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Variations in oxygen concentration cause differential antioxidant response and expression of related genes in *Beauveria bassiana*

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ABSTRACT

The entomopathogenic fungus *Beauveria bassiana* is widely used in pest biocontrol strategies. We evaluated both the antioxidant response mediated by compatible solutes, trehalose or mannitol, and the expression of related genes using oxygen pulses at three oxygen concentrations in solid state culture (SSC): normal atmosphere (21 % O_2), low oxygen (16 % O_2) and enriched oxygen (26 % O_2). Trehalose concentration decreased 75 % after atmospheric modifications in the cultures, whereas mannitol synthesis was threefold higher under the 16 % O_2 pulses relative to normal atmosphere (100 and 30 μg mannitol mg^{-1} biomass, respectively). Confirming this result, expression of the *mpd* gene, coding for mannitol-1-P dehydrogenase (MPD), increased up to 1.4 times after O_2 pulses. The expression of the *bbrgs1* gene, encoding a regulatory G protein related to conidiation, was analysed to explain previously reported differences in conidial production. Surprisingly, expression of *bbrgs1* decreased after atmospheric modification. Finally, principal component analysis (PCA) indicated that 83.39 % of the variability in the data could be explained by two components. This analysis corroborated the positive correlation between mannitol concentration and *mpd* gene expression, as well as the negative correlation between conidial production and *bbrgs1* gene expression. This study contributes to understanding of antioxidant and molecular response of *B. bassiana* induced under oxidant conditions.

Introduction

The entomopathogenic fungus, *Beauveria bassiana*, is used worldwide in biological control as an alternative to chemical pesticides because of its low environmental impact and high specificity, and because insects rarely develop resistance to it (Zimmermann 2007). Conidia are its most efficient infective propagules, and are produced mainly in solid state culture (SSC) on agricultural products and by-products such as rice (Neves & Alves 2000; Ye *et al.* 2006; Garza-López *et al.* 2012).

Beauveria bassiana is an aerobic organism that produces reactive oxygen species (ROS) through cellular metabolism such

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as mitochondrial respiration and NADPH oxidase activity (Aguirre et al. 2005). Cells operate both enzymatic and non-enzymatic antioxidant defence mechanisms, such as mannitol and trehalose (compatible solutes) biosynthesis, which prevent oxidation induced by ROS in biomolecules (Crowe et al. 1984; Smirnoff & Cumbes 1989; Singer & Lindquist 1998). In addition, oxidising states favour cytodifferentiation in fungal species of the genera *Neurospora*, *Aspergillus*, and *Metarhizium* (Miller et al. 2004; Aguirre et al. 2005).

Recently, studies on the effect of oxidising states induced by O_2 pulses in *B. bassiana*, *Metarhizium anisopliae*, and *Isaria fumosorosea* have shown strain-related differential effects on conidial production and on quality and infectivity of conidia (Tlecuitl-Beristain et al. 2010; Garza-López et al. 2012; Miranda-Hernández et al. 2014). Specifically, in *B. bassiana,* hypoxic pulses caused oxidative stress, increasing conidial production but with diminished conidial germination (Garza-López et al. 2012).

Quantification analysis of gene expression in entomopathogenic fungi has improved understanding of the infective processes (Fang & Bidochka 2006). In this sense, Wang et al. (2011) studied the relationship between mannitol synthesis-related genes (*mpd* and *mtd*) and multi-stress resistance in *B. bassiana*. The regulation of conidiation-related genes in filamentous fungi, including entomopathogenic fungi, is controlled by a G protein-mediated signalling pathway (Hamm 1998; Fang et al. 2007, 2008). Signalling through an α subunit (Gα) plays a role in regulating the balance between hyphal vegetative growth and conidiation. Regulatory G protein-signalling (RGS) proteins interact with Gα, deactivating the signal and promoting the conidiation process (Wang et al. 2013). Disruption of orthologous genes *cag8* and *bbrgs1*, which encode RGS proteins in *M. anisopliae* and *B. bassiana* respectively, reduced conidiation and virulence in these species (Fang et al. 2007, 2008).

The aim of this study was to determine the effects of different concentrations of O_2 pulses on the antioxidant response associated with trehalose or mannitol, in addition to the differential expression of related genes (*mpd* and *bbrgs1*) in *B. bassiana*.

Materials and methods

Microorganisms

The Bb 882.5 strain of *Beauveria bassiana* was used, previously isolated and described by Robledo-Monterrubio et al. (2009). This strain belongs to the fungal collection of the Universidad Autónoma Metropolitana Iztapalapa and was deposited in the ENCB-IPN (México, D. F., México) culture collection with the identification name ENCB-MG-80. Propagation was carried out in 250 ml Erlenmeyer flasks containing 50 ml of modified Sabouraud maltose agar (2 %), previously sterilised at 15 PSI for 15 min (Garza-López et al. 2012). The flasks were incubated for 8 d at 28 ± 1 °C.

Solid state culture

Serological bottles (total volume 75 ml) were used as experimental units, each containing 5 g of pre-cooked rice sterilised at 15 PSI for 15 min. Conidial suspensions were prepared, adding 20 ml of a sterile Tween 80 (Amresco, USA) (0.05 %) solution to the Erlenmeyer flasks used in the propagation. The conidial count was determined under a microscope (BM-180, Boeco, Germany) using a Neubauer chamber (Marienfeld-Superior, Germany), and the suspension was diluted to obtain a concentration of 5×10^6 conidia per millilitre (con ml^{-1}). Finally, 1 ml of this suspension was added to each bottle to obtain a final concentration of 1×10^6 conidia per gram of initial dry substrate (con gds^{-1}) and the necessary amount of a sterile solution of yeast extract (0.5 g l^{-1}) to obtain 40 % of initial moisture content.

Modified atmospheres

Three atmospheric conditions were used: normal atmosphere (NA, 21 % O_2), low oxygen (16 %) and enriched oxygen (26 %). The gaseous mixtures (16 % O_2 and 26 % O_2) were manufactured and standardised by the Praxair Company (Mexico). All experiments began with a pre-stationary phase in which the experimental units were loosely closed with cotton plugs to allow continuous gas exchange with the external environment. After 3 d of culture, hermetic rubber seals were placed on the bottles to be subjected to the 16 % and 26 % O_2 pulses, then the air in the bottles was completely flushed and replaced with the appropriate atmospheric treatment. This procedure was carried out as previously described by Garza-López et al. (2012). Three replicates of each treatment were sampled every 24 h. Biomass and conidial samples were collected after adding 20 ml of Tween 80 (0.05 %) to the serological bottles and stirring using a magnetic stirrer for 10 min.

Determination of trehalose and mannitol concentration

Compatible solutes were extracted according to the methods described by Hallsworth & Magan (1994), adding 1 ml of biomass and conidial suspension, obtained as described previously, into 1.5 ml Eppendorf tubes. Samples were centrifuged using 0.5 μm microfilters (Millipore, USA) for 5 min at 8000 ×*g* (MiniSpin, Eppendorf, Germany) to eliminate protein residues, then analysed using high performance liquid chromatography (HPLC), with an Aminex HPX-42A column (Bio-Rad, USA) attached to an Agilent 1260 chromatograph (Agilent, USA). Retention times were 15.7 min and 21.5 min for trehalose and mannitol, respectively. Concentrations of both solutes, trehalose and mannitol, were expressed as micrograms of solute per milligram of biomass (μg solute mg^{-1} biomass). Biomass was determined by difference of dry weight obtained by filtration, using 0.45 μm nylon membranes (Millipore, USA). According to the results obtained, we chose to analyse the differential expression of the encoding gene for mannitol-1-phosphate dehydrogenase (*mpd* gene).

Differential expression of mpd and bbrgs1 genes

In addition to *mpd* gene expression analysis, expression of *bbrgs1* gene was also determined. Samples were collected at 3, 4 and 6 d of culture, corresponding to the beginning of atmospheric modification, 24 h later and the time by which *Beauveria bassiana* had shown signs of oxidative stress as reported by