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Energetic metabolism of axenic promastigotes of *Leishmania (Viannia) braziliensis*

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ABSTRACT

Leishmania spp are protozoans capable of carbohydrates degradation and as energy source they can use glucose, aminoacids or lipids from the environment. The products of the metabolic pathways such as organic acids may be used as an index of their energetic metabolic profile. Therefore, in this study a metabolic profile comparison was made between promastigotes from one reference strain (MHOM/BR/1975/M2903) and two different isolates of *Leishmania (Viannia) braziliensis* (MHOM/BR/2003/IMG3 and MHOM/BR/2005/RPL5). The parasites culture was performed in complete Grace's culture media seeded in 24-well plates at 26 °C. During the growth curve performance samples were collected from the logarithmic and stationary phases of culture and therefore analyzed by high performance liquid chromatography (HPLC) and spectrophotometry assays to determine the concentrations of glucose, lactate, citrate, α-ketoglutarate, succinate, fumarate, malate, oxaloacetate and β-hydroxybutirate which are indicative of the energetic pathways. It was possible to detect an increase in the glucose from the stationary phase from the M2903 strain when compared to the logarithmic phase while in the IMG3 and RPL5 isolates there was a decrease ($p < 0.05$). The spectrophotometric and chromatographic results indicated that the logarithmic phase which presents higher energy consumption due to the intense replication rate have the energetic pathways intensified. It was also possible to note some metabolic differences between the analyzed parasites which may indicate possible adaptations of the parasite when facing different environmental and physiological conditions during its life cycle and that these differences may help in the understanding of the diversity of the host-parasite relationship from *Leishmania* parasites.

1. Introduction

Leishmania spp are protozoans capable of degrading carbohydrates through the glycolitic pathway and its first reactions occur inside the glycosome, an unique organelle from the Kinetoplastida order (Tielens and van Hellemond, 2009). These parasites use glucose and amino acids such as glutamine and proline as an energetic source to ATP production as in aerobic as in anaerobic pathways. Such substrates are sources of intermediary metabolic products that are requisite to other biosynthetic reactions (Mazareb et al., 1999; Saunders et al., 2010). These parasites can also use lipids from the culture median as energy sources and as an overall the products of the metabolism such as organic acids may act as indexes of their metabolic profiles and may indicate differences in the host-parasite relationship. These biochemical pathways are essential to the parasite's survival and the quantification of the *E/S* (excreted/secreted) metabolites may be used to characterize differences among isolates as well as to demonstrate the physiopathologic development of each isolate (Singha et al., 1996). Oliveira et al. (2010) reported the infectivity differences between two recent isolates of *Leishmania (Viannia) braziliensis* as to lesion growth in mice determining that in spite of belonging to the same species, isolates may behave differently when inside the mammalian hosts.

The biochemical analysis of *Leishmania* promastigotes cultured *in vitro* has demonstrated that this parasite presents a similar metabolism than the one observed inside the vector where aminoacids and glucose are used as energy sources. These substrates are involved in the glycolytic pathway, in the mitochondrial metabolism and in the electron transport chain reactions (Opperdoes and Coombs, 2007). Glucose is the main energy source of most stages of *Leishmania* inside vertebrate hosts. However inside the insect vectors the parasite prefers aminoacids such as proline and not glucose to energy production nevertheless the glycolytic pathway is always active (Tielens and van Hellemond, 1998). In

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promastigotes, parts of the end products are oxidized and result from the aerobic metabolism (van Hellemond et al., 1997). *In vitro* studies demonstrate the production of CO_2, succinate, acetate, pyruvate, lactate and alanine as *E/S* products from different isolates and species of *Leishmania* (Singha et al., 1996; Darling et al., 1987; Gupta et al., 1999). However there are few reports of comparative studies of the metabolism from strains and isolates from *Leishmania* species, which may indicate why they interact so differently within the hosts (Bringaud et al., 2006; Zauli-Nascimento et al., 2010). As to other aspects of the parasite we could find some descriptions such as the one from Oliveira et al. (2010) who reported the different behavior from two *L. (V.) braziliensis* isolates inoculated into C57BL/6 mice as to time course of lesion development in which one of the isolates was much more aggressive and developed a larger lesion in less time. These data presented a different pattern from the WHO reference *L. (V.) braziliensis* strain (MHOM/BR/1975/M2903) (Souza-Neto et al., 2004).

In this study an energetic metabolism comparison was made between axenic promastigotes of two recent obtained *L. (V.) braziliensis* isolates (MHOM/BR/2003/IMG3 and MHOM/BR/2005/RPL5) and the reference strain MHOM/BR/1975/M2903 *L. (V.) braziliensis* aiming the investigation of differences in the metabolism of these parasites.

2. Material and methods

2.1. L. (V.) braziliensis maintenance

L. (V.) brazilisensis (MHOM/BR/2003/IMG3 and MHOM/BR/2005/RPL5) promastigotes, refereed here as IMG3 and RPL5, were isolated, identified and provided by the Leishmaniasis Immunobiologic Bank of the Brazilian Mid-West region in the Tropical Pathology and Public Health Institute, Federal University of Goias (Oliveira et al., 2010) and the reference strain MHOM/BR/1975/M2903, refereed here as M2903 were used in this study. These promastigotes were cultured in Grace's liquid medium (Grace's Insect Medium – Gibco®) supplemented with 20% of sterile and inactivated fetal bovine serum, 2 mM of L-glutamine, 100 U/ml of penicillin and 100 U/ml of streptomycin in 24-well microplates at 26 °C incubator. The growth of the parasite was accompanied during 8 days starting with 5×10^5 parasites/ml. Samples from logarithmic (3rd day) and initial stationary (6th day) phases were collected for biochemical analysis through spectrophotometry and chromatography techniques. A sample containing the culture medium without parasites was analyzed as control. Every analysis was performed in quintuplicate.

2.2. Spectrophotometric analysis

The samples collected in logarithmic and initial stationary phases were analyzed through enzymatic spectrophometric methods using a Konelab 60i (Wiener®) equipment and the glucose concentrations were dosed: glucose (Wiener®, absorbance reading at 560 nm).

2.3. Chromatographic analysis

The organic acids excreted/secreted (*E/S*) into the culture medium by the parasites were extracted and analyzed according to methodology described by Vinaud et al. (2007, 2008). The organic acids analyzed were referring to the glycolitic and energetic metabolism such as lactate, citrate, α-ketoglutarate, pyruvate, succinate, fumarate, malate, oxaloacetate and β-hydroxibutyrate.

2.4. Statistical analysis

Data were presented as media ± standard deviation of the quintuplicate repetitions. The statistical analysis was performed through the Sigma Stat 3.2 (Microsoft Corp.) using the ANOVA/Tukey test and the statistical difference was considered when $p < 0.05$.

3. Results

The growth of *L. (V.) braziliensis* promastigotes from the M2903 strain and the IMG3 and RPL5 isolates were monitored in culture media during 8 days. It was possible to observe that the promastigotes from the M2903 strain presented a significantly higher concentration of parasites from the 3rd day of culture than the ones from the isolates (Fig. 1). No statistical difference was observed in parasites from the other days of culture.

The spectrophometric analysis showed that the glucose concentrations was increased in the stationary phase of the M2903 strain when compared to the concentrations detected in the logarithmic phase from the same strain ($p < 0.05$). On the contrary, in IMG3 and RPL5 isolates cultures it was possible to observe a decrease in the glucose concentrations in the logarithmic and stationary phases ($p < 0.05$) (Table 1).

Through the chromatographic analysis was possible to detect the following organic acids: lactate, citrate, α-ketoglutarate, succinate, fumarate, malate and oxaloacetate. These organic acids were detected in the logarithmic as well as in the stationary phase from M2903, IMG3 and RPL5 parasites. It was possible to observe that the *E/S* of citrate was significantly higher in the stationary phase than in the logarithmic one of the parasites from the IMG3 isolate ($p < 0.05$) but in parasites from the M2903 strain and RPL5 isolate there was no significant difference between the *E/S* of citrate during the *in vitro* analysis (Figs. 2–4).

The *E/S* of oxaloacetate was higher in the stationary phase than in logarithmic one of the IMG3 isolate ($p < 0.05$) while there was no statistical difference in the concentrations from the M2903 strain (Table 1). In the RPL5 isolate it was not possible to detect the *E/S* of oxaloacetate. As to the *E/S* of fumarate from the logarithmic phase of the parasites it was possible to detect that its *E/S* from the M2903 strain was significantly higher than both isolates ($p < 0.05$) and there was no difference between both of them (Table 1, Figs. 2–4).

When comparing the two isolates and the strain, it was possible to detect that the *E/S* of lactate was higher in the logarithmic phase of M2903 strain and IMG3 isolate when compared to the concentrations detected from the RPL5 isolate ($p < 0.05$). As to the *E/S* of malate in the logarithmic phase of the M2903 strain was higher than the concentrations detected from both isolates ($p < 0.05$) (Figs. 2–4).

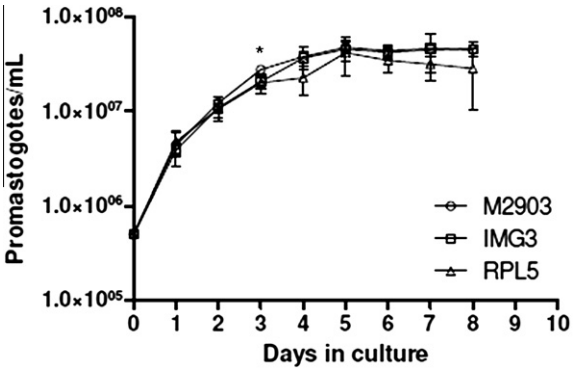

Fig. 1. *In vitro* growth of promastigotes from M2903 strain, IMG3 and RPL5 isolates of *Leishmania (V.) braziliensis*. * $p < 0.05$ (ANOVA).