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Probing the effects of TbHK2 on Trypanosoma brucei growth, social behavior, and inhibitor response

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Probing the effects of TbHK2 on *Trypanosoma brucei* growth, social behavior, and inhibitor response

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Introduction

In sub-Saharan Africa the protozoan parasite, *Trypanosoma brucei*, continues to be of major concern for the health and economic development of the region. This parasite is known to cause human African trypanosomiasis (HAT or African sleeping sickness) and nagana in livestock such as cattle. Social behaviors, such as colonization and migration, are important in the study of *T. brucei* because of the way the parasite infects its mammalian host. During the fly bloodmeal, the parasite first passes into the gut but then eventually migrates to the fly salivary glands where it will continue to develop before transmission as a parasitic form able to infect and cause disease in humans and livestock. Past research has shown that the social motility, the ability of the multitude of parasites in an infection to move in a coordinated fashion, is affected by the removal of the *T. brucei* hexokinase 2 (TbHK2) gene or expression of excess copies of the TbHK2 protein. In exploring social motility phenotypes of TbHK2-deficient insect stage (procylic form, PF)*T. brucei* parasites and parental forms complemented with excess TbHK2 gene, this project aims to understand more about the role of TbHK2 in social motility of *T. brucei*. Additionally, in order to understand how hexokinase 2 could be targeted by enzyme inhibitors, known hexokinase 1 inhibitors are explored for their effects on TbHK2 complemented cells compared to the parental strain parasites.

Recombinant strain development

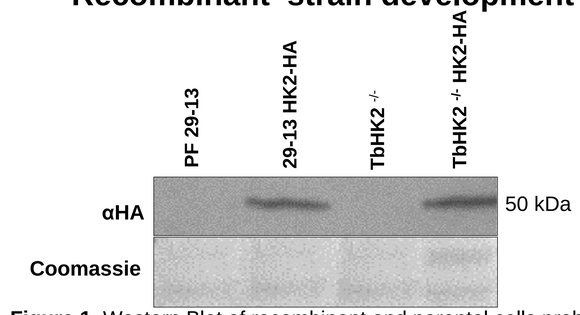


Figure 1. Western Blot of recombinant and parental cells probed with αHA. A Coomassie stained gel is used as a loading control.

Growth Curves

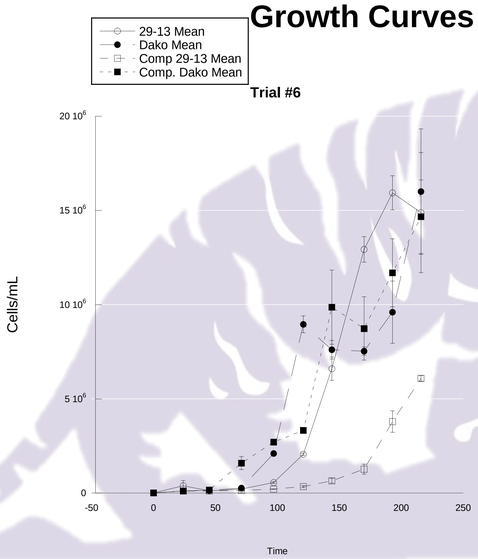


Figure 2. Growth rates of parental cells, HK2 knockouts, HK2 over-expressers, and HK2 knockouts complemented with additional HK2

Social Motility Phenotypes of parental and recombinant *T. brucei*

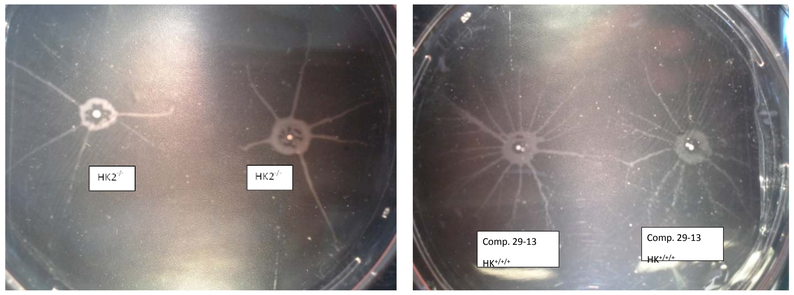


Figure 3. Difference in projection formation of colonies without expression of the hexokinase 2 enzyme and colonies with over expression of the hexokinase 2 enzyme

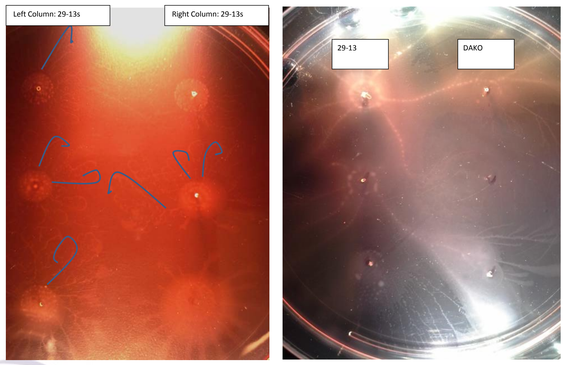
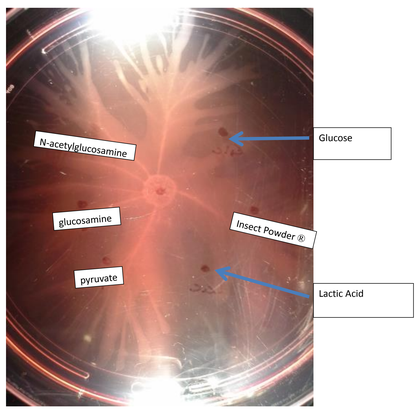


Figure 4. Projections originating from parental colonies (29-13 strain) appear to avoid running into other 29-13 colonies but collide with colonies whose ability to express hexokinase 2 have been deleted (DAKO).

Figure 5. A central colony of procyclic form trypanosomes (29-13) was plated near 1M spots of various carbon sources equidistant from the central 29-13 colony. Preliminary findings suggest the Trypanosomes migrated towards N-acetlyglucosamine, glucosamine, pyruvate, and an insect powder medium but did not migrate directly to glucose or lactic acid spots.



Lethal Dose Assays

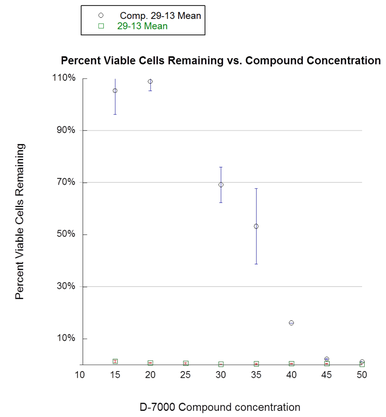


Figure 6. Parental strain procylic form *T. brucei* and over-expressers of the hexokinase 2 enzyme were assayed for their response to a known inhibitor of TbHK1 (a highly similar enzyme from the parasite), which have demonstrated anti-parasitic activity.

Conclusions & Future Directions

The genetically engineered *T. brucei* strains have shown different growth patterns and social response under various environments. An observation of note regarding projection growth is that the parental form trypanosomes (29-13 strain) start projection growth on semi-solid agarose plates between five and seven days before the over expressing strains (comp. 291-3) and those trypanosomes engineered to not express hexokinase 2. This observation may be due to the varying growth rates of the cells (Figure 2). The projection growth patterns seen between Trypanosomes over expressing hexokinase 2 and those that do not express hexokinase 2 are suggestive of the social motility effects hexokinase 2 might be exhibiting in the procyclic form of the parasite (Figure 3). Another piece of evidence suggesting the signaling power of the hexokinase 2 enzyme is the observation that parental forms of the trypanosomes forms projections which appear to avoid neighboring parental strain (29-13) colonies while growing right over colonies which do not express hexokinase 2 (Figure 4). Another possible factor in driving motility response of procyclic form trypanosomes is response to various sugar stimuli (Figure 5). The function of hexokinase 2 in procyclic form trypanosomes may also be instrumental in how the trypansome responds to inhibitor compounds (Figure 6).

Recently, hexokinase 2 has shown in vivo activity for the first time, in *Saccharomyces cerevisiae*. Testing how known inhibitors of the hexokinase 1 enzyme affect the hexokinase 2 enzyme will provide more insight into how hexokinase 2 works in the trypanosome.

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