CLONING *LEISHMANIA* TELOMERES IN YEAST BY COMPLEMENTATION

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ABSTRACT

Leishmania telemores were cloned in yeast by complementation using a half arm of the yeast artificial chromosome vector pJS97. A single copy marker associated to the telomere (YT2) located 6 kb away from one end of a megabase size chromosome of 1116c8 was cloned by end rescue and DNA sequenced. The sequence of 1479 bp did not show a significant homology with any sequence stored in GenBank. *Leishmania* telomeres are amenable to be cloned and maintained in yeast, this approach will facilitate the analysis of telomere and subtelomere regions which are usually underrepresented in genomic libraries.

1. RESULTS AND DISCUSSION

Leishmania has 36 chromosomes and the linkage of genes appears conserved accross species (Wincker et al., 1996, Ravel et al.,1996). However, the molecular karyotype of this parasite display a high degree of size polymorphism between strains and species (Bastien et al., 1992; Espinoza et al., 1995). The contribution of subtelomere/telomere variation to the karyotype polymorphism in *Leishmania* will require the characterization of repetive elements forming these regions (Ravel et al.,1995). Long range restriction mapping of chromosomes I, II and V of strain isolates of *L. infantum* showed that the size variation of these chromosomes was produced by changes in the subtelomeric region (Blaineau et al., 1992; Bastien, et al.,1992). The hexamer repeat 5' GGGATT 3' is forming the telomeres of *L. donovani* (Ellis and Crampton, 1988). In addition, the telomeric oligonucleotide (GGGATT)n hybridised the chromosomes of several *Leishmania* species (Ellis and Crampton, 1988), suggesting that this sequence is forming the telomeres of the *Leishmania* species that are pathogenic to humans. In the present work, telomeres of L. major LV39 and *L. peruviana* were cloned in yeast by complementation. The yield of cloning *Leishmania* telomeres in yeast by complementation might be increased by a rapid identification of false positives by using a negative selection screenig with plates containing 5-Fluororotic acid (5-FOA) (Boeke et al., 1987) as was the case in cloning human telomeres (Dobson and Brown, 1992) and *P. falciparum* telomeres (De Bruin et al., 1992).

A telomeric end of 1116c8 (Ytel2) was cloned in the pJS97 vector and mainatined in YPH252 yeast strain (Sikorski and Hieter, 1989). Ytel2 clone hybridised to the (TTAGGG)4 with a size of 27 kb. End rescue produced a 1.48 kb DNA fragment form the Ytel2 that hybridised to a megabase size chromosome (not shown). The YT2 hybridisation signal of the 1116c8 genomic DNA is degraded when digested with 0.25 and 0.5 units of Bal 31. The same blot was stripped and hybridised with a 3 kb anonymous probe derived from the 1116c8 genome did not show any degradation (Fig. 1). YT2 is a single copy marker located 6 kb away from one end of a megabase size chromosome of 1116c8.

The sequence of 1479 bp did not show a significant homology with any sequence stored in GenBank. There are three telomere hexamer copies dispersed in the sequence. There is a short region from 510 to 610 that has a composition of 72% A-T (GenBank accession number AF474146) BglII produced YT2 hybridising fragments of different size in *L. peruviana* strains (Fig.2.A). This size variation is not caused by a BglII polymorphism since double digestion with BglII/BamHI produced a single YT2 hybridising fragment of 1.5 kb in *L. peruviana* isolates (Fig. 2.B). These isolates were from different locations in Perú (Davies et al., 1995). The average lenght of YT2 hybridising fragments in the whole population was 9.0 kb ± 4.81 kb (n=39). Isolates from Lima has an average

length of 12.19 kb ± 2.0 kb (n=6). Isolates from Ancash has a media of 14.3 kb ± 1.98 kb (n=10), isolates from Piura has an average length of 5.57 kb ± 3.17 kb (n=22). The average length of YT2 hybridising fragment from Piura isolates was shorter than the isolates from Lima and Ancash.

The YT2 marker was not detected by hybridisation in three *L. peruviana* isolates collected from Lima (1015 and 1260) and Ancash (1060). This marker was probably lost by a genome rearrangement, however a sequence divergence, even unlikely, could also produce a loss of the hybridisation signal.

2. REFERENCES

1) Bastien, P., Blaineau, C. and Pagès, M. (1992). Molecular karyotype analysis in *Leishmania*. In: Avila, J.L. and Harris, J.R. (eds.), Subcellular Biochemistry: Intracellular Parasites. Plenum Press, New York. Vol 18. pp. 131-187.

2) Bishop, R.P. and Miles, M.A. (1987). Chromosome size polymorphisms opf *Leishmania donovani*. Molecular and Biochemical Parasitology. 24: 263-272.

3) Blackwell, J.M. (1992). Leishmaniasis epidemiology: all down to the DNA. Parasitology. 104: S19-S34.

4) Boeke J.D. , Trueheart, J., Natsoulis, G. and Fink G. (1987). 5-Fluoroacetic acid as a selective agent in yeast molecular genetics. Methods in Enzimology. 154: 164-175.

5) Burke, D.T., Carle, G.F. and Olson, M.V. (1987). Cloning of large segments of exogenous DNA into yeast by means of artificial chromosome vectors. Science. 236: 806-812.

6) Davies, C.R., Llanos-Cuentas, A., Pyke, S.D.M. and Dye, C. (1995). Cutaneous leishmaniasis in the Peruvian Andes: an epidemiological study of infection and immunity. Epidemiology and Infection. 114: 297-318.

7) De Bruin, D., Lanzer, M. and Ravetch, J.V. (1992) Characterization of Yeast Artificial Chromosomes from *Plasmodium falciparum*: Construction of a stable, representative library and cloning of telomeric DNA fragments. Genomics. 14: 332-339.

8) Dobson, M. J. and Brown, W.R.A. (1992). Cloning human telomeres in yeast artificial chromosomes. In: Anand, R. (ed.), Techniques for the Analysis of Complex Genomes. Academic Press, London. pp. 81-92.

9) Ellis, J. and Crampton, J. (1988). Chracterisation of a simple, highly repetitive DNA sequence from the parasite *Leishmania donovani*. Molecular and Biochemical Parasitology. 29: 9-18.

10) Espinoza, J.R., Skinner, A.C., Davies, C.R., Llanos-Cuentas, A., Arevalo, J., Dye, C., McMaster, W.R., Ajioka, J.W. and Blackwell, J.M. (1995). Extensive polymorphism at the GP63 locus in field isolates of *Leishmania peruviana.* Molecular and Biochemical Parasitology. 72: 203-213.

11) Lanzer, M., Fischer, K. and Le Blancq, S.M. (1995). Parasitism and chromosome dynamics in protozoan parasites: is there a connection?.Molecular and Biochemical Parasitology. 70: 1-8.

12) Shero, J.H., McCormick, M.K., Antonarakis, S.E. and Hieter, P. (1991). Yeast Artificial Chromosome vectors for efficient clone manipulation and mapping. Genomics. 10: 505-508.

13) Sikorski, R.S. and Hieter, P. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics. 122: 19-27.

14) Wincker, P., Ravel, C., Blaineau, C., Pages, M., Jauffret, Y., Dedet, J.P. and Bastien, P. (1996). The *Leishmania* genome comprises 36 chromosomes conserved across widely divergent human pathogenic species. Nucleic Acids Research. 24: 1688-1694.

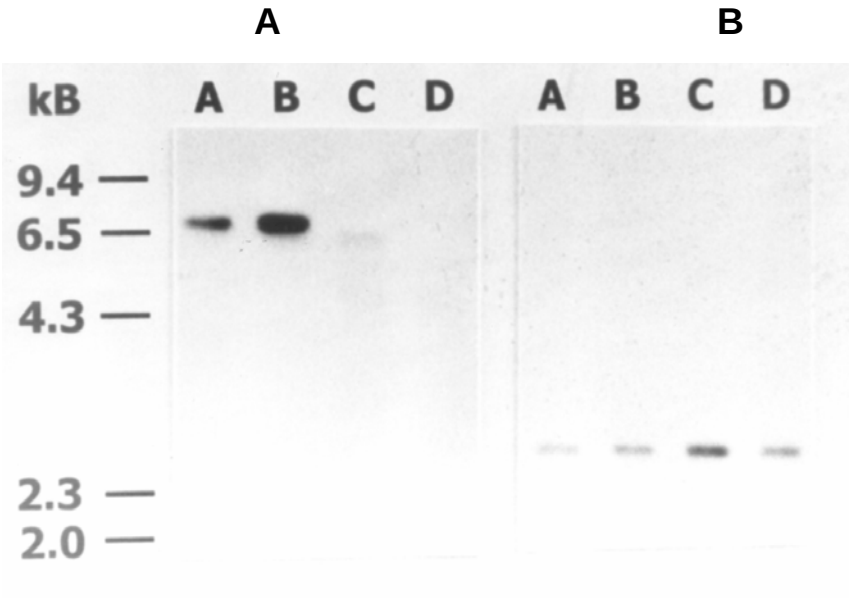

Figure 1. Telomere fragments from *L. peruviana* isolates Panel A: Digested with BglII and hybridised with YT2 probe. Panel B. Digested with *Bgl*II and *Bam*HI and hybridised with YT2 probe.

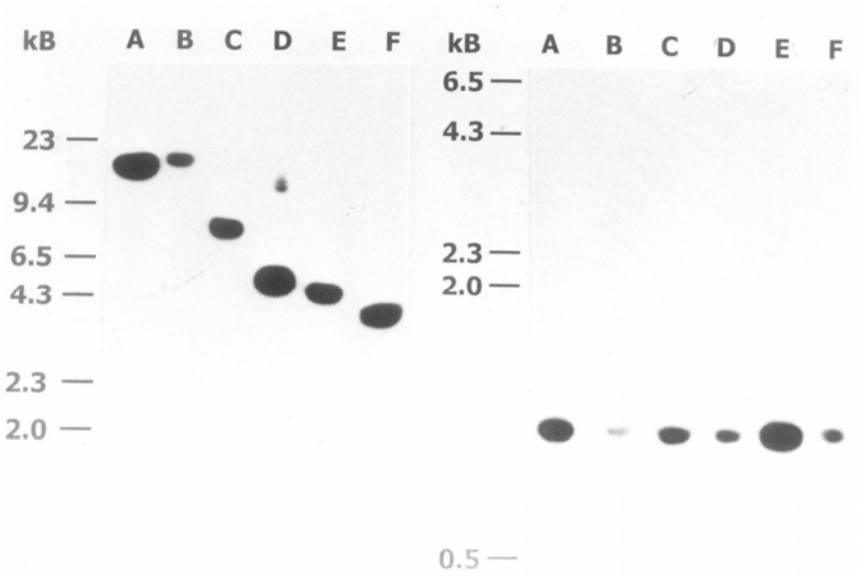

Figure 2.