DEVELOPMENT AND EVALUATION OF SENSITIVITY OF METHODOLOGIES FOR MOLECULAR IDENTIFICATION OF Trypanosoma cruzi IN AÇAI PULP

Colares, H.C.¹; Macedo, A.M.²; Bortoleto, N.D.A.¹; Valadares, H.M.S.¹

¹Laboratório de Genética Molecular, Campus Centro Oeste Dona Lindu, Universidade Federal de São João del Rei, Divinópolis, Minas Gerais, Brazil. ²Laboratório de Genética Bioquímica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

Chagas disease, a complex disease caused by parasite Trypanosoma cruzi, is still recognized as one of the most important and neglected tropical diseases in Latin America. T. cruzi is transmitted to humans primarily through contact with faeces of infected triatomines. However, alternative routes of transmission can occur, such as blood transfusion, congenital or oral transmission. Since contaminated foods, for example, the açaí pulp, are responsible, in large part, by cases of oral transmission of Chagas disease, it is evident the importance of developing a methodology for T. cruzi identification in this food source. Thus, the aim of this work is to develop and evaluate the sensitivity of methodologies for T. cruzi identification in açaí pulp employing PCR assays directed to specific markers of parasite as minicircles from kDNA, satellite DNA of 195 base pairs, mini-exon and 24Sα rRNA genes. For this, firstly, were evaluated the sensitivity of the methods employing dilutions of parasites/mL without açaí pulp. Thus, epimastigote forms were cultured in LIT medium, counted in Neubauer chamber until the concentration of 1.10^8 parasites/mL and diluted to 8.10^7, 6.10^7, 4.10^7, 2.10^7, 1.10^7, 8.10^6, 6.10^6, 4.10^6, 2.10^6, 1.10^6, 1.10^5, 1.10^4 and 1.10^3 parasites/mL. The genomic DNA was extracted employing two different methods: phenol/chloroform/isoamylalcohol protocol and the Wizard Genomic DNA Purification Kit (Promega). After this, were performed PCR assays for all molecular markers. Preliminary results revealed that the extraction method using phenol was more efficient when compared with the commercial kit. The molecular markers that showed a higher sensitivity level were 24Sα rDNA, mini-exon, minicircles and satellite DNA, respectively. Based on these results the next steps will be artificial contamination of açaí pulp with T. cruzi dilutions, DNA extraction of parasites mixed with açaí pulp, detection of parasite DNA by PCR and evaluate the sensitivity level for each methodology.

Keywords: Trypanosoma cruzi, açaí pulp, molecular markers