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Short Communication

Microsatellite genotyping reveals diversity within populations of *Sodalis glossinidius*, the secondary symbiont of tsetse flies

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ABSTRACT

The aim of this study was to develop a PCR-based microsatellite genotyping method for identifying genetic diversity in *Sodalis glossinidius*, a symbiont associated with tsetse fly infection by trypanosomes causing human and animal trypanosomiasis. Allelic polymorphism at three loci, investigated on 40 fly gut extracts, evidenced eight alleles and the existence of five genotypes. This novel approach was shown to be efficient and suitable for routine large-scale genotyping of *S. glossinidius* present in the biologically complex tsetse fly extracts; it could favor progress in the fields of diagnosis, epidemiology, population genetics, and fly/symbiont/trypanosome interactions.

1. Introduction

Tsetse flies transmit trypanosomes responsible for human African trypanosomiasis (HAT) as well as the animal disease (Nagana). HAT affects a wide range of people in sub-Saharan Africa and is fatal if untreated, and Nagana is estimated to cost African agriculture US$4.5 billion per year (Reinhardt, 2002). The available drugs are unsatisfactory (Barrett, 2006), while resistance is increasing (Matovu et al., 2001). Consequently, investigations for novel strategies must continue. Trypanosome transmission needs the parasite to establish in the tsetse fly midgut and to mature into an infective form before being transmitted to a novel mammalian host (Van den Abbeele et al., 1999). Tsetse flies harbor three different symbiotic bacteria among which *Sodalis glossinidius* is suspected of being involved in the fly's vector competence (Cheng and Aksoy, 1999; Dale and Welburn, 2001; Welburn and Maudlin, 1999). Recently, this bacterium was shown to be associated with infection of field populations of tsetse flies by trypanosomes (Farikou et al., 2010). Furthermore, genetic diversity was evidenced in *S. glossinidius* populations from insectary flies, suggesting that fly infection by trypanosomes could be favored by the presence of symbiont-specific genotype(s) (Geiger et al., 2007). Thus, a large-scale evaluation of the *S. glossinidius* genetic diversity in field populations of flies is needed. The AFLP technique previously used on DNA extracted from insectary fly hemolymph is not well adapted to routinely typing field samples because the hemolymph volume of such individual fly is too low, and the gut extracts, including the bacteria, are too complex. We therefore aimed to develop a Variable Number Tandem Repeat (VNTR) approach known to be highly discriminant (Le Flèche et al., 2006; Lindstedt, 2005; Whatmore et al., 2006). As the microsatellite approach has never been used to investigate the genetic diversity of *S. glossinidius*, we had to determine whether: (a) it would be appropriate to distinguish genetically distinct *Sodalis* strains, (b) the

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Table 1
S. glossinidius microsatellite markers, PCR primers and allelic polymorphism.

Marker	Repeat sequence[a]	Primer sequences (5′–3′)	Location at bp[b]	Number of alleles	Size of the alleles (bp)
ADNg 5/2	$(AC)_{\times\ 5,\ 7}$	GGCCGGTATTCTAACCGAC AACTGCCAGGCATCCATTAC	4115043–4115222	2	176/180
ADNg 21/22	$(GCC)_{\times\ 6,\ 7,\ 9,\ 11}$	GAGCAAATCTCCCAGCACAT TTCTTGTCCCTCAACCCATC	1450588–1450759	4	163/166/172/178
ADNg 15/16	$(AGG)_{\times\ 5,\ 7}$	ATACGGCGAAGCAATGAGAC CAGCCTCTAAGCGCTCAACTC	3250160–3250283	2	106/112

[a] Number of repeats recorded at each locus (5 or 7 at ADNg 5/2, etc.).
[b] GenBank accession number AP008232.

microsatellite approach could work on complex biological extract samples, and (c) it would be suitable for routine analyses.

2. Materials and methods

Glossina palpalis gambiensis individuals were chosen from flies that were field sampled in different areas of Burkina Faso. Pupae were collected from these flies. After adult emergence, the population was maintained in a level 2 containment insectary at 23 °C and 80% relative humidity, without any selection. Individuals used in the present work were randomly chosen. The flies were dissected in a drop of sterile 0.9% saline solution. Midguts from each fly were then separately transferred into microfuge tubes containing ethanol (95°) for further symbiont analyses. The instruments were carefully cleaned after the dissection of each fly to prevent contamination. The microfuge tubes were stored at −20 °C until use.

DNA was extracted from midgut using the classical protocol (Navajas et al., 1998). Briefly, tissues were homogenized with a pestle in a cetyl trimethyl ammonium bromide (CTAB) buffer (CTAB 2%; 0.1 M Tris, pH 8; 0.02 M EDTA pH 8; 1.4 M NaCl) and incubated at 60 °C for 30 min. The DNA was extracted from the lysis mixture with chloroform/isoamylic alcohol (24/1; V/V) and precipitated by adding isopropanol (V/V). After centrifugation (10,000 × *g*, 15 min), the pellet was rinsed with 70% ethanol, air-dried, and resuspended in distilled sterile water. The DNA samples were stored at −20 °C until PCR amplification processing.

S. glossinidius microsatellite repeat sequences were selected in a data bank (GenBank, accession number AP008232) (Toh et al., 2006) using the Vector NTI software; primer pairs were designed for the sequences flanking such regions and tested to ensure that they specifically amplify *S. glossinidius* and not host DNA. Table 1 shows the primers selected to perform the present study.

One μl of DNA was used for subsequent DNA amplification. Polymerase chain reactions (PCRs) were carried out using a DNA thermal cycler (PE Applied Biosystems, Foster City, CA, USA) in 20-μl final volumes, containing 4 pmol of each primer, 0.2 mM of each deoxyribonucleotide, 1× incubation buffer with 1.5 mM $MgCl_2$ (Quantum Appligène, Ilkirch, France) and 0.5 units of Taq DNA polymerase (Quantum Appligène). Samples were first denatured at 94 °C for 3 min and then processed through 40 cycles consisting in a denaturation step at 94 °C for 30 s, an annealing step at 55 °C for 30 s, and an extension step at 72 °C for 1 min. The final elongation step was lengthened to 5 min at 72 °C. PCR products were checked by 2% agarose gel electrophoresis and visualized under UV light after ethidium bromide staining (0.5 μg/ml). Allele bands were then resolved in nondenaturing acrylamide gels (10%), after loading 2–5 μl PCR products, and revealed by ethidium bromide staining.

In order to assess whether the amplicons actually corresponded to *S. glossinidius* microsatellites, bands representing the different alleles were purified using the Wizard DNA Clean Up System (Promega Corporation, Madison, WI, USA). Purified PCR fragments were cloned into pGEM-T Easy Vector (Promega Corporation). Clones of each cloned allele were purified with the Wizard Plus SV Minipreps DNA Purification System Kit (Promega, Charbonnière, France) and sequenced by GATC Biotech (Konstanz, Germany), allowing the allele size to be determined as well as the exact number of microsatellite repeats (GenBank accession numbers HQ842611–HQ842618).

3. Results

Fig. 1 shows an example of an electrophoretic separation on a 10% polyacrylamide gel of different alleles; it demonstrates the development of the experimental process to be successful. Table 1 summarizes the characteristics of the three microsatellite markers selected to investigate the polymorphism within the *S. glossinidius* samples from 40 *G. palpalis gambiensis* flies, and to test the genotyping method: sequence of the repeats, part of the flanking sequences used as primers, their location on the *S. glossinidius* genome, and finally the number and size of the

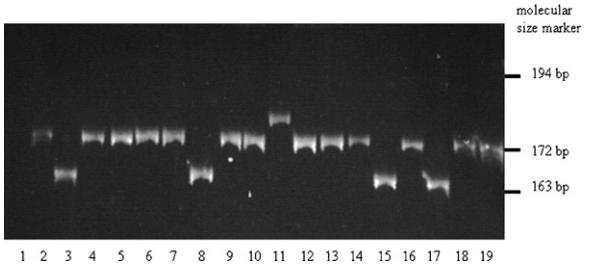

Fig. 1. Example of fragment length polymorphism at the ADNg 21/22 locus in 18 *Sodalis glossinidius* samples. The alleles have been separated by electrophoresis on a 10% polyacrylamide gel on the basis of the size of their corresponding DNA sequence. Lane 1 corresponds to the "no template" negative control. Lanes 2–19 correspond to *S. glossinidius* samples. Here three alleles have been separated into three sizes: 163 (4 strains), 172 (13 strains), and 178 (1 strain) base pairs.