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Large differences in the genome organization of different plant Trypanosomatid parasites (*Phytomonas* spp.) reveal wide evolutionary divergences between taxa

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A B S T R A C T

All currently known plant trypanosomes have been grouped in the genus *Phytomonas* spp., although they can differ greatly in terms of both their biological properties and effects upon the host. Those parasitizing the phloem sap are specifically associated with lethal syndromes in Latin America, such as, phloem necrosis of coffee, 'Hartrot' of coconut and 'Marchitez sorpresiva' of oil palm, that inflict considerable economic losses in endemic countries. The genomic organization of one group of *Phytomonas* (D) considered as representative of the genus has been published previously. The present work presents the genomic structure of two representative isolates from the pathogenic phloem-restricted group (H) of *Phytomonas*, analyzed by pulsed field gel electrophoresis followed by hybridization with chromosome-specific DNA markers. It came as a surprise to observe an extremely different genomic organization in this group as compared with that of group D. Most notably, the chromosome number is 7 in this group (with a genome size of 10 Mb) versus 21 in the group D (totalling 25 Mb). These data unravel an unsuspected genomic diversity within plant trypanosomatids, that may justify a further debate about their division into different genera.

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1. Introduction

Plant trypanosomatids of the genus *Phytomonas* can be found in latex, phloem, fruits and seed of many plant families with a wide geographical distribution, and are thought to be transmitted by phytophagous insects (Dollet, 1984; Camargo, 1999). Thus, *Phytomonas* spp. have been described as digenetic trypanosomatids found in plants with a typical 'promastigote' appearance (Vickerman, 1976). In plants, trypanosomatids multiply in different kinds of tissues and organs. Those parasitizing latex tubes, fruits or seeds as well as flowers, generally are not harmful. Only those parasitizing the phloem sap are specifically associated with lethal syndromes in Latin America, such as, phloem necrosis of coffee (Stahel, 1931), 'Hartrot' of coconut (Parthasarathy et al., 1976) and 'Marchitez sorpresiva' of oil palm (Dollet, 1984). These infections inflict considerable economic losses and have important ecological implications because of intensive crop treatment with insecticides.

From the 1930s, the criteria used to classify isolates as *Phytomonas* spp. were based on morphological features as well as host and geographical origin (Camargo, 1999). Actually, the creation of *Phytomonas* as a genus may seem arbitrary as it essentially grouped trypanosomatids found in plants (Donovan, 1909), whereas it has been demonstrated that so called "monoxenous insect trypanosomatids" of the genera *Crithidia*, *Leptomonas* and *Herpetomonas* can also be found in plants and easily be mistaken as *Phytomonas* (Conchon et al., 1989; Jankevivius et al., 1993; Catarino et al., 2001; Fiorini et al., 2001; Godoi et al., 2002; Marín et al., 2007). The matter is important at all levels because studies on circulation of parasites among hosts, identification of natural reservoirs of pathogenic isolates, demonstration of infection and virulence, metabolism, etc. require a precise identification of the pathogen. The first biochemical study comparing plant trypanosomatids, isolated from different continents and different tissues, suggested that the term *Phytomonas* could not alone reflect their genetic diversity (Guerrini et al., 1992). Subsequent comparison of nucleotide sequences of the spliced-leader (SL) RNA (Sturm et al., 1995; Serrano et al., 1999; Dollet et al., 2001), 5S rRNA genes (Dollet et al., 2000), kinetoplast minicircle conserved region (Sturm et al., 2007) and more recently the internal transcribed spacer of the ribosomal operon (Dollet, Sturm and Campbell, unpublished) improved the classification of plant trypanosomatids, showing 8, and then 10, strong groups. Still, a century after its discovery, an established objective criterion does not exist for species determination within a questionable single genus *Phytomonas*.

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Leaving aside this taxonomic dubiousness, the genetics and molecular biology of *Phytomonas* are almost completely ignored. Taking isolates from latex as representative, our group has recently published for the first time the genomic organization of *Phytomonas* spp. (Marín et al., 2008). The molecular karyotype of this latex group (group D) consists of 21 chromosomal linkage groups sizing between 0.3 and 3 Mb, with a genome size estimated at 25 Mb. In view of the above-described genetic diversity, we decided to further explore the genomic organization of *Phytomonas* spp. by analyzing the most pathogenic group of this parasite, also the most difficult one to grow *in vitro*.

The term "molecular karyotype" (MK) was forged when chromosomes from single-celled organism, that do not condense during mitosis, could be separated and visualized using pulsed field gel electrophoresis (PFGE) (Schwartz and Cantor, 1984; Bastien et al., 1992). In protozoa, MKs usually show a high degree of polymorphism between isolates of the same species (Pagès et al., 1989; Janse et al., 1994; Kanmogne et al., 1997; Vargas et al., 2004), this being due to size variations among homologous chromosomes. But the large-scale genomic organization, as seen from the number and approximate size of chromosomes, remains highly conserved at this taxonomic level. Inter-species comparisons of MKs may reveal much wider divergences than the intra-specific level, as in the case of *Trypanosoma* spp. (Kanmogne et al., 1997; Vargas et al., 2004), or a similar level of polymorphism as between *Leishmania* species (Wincker et al., 1996).

In the present work, we elucidated the complete molecular karyotype of two representative *Phytomonas* isolates from the phloem of coconut palm (group H). The results reveal a chromosomal organization largely different from that of the first latex-specific group examined previously (Marín et al., 2008).

2. Materials and methods

2.1. Parasites

The phloem-restricted *Phytomonas* isolates analyzed were isolated from *Cocos nucifera* in French Guiana: Hart1 in 1986 and Hart4 in 1987; both belong to group H (Dollet et al., 2001). These isolates as well as and one reference isolate (EM1) from the latex of *Euphorbia pinea* (group D), isolated in Montpellier, France, in 1980 (Marín et al., 2008), were routinely cultured in Grace's Insect medium (Gibco®) supplemented with 10% heat inactivated foetal bovine serum at 28 °C. The identification of all strains was checked by sequencing the SL RNA and 5S ribosomal RNA (rRNA) genes and comparing them against the sequences published previously (Dollet et al., 2000, 2001). *Leishmania major* "Friedlin" (LmjF) (MHOM/IL/81/FRIEDLIN) was grown in RPMI 1640 supplemented with 10% heat inactivated foetal bovine serum at 28 °C.

2.2. Preparation of samples and PFGE

For preparation of chromosomal DNA, all the parasites were cultivated in large volumes up to a final total amount of 7×10^8 cells. DNA agarose blocks were prepared and processed and PFGE carried out on home-made devices as described in Pagès et al. (1989). PFGE and Southern-blotting were performed as described previously (Marín et al., 2008). The voltages and pulse conditions necessary to resolve every chromosomal size class are described in the legend to Fig. 1. As chromosome size markers, we used the molecular karyotype of the reference *L. major* Genome project, *L. major* "Friedlin"(LmjF), available at http://www.ebi.ac.uk/parasites/LGN/chromsum.html.

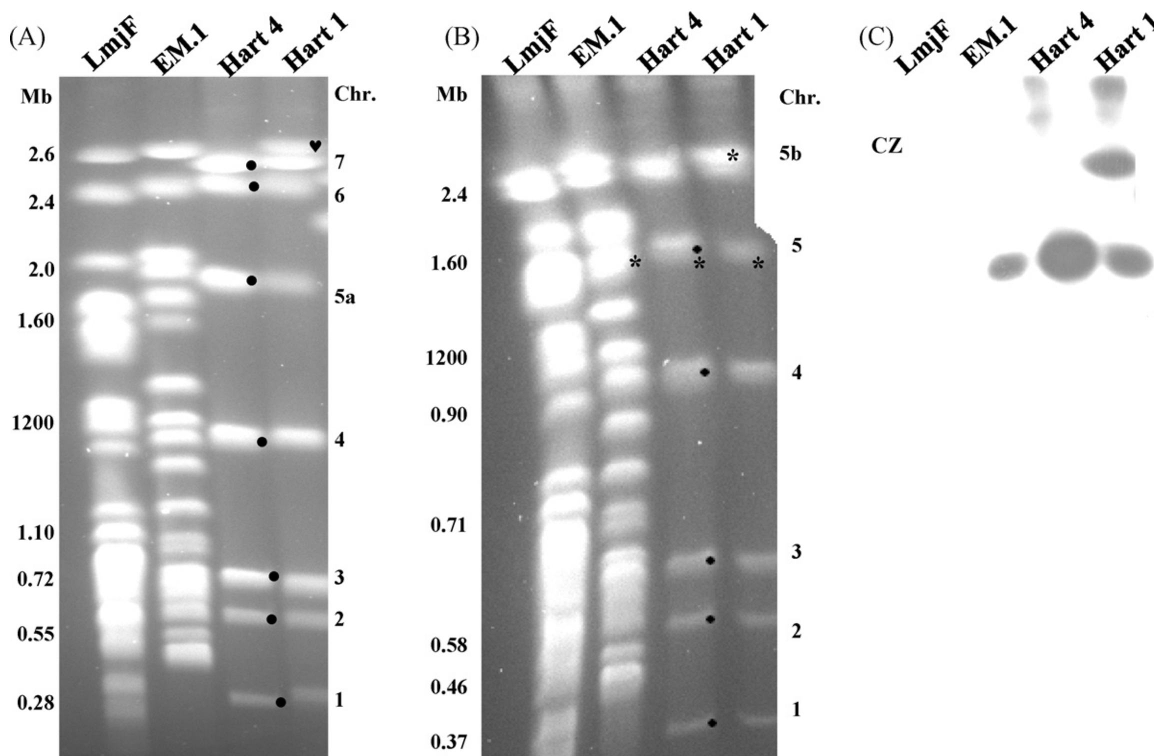

Fig. 1. Complete resolution of the PFGE karyotype of *Phytomonas* isolates from phloem. The chromosomes of *Leishmania major* Friedlin (LmjF), one reference isolate from the latex-restricted group of *Phytomonas* (EM1) (Marín et al., 2008), and two isolates of phloem-restricted *Phytomonas* (Hart4 and Hart1) were separated in PFGE gels A and B. The pulse/migration time conditions were 500 s/48 h, 350 s/48 h and 200 s/36 h in a 1.2% agarose gel at 7.5 V/cm (in A), and 320 s/24 h, 180 s/24 h and 1200 s/24 h in a 1.5% agarose gel at 7.5 V/cm (in B). The 'Hartrot' *Phytomonas* chromosomes are indicated for strain Hart4 by dots on the gels and their attributed numbers are shown on the right. C.Z. compression zone. The chromosome sizes of LmjF are indicated on the left in megabase pairs (Mb) [from http://www.ebi.ac.uk/parasites/LGN/chromsum.html]. A better resolution of the megabase chromosomes was achieved using pulse/migration times of 800 s/60 h, 600 s/60 h and 400 s/40 h in a 1% agarose gel at 5 V/cm (not shown). (C) The ethidium bromide-stained gel in B was blotted and hybridized with a ^{32}P-labeled chromosome 5-specific probe (CMS60). The chromosomes recognised by the probe are indicated by asterisks in B. The largest homologue of chromosome 5 (5b) in Hart1 is also indicated by ♥ in A.

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