

Gene expression and nutrient utilization in the basidiomycete *Moniliophthora perniciosa* suggest the existence of regulatory mechanisms of catabolite repression

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Moniliophthora perniciosa, a hemibiotrophic fungus (characterized by biotrophic and saprotrophic phases in its life cycle), is the causal agent of witches' broom disease (WBD), which negatively affects the Latin American cocoa (*Theobroma cacao*) production. The southern region of Bahia, in Brazil, had the first occurrence of WBD in 1989, which led to a great drop in cocoa production in the following years. During the biotrophic phase, the infecting fungus promotes stem enlargement, hyperplasic and hypertrophic tissue growth, loss of apical dominance, and green broom formation in *T. cacao*. During the saprotrophic phase of fungal infection, the green broom turns into a dry one, due to an intense programmed cell death that leads to necrosis of infected parts of the plant. Studies have suggested the presence of a carbon repression (CR) mechanism in *M. perniciosa*. CR is well characterized in the yeast *Saccharomyces cerevisiae* and consists in the repression or induction of a set of genes according to the type of carbon source present in the environment. *MIG1* and *SNF1* genes encode transcription factors that are involved in the regulation of the CR process. The aim of this study was to demonstrate the existence of CR and to characterize its process in *M. perniciosa* through physiological studies and analysis of gene expression. The fungus was grown in media with different glucose and glycerol concentrations, and mycelial growth, carbon and peptone consumption profile of the culture medium, pH variation, and resistance to oxidative stress generated by external hydrogen peroxide (H_2O_2) were monitored. Real-time polymerase chain reaction (qPCR) was used for expression analysis of selected genes of CR, energy metabolism, autophagy, and protection against oxidative stress agents. The main results were: i) peptone seems to be important in fungal nutrition and growth, being preferentially consumed, even in the presence of glucose or glycerol; ii) growth in medium with glycerol as the main carbon source induced a higher resistance to H_2O_2 , a slower growth of mycelia and a lower carbon consumption, as compared to glucose-supplemented medium, during 35 days of

culture; iii) increasing glucose concentrations led to a proportional delay in mycelial growth, a higher resistance to H₂O₂ and an increased expression of cytoplasmic catalase-encoding gene Mp*CTT1*; iv) there was a shift to alkaline conditions (pH >8.0) in the culture medium during growth of the fungus; v) time of culture of *M. pernicioso* did not alter the cells' H₂O₂ resistance profile; vi) there was no change in the expression of Mp*PCK* (phosphoenolpyruvate carboxykinase) and Mp*PYK* (pyruvate kinase) genes; vii) Mp*PDH* (pyruvate dehydrogenase) gene expression increased with rising glucose concentration; viii) expression of the transcription factors Mp*Cre1* and Mp*Snf1* (here shown for the first time to exist in *M. pernicioso*) was compatible with a predicted CR mechanism, as we found higher Mp*CRE1* than Mp*SNF1* gene expression in the presence of glucose, but this expression was not proportional to glucose concentration as would be expected; ix) sequencing and bioinformatics analysis of Mp*SNF1* and Mp*CRE1* PCR products revealed an amino acid identity of 68 and 77.5% to homologous Snf1p and Mig1p sequences of *S. cerevisiae*, respectively. These results show that in a complex medium, peptone is primarily consumed at the expense of a carbon source, but having the choice between glycerol and glucose, the latter is preferred. Considering all the results, the existence of a carbon repression mechanism in *M. pernicioso* cannot be ruled out. Consumption of glucose or glycerol in *M. pernicioso* is, however, not regulated in the same manner as in the yeast *S. cerevisiae*.

Key words: Carbon repression; Energy metabolism; Glucose; Glycerol; Peptone