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# Recombinant expression of trypanosome surface glycoproteins in *Pichia pastoris* for the diagnosis of *Trypanosoma evansi* infection

S. Rogé[a,h,*], N. Van Reet[a], S. Odiwuor[a,b], T. Tran[a], K. Schildermans[c], S. Vandamme[c], I. Vandenberghe[d], W. Vervecken[e,f,1], K. Gillingwater[g], F. Claes[a], B. Devreese[d], Y. Guisez[c,h], P. Büscher[a]

[a] *Department of Biomedical Sciences, Unit of Parasite Diagnostics, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium*
[b] *Department of Medical Laboratory Sciences, School of Health Sciences, Mount Kenya University, Thika, Kenya*
[c] *Centre for Proteome Analysis and Mass Spectrometry, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp, Belgium*
[d] *Laboratory for Protein Biochemistry and Protein Engineering, Ghent University, K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium*
[e] *Department for Molecular Biomedical Research, VIB, Technologiepark 927, B-9052 Ghent-Zwijnaarde, Belgium*
[f] *Department of Biomedical Molecular Biology, Ghent University, Technologiepark 927, B-9052 Ghent-Zwijnaarde, Belgium*
[g] *Swiss Tropical and Public Health Institute, Department of Medical Parasitology and Infection Biology, Socinstrasse 57, CH-4002 Basel, Switzerland*
[h] *Laboratory for Molecular Plant Physiology and Biotechnology, Department of Biology, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp, Belgium*

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ABSTRACT

Serodiagnosis of surra, which causes vast economic losses in livestock, is still based on native antigens purified from bloodstream form *Trypanosoma (T.) evansi* grown in rodents. To avoid the use of laboratory rodents in antigen preparation we expressed fragments of the invariant surface glycoprotein (ISG) 75, cloned from *T. brucei gambiense* cDNA, and the variant surface glycoprotein (VSG) RoTat 1.2, cloned from *T. evansi* gDNA, recombinantly in *Pichia (P.) pastoris*. The M5 strain of this yeast has an engineered N-glycosylation pathway resulting in homogenous $Man_5GlcNAc_2$ N-glycosylation which resembles the predominant $Man_{9\text{-}5}GlcNAc_2$ oligomannose structures in *T. brucei*. The secreted recombinant antigens were affinity purified with yields of up to 10 mg and 20 mg per liter cell culture of rISG $75_{29\text{-}465\text{-}E}$ and rRoTat $1.2_{23\text{-}385\text{-}H}$ respectively. In ELISA, both recombinant proteins discriminated between pre-immune and immune serum samples of 25 goats experimentally infected with *T. evansi*. The diagnostic potential of rRoTat $1.2_{23\text{-}385\text{-}H}$ but not of rISG $75_{29\text{-}465\text{-}E}$ was confirmed with sera of naturally infected and control dromedary camels. The results suggest that rRoTat $1.2_{23\text{-}385\text{-}H}$ expressed in *P. pastoris* requires further evaluation before it could replace native RoTat 1.2 VSG for serodiagnosis of surra, thus eliminating the use of laboratory animals for antigen production.



## 1. Introduction

Surra is an infectious disease in domestic (buffaloes, cattle, horses, camels etc.) and pet animals (dogs and cats) caused by the protozoan parasite *Trypanosoma (T.) evansi*. It occurs in Africa, the Middle East, Asia and South and Central America with sporadic outbreaks in Europe (Desquesnes

* Corresponding author at: Department of Biomedical Sciences, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium. Tel.: +32 32476534; fax: +32 32476373.
*E-mail address:* sroge@itg.be (S. Rogé).
[1] Current address: Oxyrane Belgium nv, Technologiepark 3, B-9052 Ghent-Zwijnaarde, Belgium.

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et al., 2008; Gutierrez et al., 2010; Tamarit et al., 2010). The parasite is transmitted mechanically by bloodsucking flies such as *Tabanidae* and *Stomoxys* species and occasionally by vampire bats in Latin America (Stephen, 1986). It causes severe anemia, edema, immunosuppression and various neurological disorders resulting eventually into the death of the affected animals. Hence, surra leads to serious economic losses to the farmers in terms of morbidity, mortality, abortion, infertility, reduced milk yield and costs for trypanocides (Dobson et al., 2009).

The surface of trypanosomes is covered with a densely packed layer of about $5 \times 10^6$ dimers of one type of variant surface glycoprotein (VSG). This VSG is a strong immunogen, but the parasite avoids eradication by the host immune system by antigenic variation, i.e. changing the variant antigen type (VAT) of its VSG coat. Each parasite genome contains a large repertoire of different VSG genes, supplemented by recombination and gene conversion events, but only one is predominantly expressed at a time (Berriman et al., 2005; McCulloch and Horn, 2009). Switching the expression of one VSG gene to another results in a trypanosome bearing a different VAT that may escape immune destruction as long as the infected host has no antibodies against this particular VAT (Barry and McCulloch, 2001). VSG RoTat 1.2 is found to be predominantly expressed in *T. evansi* except in *T. evansi* type B strains circulating in Kenyan and in Ethiopian camels (Bajyana Songa and Hamers, 1988; Hagos et al., 2009; Ngaira et al., 2005; Payne et al., 1991; Verloo et al., 2000, 1998). In addition, other *Trypanosoma* sp. do not express this particular VAT (Claes et al., 2004). Current diagnostic tests for surra are therefore based on antibody detection against *T. evansi* RoTat 1.2. The card agglutination test for trypanosomiasis CATT/*T. evansi* is one of the OIE reference tests for antibody detection in surra diagnosis (Manual of Diagnostic Tests and Vaccine for Terrestrial Animals (OIE, 2012)).

Buried within the VSG layer, some receptors and invariant surface glycoproteins (ISGs) such as the ISG 75 are expressed in all *Trypanozoon* species with an amino acid homology of 92% and 90% between group I and II ISG 75 sequences respectively (Tran et al., 2006). The trypanosome surface contains $5 \times 10^4$ copies of ISG 75 that are believed to be evenly distributed over the surface (Jackson et al., 1993; Ziegelbauer and Overath, 1992). Since ISG 75 is strongly immunogenic and invariant, it is a good candidate antigen for diagnosis of *Trypanozoon* infections (Büscher and Lejon, 2004; Hutchinson et al., 2004; Tran et al., 2008).

Up to now, the available antibody tests for surra are all still based on native proteins purified from bloodstream form trypanosomes grown in rodents. To eliminate the use of laboratory rodents, VSG RoTat 1.2 has been recombinantly expressed in *Spodoptera frugiperda* with clear diagnostic potential but poor expression reproducibility (Lejon et al., 2005; Urakawa et al., 2001). Later, the diagnostic value of ISG 75 recombinantly expressed in *Escherichia coli* was demonstrated in camels and goats (Tran et al., 2008, 2009). To avoid the difficult and tedious purification of intracellular, recombinant proteins, we aspired a model for secreted expression also taking into account that glycosylation of a protein has a profound effect on its folding, thus on its potential to react with antibodies elicited by its native counterpart. Therefore, in the present study, we expressed VSG RoTat 1.2 and ISG 75 recombinantly in *Pichia pastoris*, a yeast that has proven to be successful for the recombinant expression of several trypanosomal proteins such as acid α-mannosidase and trans-sialidase from *T. cruzi*, rhodesain from *T. brucei rhodesiense* and congopain from *T. congolense* (Caffrey et al., 2001; Huson et al., 2009; Laroy and Contreras, 2002; Vandersall-Nairn et al., 1998). Glycoproteins produced in *P. pastoris* contain high mannose glycan structures. Those structures can hamper downstream processing, might be immunogenic and can cause rapid clearance from the circulation. To prevent hypermannosylation, our proteins are expressed in the M5 strain of *P. pastoris*. This strain has an engineered N-glycosylation pathway resulting in homogenous $Man_5GlcNAc_2$ N-glycosylation which resembles the predominant $Man_{9\text{-}5}GlcNAc_2$ oligomannose structures in *Trypanosoma brucei* (Acosta-Serrano et al., 2004; Jones et al., 2004; Mehlert et al., 1998; Vervecken et al., 2004; Zamze et al., 1991, 1990).

The expressed and secreted recombinant proteins are affinity purified and tested for their diagnostic potential against experimentally *T. evansi* infected goat sera and against uninfected and naturally infected dromedary camel sera.

## 2. Materials and methods

### 2.1. Yeast strain

For recombinant expression of the trypanosome proteins, the *P. pastoris* GS115 M5 strain was used in view of homogenous $Man_5GlcNAc_2$ N-glycosylation of the secreted proteins (Vervecken et al., 2004).

### 2.2. Construct engineering

The ISG 75 sequence was amplified from cDNA collected from *T. brucei gambiense* LiTat 1.3 (clone cb4; GenBank accession no. DQ200254) with a primer set starting from the first residue of the mature polypeptide (amino acid 29) and ending immediately upstream of the transmembrane domain (at amino acid 465 of a total of 523 amino acids). This clone exhibits a sequence and protein identity of >99% with an ISG 75 clone from *T. evansi* RoTat 1.2 (clone g21; GenBank accession no. DQ200175). A *PvuII* site was incorporated in the forward primer (ISG 75_FP). An E tag coding sequence (GE Healthcare), a stop codon and an *AvrII* site were added to the reverse primer (ISG 75_RP) (Table 1). The RoTat 1.2 VSG sequence was amplified from *T. evansi* RoTat 1.2 gDNA (GenBank accession no. AF317914) (Urakawa et al., 2001) by PCR. A *XhoI* site was incorporated in the forward primer (RoTat 1.2_FP) followed by 18 nucleotides reconstituting the α-mating factor signal sequence necessary for secreted expression in yeast (Daly and Hearn, 2005). Since the first amino acid codon of RoTat 1.2 showed self-complementarity with the reconstituted signal sequence, we started translation at the second residue of the ORF (amino acid 23). In order to obtain soluble RoTat 1.2 VSG, the coding region of the RoTat 1.2 VSG cDNA was truncated at the C-terminal end