Intricate balance of dually-localized catalase modulates infectivity of *Leptomonas seymouri*

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Introduction

The evolutionary history of catalase in trypanosomatids is unexpectedly complex: the enzyme is absent from other kinetoplastids implying that their common ancestor lacked it. Hence, the genes encoding this enzyme must have been independently acquired by different lineages of monoxenous (one host) trypanosomatids from different bacteria at least three times. This observation posited an obvious question: why was catalase so "sought after" if many trypanosomatid groups do just fine without it? The overexpression of catalase in dixenous (two hosts) *Trypanosoma brucei* and *Leishmania mexicana* impaired the development in both insect and vertebrate hosts (Horáková, 2020; Sádlová, 2021). To further dissect the role(s) of catalase in trypanosomatids, we decided to investigate its function in *Leptomonas seymouri*, a monoxenous parasite that is often found in coinfections with *Leishmania*. This species is predisposed to the dixenous life style due to its ability to withstand elevated temperatures (Kraeva, 2015) and, thus, represents a suitable model for a thorough characterization of this intriguing enzyme in Trypanosomatidae.

Results

1) Dual localization of catalase is sensitive to H_2O_2 treatment.

We endogenously tagged catalase and explored its localization by immunofluorescent microscopy. Under normal conditions, the enzyme has a dual localization being diffusely distributed in the cytoplasm and concentrated in a presumably membranous compartment in front of the nucleus and close to the kinetoplast. The compartment in question did not co-localize with aldolase, a bona fide glycosomal marker (Fig. 1A). Since glycosomes of trypanosomatids are known to be heterogeneous, we also investigated co-localization of catalase with another glycosomal marker, phosphomevalonate kinase (MVAK), an enzyme of the isoprenoid biosynthesis pathway (Fig. 1B). The colocalization of MVAK and catalase suggests that the latter is confined to a subset glycosomes, which are not involved in glycolysis.

To complement our localization data, we employed digitonin permeabilization technique followed by Western blotting analysis. Confirming previous results, we documented a low abundance of catalase in the cytoplasmic fraction, while most of the signal was associated with a membranous compartment of the sterol composition reminiscent of the glycosomes (Fig. 2A). The treatment with H_2O_2 resulted in an increased level of catalase in the cytoplasm (Fig. 2B), while the total amount of the enzyme was not affected (Fig. 2C) further confirming that its dual localization and cytoplasmic abundance are H_2O_2-dependent.

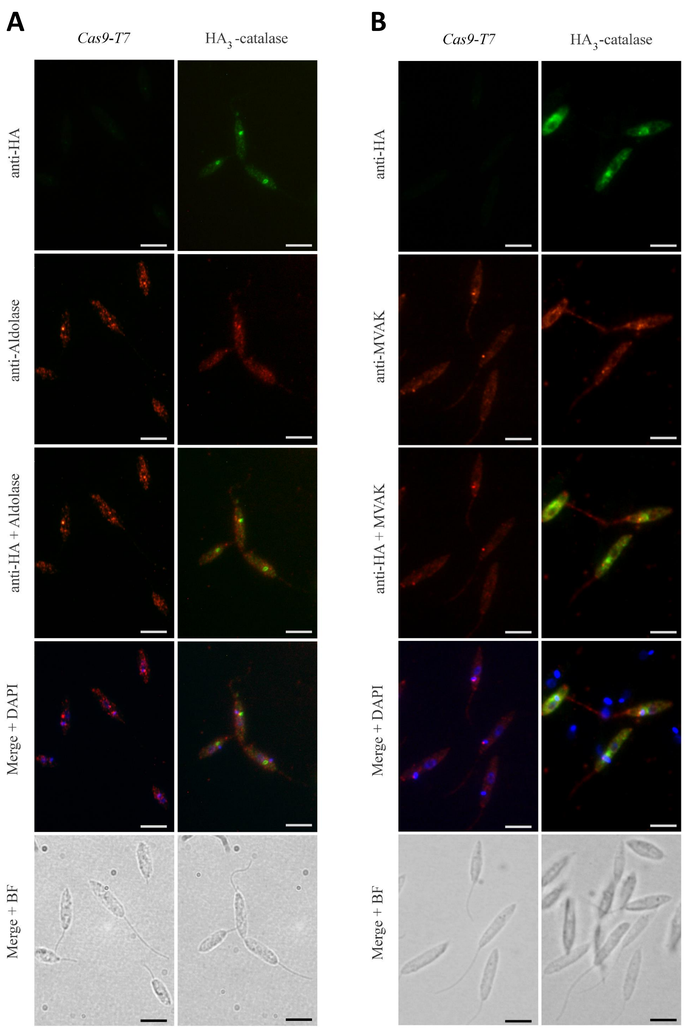

Fig. 1. Localization of *L. seymouri* catalase analyzed by microscopy. Immunofluorescence analysis of N-terminally tagged *L. seymouri* catalase (green). Co-staining with anti-Aldolase antibody (red, panel A) and anti-MVAK (red, panel B) was used to assess colocalization. Nuclei and kinetoplasts are stained with DAPI. Scale bar 5 µm.

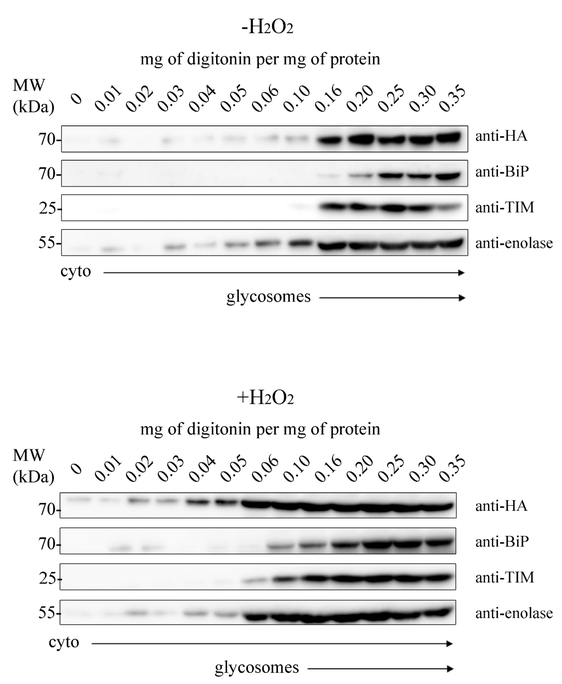

Fig. 2. Localization of *L. seymouri* catalase analyzed by differential digitonin permeabilization method. A, B) HA-tagged catalase localization after digitonin fractionation in untreated (A) and 0.2 mM hydrogen peroxide-treated (B) cultures of *L. seymouri* analyzed by Western blotting with anti-HA, -BiP (an ER marker), -TIM (a glycosomal marker), and –enolase (a cytoplasmic marker) antibodies. Molecular weights in kDa are indicated on the left. C) The total amount of HA-tagged catalase in treated- and untreated samples analyzed by Western blotting with anti-HA antibody. Tubulin was used as a loading control in A)-C).

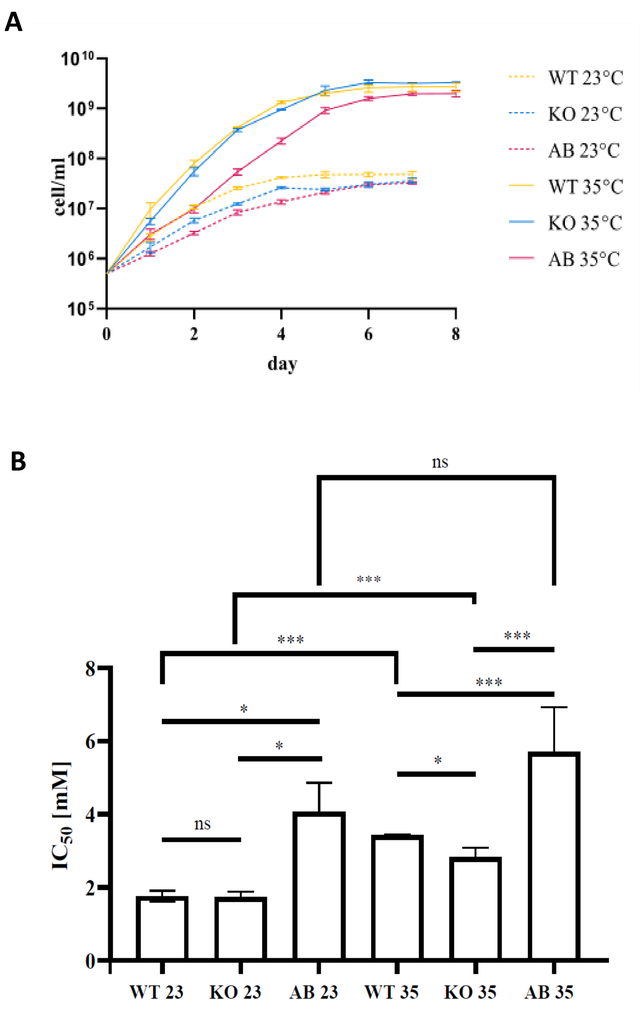

Fig. 3. The effect of catalase ablation in *L. seymouri in vitro*. A) Comparison of growth rates of WT, KO, and AB of *L. seymouri* at 23°C and 35°C. B) Sensitivity of WT, KO, and AB of *L. seymouri* (IC50) to hydrogen peroxide at 23°C and 35°C measured by alamarBlue assay. The data and statistical analyses represent three independent biological replicates. The error bars indicate standard deviation; *, **, and *** denote p-values ≤ 0.05, ≤ 0.001, and ≤ 0.00001, respectively; ns – not statistically significant.

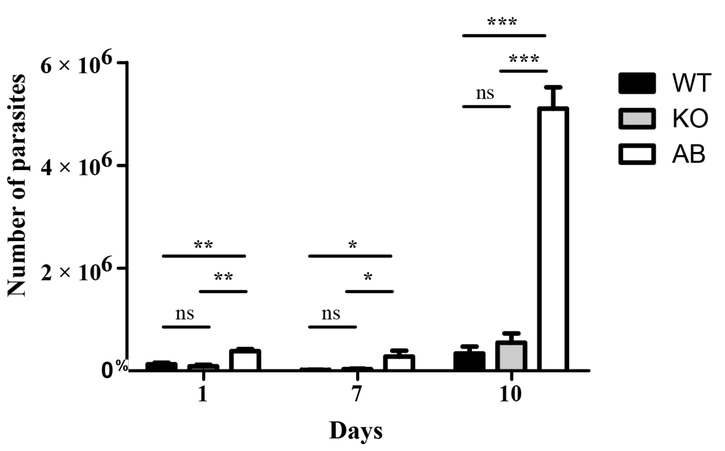

Fig. 4. Development of *L. seymouri* in experimental infection of *D. peruvianus*. Intensity of infection was assayed on days 1, 7, and 10. The error bars indicate standard deviation; *, **, and *** denote *p*642 values ≤ 0.05, ≤ 0.001, and ≤ 0.00001, respectively; ns – not statistically significant.

2) Catalase is dispensable *in vitro* but enhances resistance to H_2O_2 when overexpressed.

To further dissect the role of catalase, we ablated the corresponding gene (*Lsey_0026_0490*) using the CRISPR/Cas9 system and established the add-back (AB) cell line (integration into the 18S rRNA). *L. seymouri* multiplies faster at 35°C than at 23°C. Moreover, AB cells divided slower, a difference that was more pronounced at 35°C (Fig. 3A). At 23°C, the ablation of catalase had little effect (if any), while its overexpression provided a significant survival advantage after cytotoxic effects of H_2O_2. At 35°C, ablation and overexpression of catalase in the KO and AB cell lines had a negative and a positive influence on *L. seymouri* survival, respectively (Fig. 3B).

3) Catalase is dispensable *in vivo* but enhances resistance to H_2O_2 when overexpressed.

Experimental infections of the cotton stainer *Dysdercus peruvianus* (Hemiptera: Pyrrhocoridae) with the WT, KO, and AB strains of *L. seymouri* demonstrated a situation similar to that observed *in vitro*: the ablation of catalase had no detectable effect on infectivity, while its overexpression resulted in a significant increase in the parasite abundance (Fig. 4).

Conclusions

In conclusion, we show that in *L. seymouri* catalase is present in the cytoplasm and a subset of glycosomes, and that its cytoplasmic retention is H_2O_2-dependent. The ablation of catalase in *L. seymouri* is not detrimental *in vivo*, while its overexpression resulted in a substantially higher parasite load in the experimental infection of *D. peruvianus*. We propose that the capacity of studied flagellates to modulate the catalase activity in the midgut of its insect host facilitates their development and protects them from the oxidative damage at elevated temperatures.

Acknowledgements

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