Calpain in *Phytomonas serpens*: Detection and Effect of Inhibitors on Parasite Proliferation

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Since trypanosomatids like *Trypanosoma cruzi* and *Leishmania amazonensis* showed a susceptible behavior to the treatment with calpain inhibitors (CIs), the purpose of the present study was to determine the effect of CIs on the proliferation of the tomato parasite *Phytomonas serpens*, as well as to evaluate the presence of calpain homologues. The parasite was cultured either in the presence of the calpain inhibitor III (MDL28170) or the calpain inhibitor V at 10 - 70 µM. Our results showed that both CIs arrested the growth in a dose-dependent manner: CI III at 60 µM promoted a reduction in the cellular growth rate of 91% after 96 h, while CI V at 70 µM decreased the growth rate by 68%. The anti-phytomonad activity of both inhibitors was reversible, since cells pre-treated for 72 h with each CI at resumed growth when subcultured in a drug-free fresh medium. Optical microscopy of treated cells revealed an increase in the cell volume, with the flagellates becoming round. MDL28170-resistant promastigotes were obtained by exposing *P. serpens* to a gradual increase in drug concentration (up to 70 µM). Flow cytometry analysis showed that the polyclonal antibody raised against *Drosophila melanogaster* calpain was found to react with both MDL28170-sensitive parental and the resistant cells, although the antibody binding was stronger in the former. These results suggest that calpain homologues are also present in *P. serpens*, and that exposition to a CI led to altered levels of expression or secretion.

Key words: Trypanosomatid; *Phytomonas*; Peptidase; Calpain

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