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Initial steps in the study of catalase function and localisation in *Leptomonas seymouri*

Catalase is a ubiquitous enzyme involved in the protection against reactive oxygen species (ROS). Its main function is the decomposition of hydrogen peroxide to water and oxygen. Hydrogen peroxide is not only a harmful molecule, it is also known to participate in redox signalling and regulation of biological activities by the oxidation of thiolate groups. Therefore, it is important to keep the level of this molecule in the nM range in a cell. This task is accomplished by a set of different enzymes (peroxidases, catalase, peroxiredoxins). Despite its wide distribution, some eukaryotic lineages lack catalase. The catalase-encoding gene is, for instance, absent in species inhabiting anoxic conditions, parasitizing in blood, or photosynthetic eukaryotes with secondary plastids. An interesting pattern of catalase distribution can be found in the family Trypanosomatidae. It has been shown that the gene encoding catalase was acquired three times independently from three different bacterial lineages *via* horizontal gene transfer by the monoxenous Leishmaniinae (from *Brachyspira* spp.), Blastocrithidiinae (from *Snodgrassella* spp.), and *Vickermania* spp. (from *Acinetobacter* spp.). Subfamily Leishmaniinae is an intriguing example to study catalases of Trypanosomatidae. It unites dixenous (with two hosts in the life cycle), medically important species (*Leishmania sensu lato*) and monoxenous (one host in the life cycle) species. The catalase gene is present in the genomes of monoxenous relatives and was secondarily lost in dixenous species suggesting that presence of this enzyme is incompatible with dixeny. Here we investigated the role of catalase in *Leptomonas seymouri*, a monoxenous trypanosomatid of the subfamily Leishmaniinae. This species is thermotolerant and was often documented in immunocompromised patients or co-infections with *Leishmania donovani.* We report that *L. seymouri* is amenable to genetic manipulations using conventional and CRISPR/Cas9-mediated approaches by establishing lines with catalase ablation (KO) and add-back (AB). Three investigated cell lines (WT, KO, and AB) were similar in the growth kinetics and morphology, while the cytotoxicity assay revealed an increased resistance to hydrogen peroxide in case of AB compared to WT and KO counterparts (EC_{50}: 1,76; 1,73; 4,07 mM H_2O_2, respectively). The cell line with endogenously tagged catalase was used to study its localization (by IFA and biochemically). We demonstrated that this enzyme has dual localization – glycosomes and cytoplasm – unifying previously reported contradicting results in trypanosomatids. Moreover, the proportion of cytoplasmic catalase increased significantly upon treatment with hydrogen peroxide.