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Temperature Effect on *Rhizoctonia solani* Analyzed by Microcalorimetry

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ABSTRACT

Temperature is one of the factors playing an important role in fungi growth and spread. The aim of this study was to determine the effect of temperature on the growth of ten *Rhizoctonia solani* isolates. Colony Growth Rate (GR) was measured in potato-agar-dextrose cultures and Metabolic Efficiency (ME) by isothermal microcalorimetry in *R. solani* isolates growing in a temperature range of 10 to 40°C at 5°C intervals. The apparent activation Energy (Ea) was determined in the range of 15 to 30°C. Different values of Ea were found for each of the strains analyzed. GR increased as temperature increased up to 30°C, showing the highest values between 25°C. ME decreased as temperature increased in nine out of ten isolates, reaching an optimum for the different isolates between 15 and 25°C. Both GR and ME analyses showed different behaviors for each isolate. *R. solani* AG4 isolates showed a variable response to the same temperature of exposure and it appears that microcalorimetry is more sensitive in detecting early effects of heat stress.

Keywords: *Rhizoctonia Solani*, Growth Rate, Microcalorimetry, Metabolic Heat

1. INTRODUCTION

Rhizoctonia solani is a soil fungus with a worldwide distribution that attacks a wide range of hosts under different environmental conditions. This fungus is considered a complex species composed of individuals that showed differences in morphological, physiological and genetic characteristics. They are grouped according to their compatibility reaction in hyphal Anastomosis Groups (AG) (Tsror, 2010). It has been observed that a single host can be infected by individuals of different AG and that these vary according to the region where they develop. This behavior is attributed to the temperature effect on the pathogen distribution, growth and persistence (Goswami *et al*., 2011).

The temperature effect on fungi metabolic activity has been analyzed based on substrate mass loss, fungal biomass increase, hyphae linear growth, ergosterol content, CO_2 production and oxygen consumption, among others (Bjurman and Wadso, 2000). However, considering that all of them evaluate different phenomena related to fungal metabolic activity; it is difficult to select the best indicator. Since the energy required to maintain metabolic processes generates heat, then, its determination is a direct and objective indicator of the level of the organism activity. Metabolic heat determination can be accomplished by isothermal Microcalorimetry (MC) techniques, which have been used in a variety of microbiological studies focused on soil (Critter *et al*., 2001; Vor *et al*., 2002; Barros *et al*., 2003) and food (Gardea *et al*., 2002) organisms, as well as bacteria and yeast (Critter *et al*., 2004; Menert *et al*., 2004).

Calorimetry of living systems is based on the first law of thermodynamics, considering the individual or a population as an open system that exchanges matter and energy with their environment. When a body is living

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under constant temperature and pressure, there is no loss of energy for work performed and under those conditions the heat generated by such living system is equal to the enthalpy change associated with metabolic changes undergoing at that time. If the metabolic process is constant, the heat released is proportional to the growth and nutrient intake (Gustafsson, 1991).

Bjurman and Wadso (2000) conducted a MC study of the metabolic activity based on the temperature of six wood-degrading fungi and concluded that the observed effect of this factor on metabolism is consistent with the behavior determined by other methods, with the advantage that MC allows monitoring of fungal activity in real time. Since the data provided by this technique is related with the general behavior of metabolism, they recommended further studies with more specific methodologies, especially when dealing with unfamiliar processes.

Previous experiments conducted by our research group (Meza-Moller *et al*., 2007) studied the pathogenicity, anastomosis reaction and morphological and genetic variability of *Rhizoctonia solani* strains isolated from the rhizosphere of *Vitis vinifera* L. var. 'Perlette' in two of the most important table grape producing regions of Sonora Mexico, where summer high temperatures are within a range from high 40's to low 50's°C.

In an attempt to learn more about the behavior of this pathogen under heat stress, the present study was conducted to determine the influence of temperature on the growth and metabolic activity of *Rhizoctonia solani* isolates by growth on plate and microcalorimetry.

2. MATERIALS AND METHODS

2.1. Sample Preparation

The analysis was performed with *Rhizoctonia solani* strains from each of the anastomosis groups AG1 and AG4 isolates from chilli pepper crops in Jalisco, Mexico and 8 strains of AG4, identified as M99, M109, M131, M142, M166, M189, M200 and M211, isolated from the rhizosphere of *Vitis vinifera* L. var. 'Perlette' grown in Sonora, Mexico (Meza-Moller *et al*., 2007). Each isolate was developed in glucose-yeast-extract agar in 90 mm Petri dishes at 25°C for 24 h. After completing this period, from the colony edge 8×5 mm cylinders were taken, placed in sterile Petri dishes and incubated at 25°C for 12 to 18 h, allowing mycelia to fully invade cylinders.

2.2. Growth Rate on Plate

The ten isolated strains were inoculated one by one in Petri dishes with potato-dextrose-agar and incubated at 10, 15, 20, 25, 30, 35 and 40°C. Mycelial growth diameter was recorded every 12 h from 12 to 96 h after inoculation in two perpendicular directions. Growth rate was determined by a linear regression analysis (Harikrishnan and Yang, 2004).

2.3. Isothermal Calorimetry

The isothermal calorimetry was done with a CSC 4100 differential scanning calorimeter (Calorimeter Science Corporation, Pleasant Grove, Utah). The instrument has four 1 cm^3 hermetic cells, one of which is used as reference. To prevent moisture condensation inside the instrument, a N_2 flux at constant pressure of 1.75 kg cm^{-2} was kept. Chamber temperature was maintained at 15°C with a cool-circulating bath (PolyScience, Niles, Illinois, USA). In each of the three cells, a cylinder of culture medium covered with micellium was placed, while the reference cell kept a non-inoculated culture medium cylinder as control. Temperature effect on isolates metabolic activity was determined by the apparent activation Energy (Ea), which was calculated from the linear section of the graph plotting temperature against metabolic heat production in an Arrhenius fashion (Criddle *et al*., 1990; Carbajal-Millan *et al*., 2000). The metabolic efficiency or amount of heat generated per mol of CO_2 formed (Rq/RCO_2, $KJmoL^{-1}$) was calculated from the reported metabolic heat in Watts per milligram of dry weight (q, Wmg^{-1} dw) divided by the production of CO_2 (RCO_2, nmoL sec^{-1}) (Criddle *et al*., 1990; Hansen *et al*., 1998). Heat and CO_2 production was estimated at 5°C intervals, ranging from 10 to 40°C at intervals of 2000 sec for each temperature. For data adjustment, a baseline was determined for each temperature treatment, by generating respective isotherms with culture medium in the four cells. Sample dry weights were obtained by heating the cylinder of medium including the fungus mycelium in 100 mL of boiling water to eliminate agar. The mycelium obtained was dried at 60°C for 30 min. The data were fitted with their respective baselines and normalized based on the sample dry weight.

2.4. Statistical Analysis

Each isolate data were independently analyzed as a completely randomized design, with six replicates in the calorimetric study and three for the on-plate growth. The significance level was set at 5%. When statistical significant differences were found, Tuckey's comparison

of means was performed. All the analysis were performed using SAS version 6.08 (SAS, 1994).

3. RESULTS

3.1. Rate of Growth on Plate

The growth rate of all isolates was affected by changes in temperature. The greater effect was observed by increasing the temperature from 20 to 25°C. At 30°C, only isolates AG1, M99, M109, 166, M189 and M211 significantly increased their growth rate. It was also noted that when isolates were subjected to a temperature of 35°C, a decrease in growth was found, with variable behavior depending on the strain. Growth was neglectable at 40°C (**Table 1**).

3.2. Isothermal Calorimetry

Table 2 shows that all strains analyzed demonstrated different Ea values with a wide range from 21 up to 99 Joules*mol °K^{-1}. **Figure 1** shows the metabolic efficiency of the isolates analyzed at various temperatures. In the same graph the Thornton's constant (455±15 kJ moL^{-1}) is shown (Criddle *et al*., 1990; Hansen *et al*., 1998), which is used as a criterion to classify metabolic efficiency. In this context, values higher than this constant are considered at low-efficiency, while values less than or equal to the constant are determined as of high-metabolic efficiency.

While the optimum temperature for isolate growth, determined by both on-plate growth and by the Arrhenius plot was found between 15 and 30°C, when determining the metabolic efficiency (q/R_{CO2}) of the isolates, they showed a different pattern of carbon substrate conversion to structural biomass in response to changes in temperature.

Metabolic efficiency of most of the isolates increased significantly from 15 to 25°C. However, in this temperature range, the M131 isolate lost efficiency at 20°C, while isolates M142 and M166 showed low metabolic efficiency at 25°C. Also, it was observed that above 30°C most of the isolates exhibited a low metabolic efficiency, being undetectable in some cases (strains AG1 and M142), exception being isolate M200, which showed a high metabolic efficiency up to 40°C.

Table 1. Growth rate (mm h^{-1}) of *Rhizoctonia solani* at different temperatures[a]

	Temperatures						
Isolate	10º	15º	20º	25º	30º	35º	40º
AG1	0.03 e	0.04 d	0.08 c	0.16 b	0.18 a	0.01 f	0.00 f
AG4	0.03 c	0.03 c	0.10 b	0.15 a	0.16 a	0.04 c	0.01 d
M99	0.00 f	0.02 e	0.06 d	0.10 c	0.16 a	0.11 b	0.00 f
M109	0.01 e	0.02 d	0.08 c	0.14 b	0.16 a	0.08 c	0.00 f
M131	0.02 de	0.02 d	0.08 c	0.16 a	0.17 a	0.12 b	0.00 e
M142	0.02 d	0.03 c	0.08 b	0.14 a	0.14 a	0.02 d	0.00 e
M166	0.01 e	0.01 ef	0.07 d	0.13 b	0.15 a	0.11 c	0.00 f
M189	0.01 e	0.04 d	0.06 c	0.08 b	0.10 a	0.08 b	0.00 f
M200	0.03 c	0.02 c	0.06 b	0.10 a	0.10 a	0.02 c	0.00 d
M211	0.01 f	0.02 e	0.06 d	0.09 b	0.10 a	0.08 c	0.00 g

[a]Means followed with same letter along the same row are statistically equal by Tukey ($p>0.05$). Growth rates were calculated by regression analyses

Table 2. Apparent activation energy (Ea) of *Rhizoctonia solani* isolates determined in the range of 15 to 30°C

Isolates of *Rhizoctonia solani*	Apparent activation energy (Joules * mol ºK^{-1})
AG-1	21
AG-4	53
M109	77
M200	46
M99	92
M211	99
M131	43
M142	65
M166	31
M189	95

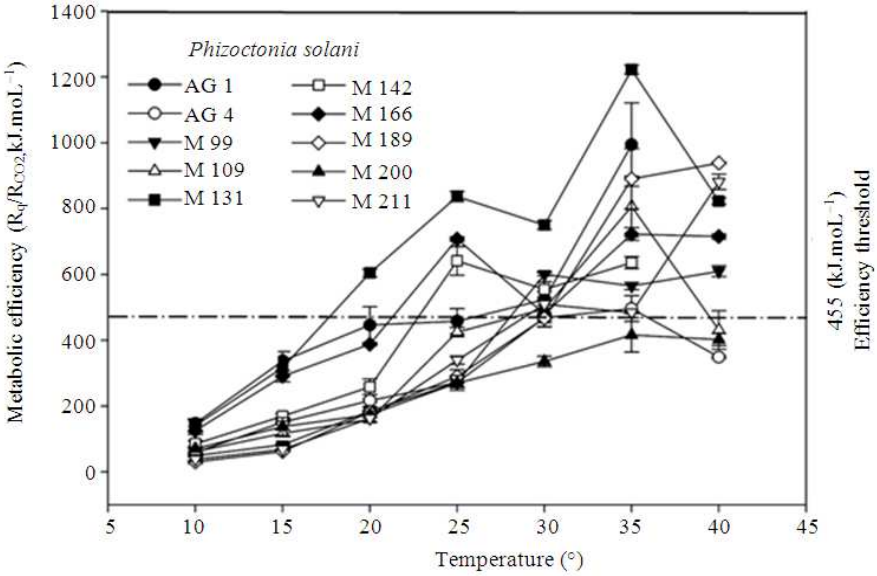

Fig. 1. Temperature effect on metabolic efficiency (q/R_{CO2}) of *Rhizoctonia solani* isolates. The dotted line in the graph shows the Thornton constant value. Means are the average of 6 replicates and vertical lines correspond to the standard deviations

4. DISCUSSION

Although the results of the on-plate growth rate, apparent activation energy and metabolic heat production of the *R. solani* isolates indicated that the optimum growth temperature lays between 15 and 30°C, the metabolic efficiency analysis showed that the optimum temperature for most of the isolates is actually between 15 and 25°C, since at temperatures above 30°C, isolates increased energy expenditure to incorporate CO_2 into their biomass structure. Similar results were reported with five isolates of *R. solani* representing five clusters group. The rates of growth and sclerotial formation were not uniform at different levels of temperature and pH, but the maximum mycelial growth of all isolates was found at 30°C (Goswami *et al*., 2011).

In response to severe environmental stress conditions, all available energy in the organism is used to respond to increasing demands for maintenance processes and as a result it produces a lack of energy for biomass synthesis (Gustafsson, 1991). According to this explanation, the growth of isolates at temperatures at or above 30 °C was minimal and clearly there was a greater loss of energy as heat, which supports the explanation that little energy was transferred to biomass.

The anastomosis group AG4 is characteristic from warm climates (Tsror, 2010); therefore, this feature may explain the results on metabolic efficiency at high temperatures found in strain M200. Even though all evaluated strains belonged to AG4 (Meza-Moller *et al*., 2007), the variation in the pattern of metabolic efficiency of the isolates could be related to the large genetic variability (Goswami *et al*., 2011; Strausbaugh *et al*., 2011).

With the exception of strain AG4 from temperate regions in Mexico (Lopez-Olmos *et al*., 2005), all the isolates evaluated in this assay are part of the rhizosphere of *V. vinifera* L. var. 'Perlette' growing in the soils of the Sonoran Desert. Furthermore, all of them infected grapevine roots. Considering that the isolates are from a region characterized by a dry, warm climate with an average annual temperature of 22°C and a maximum monthly average of 37°C in July and August, with daily temperature up to 47°C in summer and in winter with a monthly average temperature of 12°C (with little chances of frosts), it can be predicted that several of the strains analyzed in this study could be potentially active throughout the year.

5. CONCLUSION

Although the growth rate of *R. solani* AG4 was favored between 15 and 30°C, in an overall fashion, metabolism was more efficient between 15 and 25°C, depending on the isolate. In general, temperatures above 30°C reduced metabolic efficiency. The optimum growth temperatures for *R. solani* isolates was different, as determined by microcalorimetry and plate growth. However, microcalorimetry would be more sensitive in

detecting early effects of heat stress and because of it; an optimum growth range at lower temperature was determined by this technique. Based on the variabiliy observed, seems clear that anastomosis groups include genotyps with a wide variety of physiological responses due to temperature conditions.

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