SHORT COMMUNICATION

The 245 kb Amplified Chromosome of *Leishmania (V.) braziliensis* Contains a Biopterin Transporter Gene

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Leishmania (V.) braziliensis M2903 *presents a small linear and stable 245 kb chromosome originating from a genomic amplification. Similar amplifications present in other species of* Leishmania *contain a gene coding for a biopterin transporter. Since* Leishmania *is auxotrophic for this metabolite, this amplification could result from the need to better capture biotpterin from growth media under specific circumstances. In this paper we show that this gene is also present in* L. (V.) braziliensis *small chromosome, which shares sequences with other genomic amplifications already described.*

Key words: *Leishmania (V.) braziliensis* - small chromosome - genomic amplification

Leishmania (V.) braziliensis is responsible for mucocutaneous leishmaniasis, present in the New World. *Leishmania* genome does not compact during the cell cycle, so a karyotype can only be obtained through pulsed field gel electrophoresis (PFGE). *Leishmania* karyotypes show polymorphism among species and strains (Lighthall & Giannini 1992). The appearance of genomic amplifications is common, in the form of linear or circular small chromosomes. These amplifications can appear spontaneously, or as a result of drug pressure, being stable or not after appearance (Beverley 1991). Spontaneous amplifications are seen in many *Leishmania* species, the best studied ones being from the LD1 amplicon type (Segovia & Ortiz 1997). The 245 kb small chromosome of *L. (V.) braziliensis* M2903 falls into this group (Scholler et al. 1986, Fu et al. 1998, Stiles et al. 1999). The functions for these amplifications are still not clear. The finding of a gene coding for a biopterin transporter (BT1) in small amplified chromosomes, and since *Leishmania* are auxotrophic for this metabolite, indicates that biopterin uptake could be the underlying reason for the appearance of these amplifications (Moore & Beverley 1996). In the present work we show the presence of BT1 in the small chromosome of *L. (V.) braziliensis*, and that it shares sequences with similar genomic amplifications present in *Leishmania (L.) donovani* and *Leishmania (L.) major*.

L. (V.) braziliensis strain M2903 (MHOM/BR/75/M2903) isolated in Serra dos Carajás, State of Pará, was used in these studies. Two lineages from different sources were obtained: one containing the small 245 kb chromosome, here denominated M2903(+), was obtained from Dr Gabriel Grimaldi (Instituto Oswaldo Cruz, Rio de Janeiro, RJ); another, not containing the small chromosome, here denominated M2903(-), was obtained from Dr Diane McMahon-Pratt (Yale University, New Haven, CT). Both were maintained as culture promastigotes in modified M199, with 10% fetal bovine serum at 24ºC (Kapler et al. 1990). PFGE was carried out in a CHEF DRII apparatus (BioRad) (Chu et al. 1986) and electrophoresis conditions are described in the figures legends. Gels were transferred to nylon membranes and hybridized according to standard conditions (Tavares et al. 1992). We used a BT1 probe

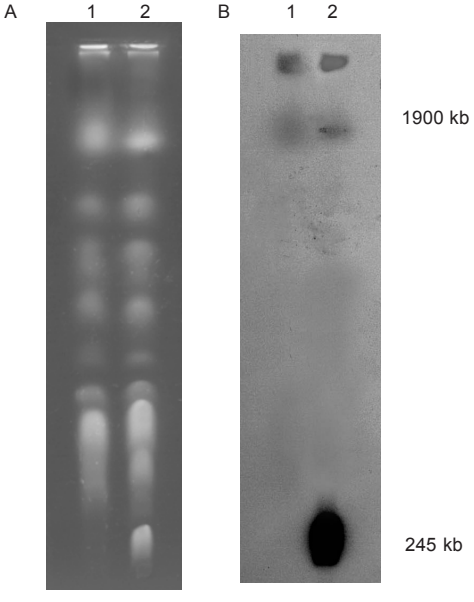

Fig. 1 - A: pulsed field gel electrophoresis; 1: *Leishmania (V.) braziliensis* M2903(-) and, 2: *L. (V.) braziliensis* M2903 (+). Electrophoresis conditions: V = 175 V; 1.5% agarose in 0.5 x TBE (45mM Tris; 45 mM boric acid; 1mM EDTA pH 8.3); T = 14ºC; run length 24 h; B: autoradiography after hybridization against the small chromosome itself. The probe was prepared according to Beverley (1990).

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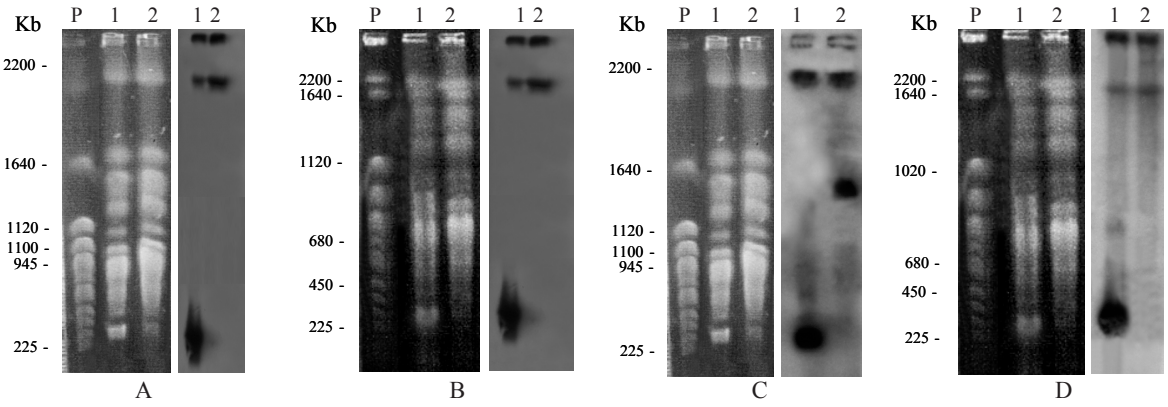

Fig. 2 - P: yeast chromosomes molecular weight markers (sizes indicated in the figure) 1: *Leishmania (V.) braziliensis* M2903(+) and, 2: *L. (V.) braziliensis* M2903 (-). In A and C, pulsed field gel electrophoresis (PFGE) in ideal conditions for resolution above 1000 kb chromosome sizes (V = 175 V; T = 14ºC; agarose 1% in 0.5 x TBE; pulses from 100 to 200 sec; run length: 24 h). In B and D, PFGE in ideal conditions for resolution of chromosomes below 1000 kb (V = 175 V; T = 14ºC; agarose 1% in TBE 0.5 x; pulses from 50 to 100 sec; run length: 24 h). A and B were hybridized against BT1 probe and C and D were hybridized against p7R50-19 probe. The hybridization was done at 65ºC and three washes at 1X SSC and 0.5% SDS.

[1.4 kb BamHI-BglII fragment within the BT1 gene of *L. (L.) donovani*, kind gift of Dr Jeffrey Moore] and p7R50-19 probe, a random 1.9 kb *Pst*I fragment from the *L. (L.) major* 715 class small chromosome (Beverley & Coburn 1990) against chromoblot obtained by PFGE.

The hybridization in Fig. 1, using the small chromosome itself as probe, shows the recognition of the large chromosome (1900 Kb) that gives rise to the 245 kb linear amplification, and confirms the presence of the genomic amplification in lineage M2903(+). Similar results are seen in Fig. 2 where BT1 and p7R-50-p19 probes from *L. (L.) donovani* and *L. (L.) major* small chromosomes were used, showing that these species share common genes with the *L. (V.) braziliensis* M2903 small chromosome.

Hybridization with BT1 (Fig. 2) confirms the presence of the biopterin transporter gene in the small 245 kb chromosome, and in the large source chromosome, as had been previously observed in *L. (L.) donovani* (Lemley et al. 1999). Hybridization with p7R-50p19 reveals not only the amplified small chromosome and the source chromosome, but also, in the case of M2903(-), labeling of a chromosome of approximately 1600 kb. This might be due to the presence of repetitive sequences present in more than one chromosome, since this probe is a random fragment of a small chromosome. The presence of BT1 gene in the amplicon, and preliminary results indicating higher BT1 RNA levels in M2903(+), suggest that the amplification of this essential gene might provide a nutritional advantage to lineage M2903(+). This was corroborated by growth curves of the two strains, in the presence of the metabolite (manuscript in preparation).

Our results point to the consistent presence of the BT1 transporter in amplified linear chromosomes of various *Leishmania* species. This most probably indicates the essentiality of this gene in conditions of nutrient deprivation. We are presently investigating this question by comparing metabolic and biological features of the lineages containing or not the small amplified chromosome.

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