACCACGGCCGCCTGAACCGTCTGCCCTCTGCGGCCCTCGGCGATATGGTG
 V  K  G  Y  H  G  R  L  N  R  L  P  S  A  A  L  G  D  M  V

        190       200       210       220       230       240
....|....|....|....|....|....|....|....|....|....|....|....|
ATGTGCTCCGTGAAGAAAGGCAAGCCGGAACTCCGCAAGAAGGTGCTCAACGCTGTCATC
 M  C  S  V  K  K  G  K  P  E  L  R  K  K  V  L  N  A  V  I

        250       260       270       280       290       300
....|....|....|....|....|....|....|....|....|....|....|....|
ATCCGTCAGCGCAAGAGCTGGCGCCGCAAGGACGGCACCGTCATCTACTTTGAGGATAAC
 I  R  Q  R  K  S  W  R  R  K  D  G  T  V  I  Y  F  E  D  N

        310       320       330       340       350       360
....|....|....|....|....|....|....|....|....|....|....|....|
GCTGGCGTCATCGTTAACCCCAAGGGTGAGATGAAGGGCTCCACCATTGCCGGCCCCGTC
 A  G  V  I  V  N  P  K  G  E  M  K  G  S  T  I  A  G  P  V

        370       380       390       400       410       420
....|....|....|....|....|....|....|....|....|....|....|....|
GCGAAGGAATCTGCGGACCTCTGGCCCAAGATCTCCACCCACGCCCCTGCCATCGTCTAA
 A  K  E  S  A  D  L  W  P  K  I  S  T  H  A  P  A  I  V  *
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Fig. 1. Nucleotide and amino acid sequences of *Blastocrithidia culicis* L17 ribosomal protein (BcL17). The nucleotide sequence is given in the 5' to 3' direction. Grey circle - methyonine (start codon); grey rectangle - L17 signature; circle - PKC sites; rectangle - N-myristoylation sites; * - stop codon.

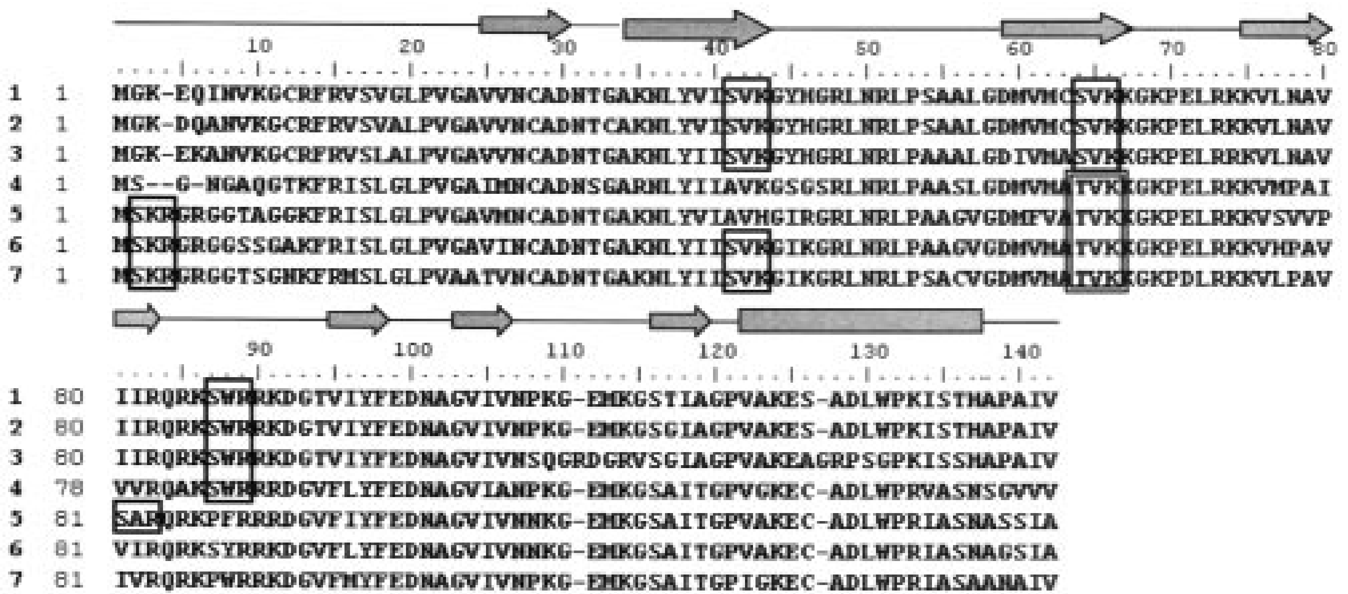

Fig. 2. Comparison of the amino acid sequences of L17 ribosomal proteins among eukaryotic species. 1 - *Blastocrithidia culicis* (AAY78546); 2 - *Leishmania infantum* (AF097022); 3 - *Trypanosoma cruzi* (gi 2500266); 4 - *Saccharomyces cerevisae* (gi 13274); 5 - *Drosophila melanogaster* (gi 1350673); 6 - *Homo sapiens* (gi 266927); 7 - *Arabidopsis thaliana* (gi 2459420). Single line boxes, PKC phosphorylation sites associated with serine; double line boxes, threonine phosphorylation sites. Regions of secondary structure in *Blastocrithidia culicis* L17 are indicated above the alignment. The α-helices are displayed as open bars; β-strands as large arrows and coil as lines.

(NLS) was detected, which facilitates protein transport between nucleus and cytoplasm (Miyamoto *et al*. 1999).

Figure 2 shows the comparison between L17 from *Blastocrithidia culicis* and the corresponding L17 ribosomal proteins from different sources, representing both lower and higher eukaryotes. In the protozoan proteins there are three PKC phosphorylation sites, all of which are associated with serine. In all other species considered, threonine is present in the most highly conserved site, at position 63. Protozoan proteins do not possess the phosphorylation sites at position 2, whilst *Saccharomyces cerevisae* and *Drosophila melanogaster*, lack the site at position 40. On the other hand, *D. melanogaster* presents an additional site at position 81. The secondary structure prediction of *Blastocrithidia culicis* L17 protein showed that there is a predominance of b-strand and coil, with the prediction of only one α-helix in the C-terminal region. The conservation of this region among the different organisms analyzed indicates that the helix is probably maintained in all such species.

The multiple sequence alignments that were used for the phylogenetic analyses revealed that the first ten amino acids are only conserved among the trypanosomatids (data not shown). The phylogenetic reconstruction showed the presence of well-divided groups (Chordata, Nematoda, Arthropoda, Kinetoplastida, Coccidia, Fungi and Plantae). Among Trypanosomatidae, the L17 protein of *Blastocrithidia culicis* is closer to *Leishmania infantum* than *Trypanosoma cruzi*, as shown in Figure 3.

Southern blot analysis of *Blastocrithidia culicis* genomic DNA (Fig. 4) digested with two restriction enzymes, which did not cleave inside the ORF of the BcL17 probe, suggested that this parasite presents only a single copy of the gene.

Western blot results demonstrate that the anti-BcL17 antibody recognizes the presence of the same ribosomal protein (L17) either in *Blastocrithidia culicis* and *T. cruzi* strains nuclear extracts (Figs 5A and B: lane 4, Blas, Bol, Y), even slightly different patterns of molecular weight size as compared with the *T. cruzi* purified ribosomal proteins (Rib). An arrow indicates the 17 kDa recombinant protein (Figs 5A, lane 3 and B, L17). The reactivity bands that appeared in pre-immune serum are due to the lack of absorption with *E. coli* proteins, since there was not any reaction with trypanosomatid total and nuclear extracts (data not shown). As expected, no reaction occurred after second conjugate incubation

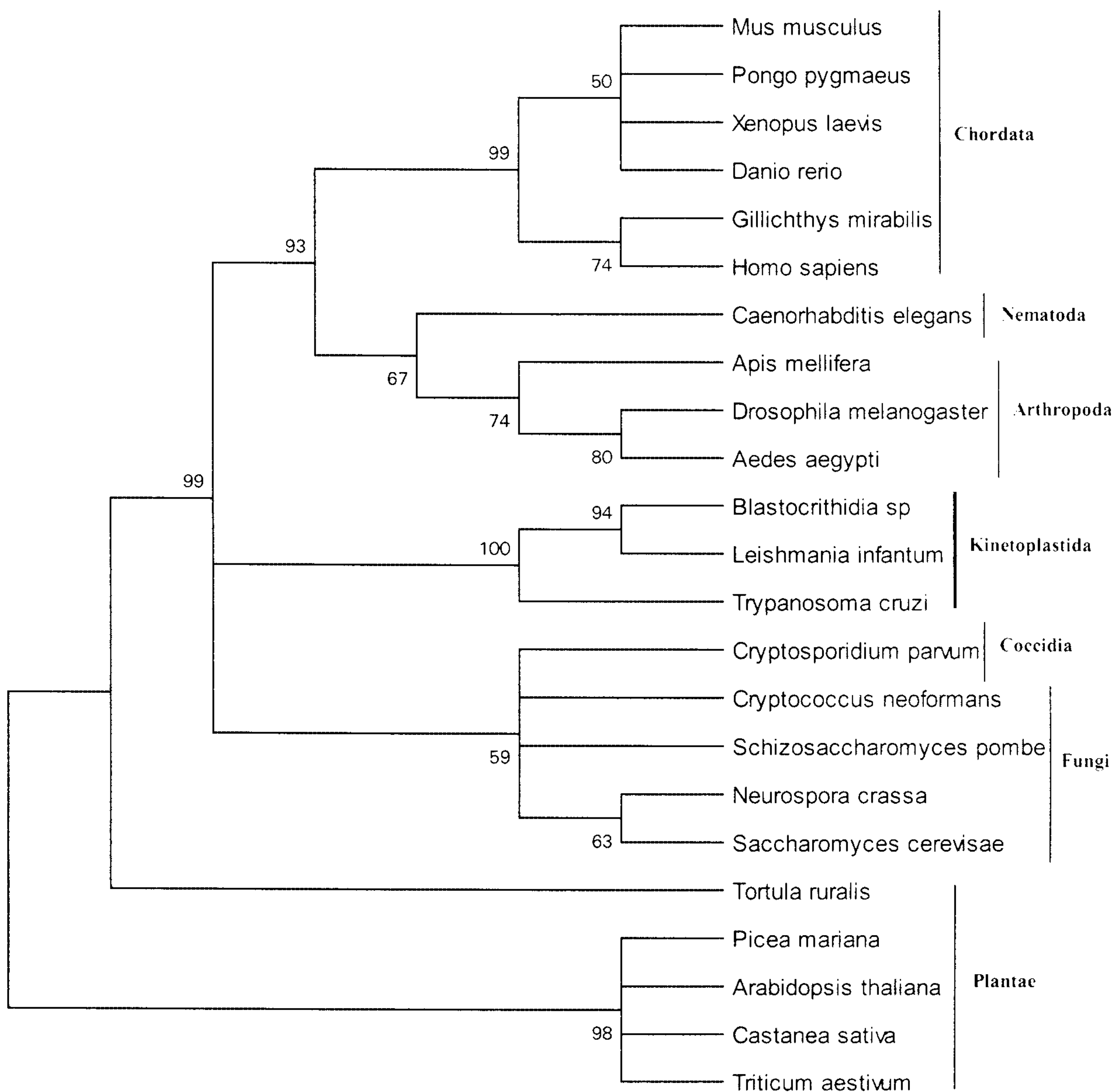

Fig. 3. Molecular phylogenetic tree based on comparison of amino acid sequences of *Blastocrithidia culicis* (AAY78546) L17 ribosomal protein with those of others species: *Leishmania infantum* (gi 3851618); *Trypanosoma cruzi* (gi 2500266); *Arabidopsis thaliana* (AA042332); *Caenorhabditis elegans* (AAK18857.1); *Castenea sativa* (AAK25758.1); *Apis mellifera* (XP_392812.1); *Triticum aestivum* (AAW50991.1); *Picea mariana* (AAC32130.1); *Danio rerio* (NP_957026.1); *Mus musculus* (NP_075029.1); *Gillichthys mirabilis* (AF266222); *Cryptococcus neoformans* (CNBK0380); *Homo sapiens* (AAH62716.1); *Pongo pygmaeus* (CAH89715.1); *Drosophila melanogaster* (NP_523813.1); *Tortula ruralis* (AAD23966.1); *Aedes aegypti* (AAL85622.1); *Schizosaccharomyces pombe* (CAA15912.1); *Cryptosporidium parvum* (EAK90115.1); *Neurospora crassa* (XP_330093.1), *Saccharomyces cerevisae* (gi 132744); *Xenopus laevis* (AAH73541.1). Outgroup: *Triticum aestivum*.

(Fig. 5A, lane 5). Two ribosomal proteins (40 and 47 kDa, approximately, indicated by arrows in the Fig. 5B, Rib) appeared either in *T. cruzi* isolated ribosomal proteins and *B. culicis* total (Fig. 5A, lane 4) and nuclear (Fig. 5B, Blast) extracts corroborating with the findings showed in the phylogenetic reconstruction (Fig. 3). The differences on the reactivity among the trypanosomatid strains are still unknown.

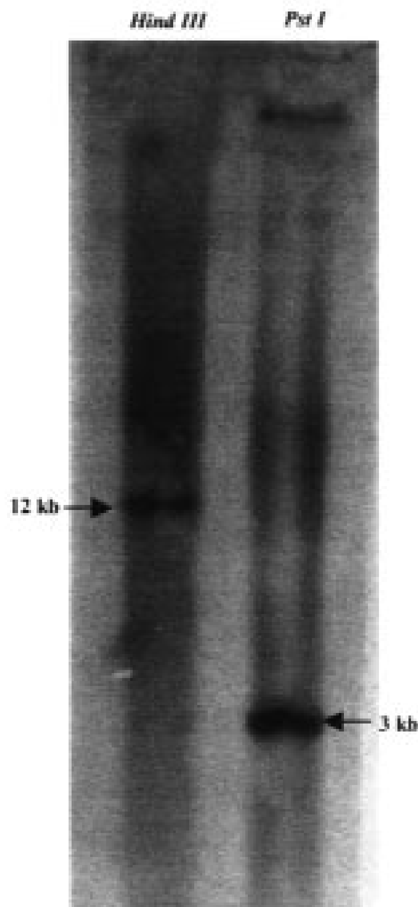

Fig. 4. Southern blot analysis of BcL17 encoding gene in *Blastocrithidia culicis* genomic DNA using *Hind*III and *Pst*I as restriction enzymes. The molecular weight (kb) of the bands is indicated.

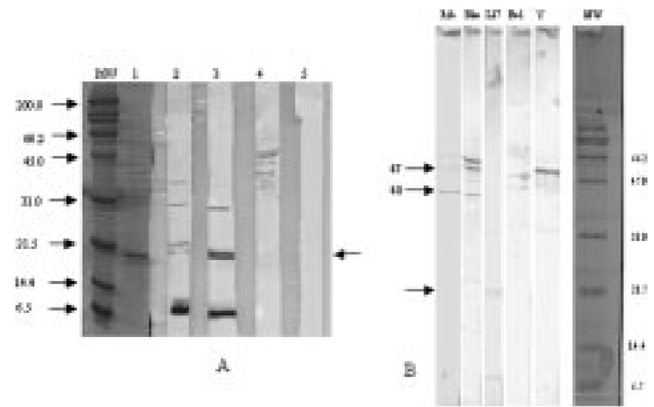

Fig. 5. Western blot analysis of BcL17-recombinant protein (A), *Blastocrithidia culicis* and *T. cruzi* native proteins (B) with anti-BcL17 antibody. **A:** 1 - SDS-PAGE (15%) of recombinant protein lysate (soluble fraction); 2, 3, 5 - semi-purified BcL17 (17 kDa) assayed, respectively, with pre-immune serum (1:400, non-*E. coli* adsorbed), anti-BcL17 antibody (1:50, *E. coli*-adsorbed) and protein A-peroxidase conjugate as a negative control; 4 - *B. culicis* total extract. **B:** *T. cruzi* (Tulahuen strain) purified ribosomal proteins (Rib), *T. cruzi* nuclear extracts (Bol and Y strains) and *Blastocrithidia* nuclear extract (Blas) and BcL17-recombinant protein (L17). The arrows indicate the BcL17-recombinant protein and the putative native proteins present in the parasite extracts comparing with purified ribosomal proteins (40 and 47 kDa). MW - molecular weight standards in kDa.

DISCUSSION

This work presents results of the cloning and sequencing of the gene encoding the L17 ribosomal protein from the parasite *Blastocrithidia culicis*. The analysis of sequence alignments showed that the nucleotide sequence of the *Blastocrithidia culicis* L17 gene is very similar to those of the L17 ribosomal proteins from *Leishmania infantum* (96 %) and *Trypanosoma cruzi* (82 %) (gi: D87216 and gi: AF097022, respectively). The BcL17 amino acid sequence also showed high levels of identity with ribosomal proteins present in other higher eukaryotes, but presented structural differences when compared with homologous proteins from other organisms. These differences include the length of the amino acid sequence, 139 residues in the case of BcL17 protein, which is smaller than that found in *Sacharomyces cerevisae, Drosophila* or humans (Mukhopadhyay *et al*. 1988, Suzuki and Wool 1991).

The positions of the PKC phosphorylation sites are identical among the trypanosomatids. Although these are also highly conserved in higher eukaryotes, even the most conserved site presents the substitution of a serine in Trypanosomatidae by a threonine in the other organisms analyzed. *N*-miristylation and a bipartite nuclear localization sequence (NLS) were also identified. These regions are also conserved in other ribosomal proteins, for example, L14 of *Leishmania brasiliensis* (González *et al*. 2004).

The phylogenetic proximity among the trypanosomatids was confirmed by analysis of the amino acid sequences, demonstrating that *Blastocrithidia culicis* is closest to *Leishmania infantum*. However, the Western blot results also suggest that the BcL17 ribosomal proteins share common epitopes with isolated *T. cruzi* ribosomal proteins, even in TriTryp genomes (*T. brucei*, *T. cruzi* and *Leishmania*), this protein has been described as a 50S putative ribosomal protein L17.

Southern blot analysis demonstrated the presence of only one copy of L17 in *Blastocrithidia culicis*. Similar experiments with *Leishmania infantum* also demonstrated the presence of only one copy in genomic DNA, despite the fact that Northern blot analysis suggested the existence of two non-identical copies. However, in this latter case allele polymorphism of the gene cannot be discharged (González-Aseguinolaza *et al.* 2000).

The affinity of the L17 ribosomal protein for spiramycin and derivatives, also observed in other organisms, may represent a useful means to investigate the structural components of the peptidyl-transferase center of the ribosome, because the proteins that interact strongly with the spiramycin molecule have been shown to be close to or part of this region (Walleczek *et al.* 1988, 1990). Analysis of a multiple alignment involving ribosomal proteins from different organisms which vary from lower eukaryotes to man demonstrated that a lysine residue is possibly involved in macrolide binding. This was predicted to be position Lys^{35} in *Blastocrithidia culicis*, which is highly conserved. Among the 23 organisms used in these analyses, only a hypothetical protein from *Neurospora crassa* (XP_330093.1) and a ribosomal L23 (L17) of *Saccharomyces cerevisae* (gi 132744) presented an arginine substituting the lysine residue at this position (data not shown).

The mode of action of the macrolides has been mainly attributed to the different sugars moieties, which are part of the lactone-ring (Vazquez 1979). The knowledge of the possible mechanism of action of this therapeutic class of compounds associated with the understanding of the structure and function of each structural component of the ribosome may represent an important tool for the discovery of new and more specific therapeutic agents. The findings about BcL17-common epitopes sharing with *T. cruzi* ribosomal protein would open a possibility to better evaluate this issue.

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