AGROSAVIA

Corporación colombiana de investigación agropecuaria

El campo es de todos | Minagricultura

Effect of abiotic factors on viability and characterization of *Metarhizium rileyi* Nm017

Johana Sanabria[1], Ginna Quiroga[1]*, Cindy Mejía[2], Erika Grijalba[1], Martha Gómez[1]

[1] Corporación colombiana de investigación agropecuaria AGROSAVIA, Depto de Bioproductos, Sede Central, Kilómetro 14 vía Mosquera-Bogotá, Cundinamarca, Colombia, 250040
[2] Corporación colombiana de investigación agropecuaria AGROSAVIA, Centro de Investigación Tibaitatá, Kilómetro 14 vía Mosquera-Bogotá, Cundinamarca, Colombia, 250040
* gquiroga@agrosavia.co

Introducción

The species *Chloridea virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae) are declared agricultural pests with a high economic impact worldwide (Angulo *et al*. 2008). They are widely distributed on the American continent, and agrochemical are the most common method to control, which can cause environmental, social, economic and public impacts. A strain of *Metarhizium rileyi* Nm017 [AGROSAVIA - Orinoquia area (Col.)], demonstrated an efficacy of 75.8% on *C. virescens*, and 92.5% on *H. zea* on laboratory conditions. Mass production and virulence of *Metarhizium* sp. are susceptible to stress conditions such as temperature, UVB radiation and pH, affecting conidial vigor, germination, and sporulation (Rangel *et al*. 2008, Oliveira *et al*. 2016). Likewise, the culture medium can affect the infection processes measured through hydrophobicity and enzymatic activities (Ortiz 2013). The identification of these parameters allows selecting the most favorable conditions for its production and the challenges that must be assumed in downstream processes.

Keywords: *Metarhizium rileyi*, ecophysiological characterization, hydrophobicity, enzymatic activities

Material and methods

1. Ecophysiological characterization

M. rileyi Nm017

Conidial suspension (10^8 conidia/mL)

UVB radiation 10^8 conidia/mL

Treatment	Exposure Time in laboratory*	Field equivalency time **
T1	2 min 6 sec	15 min
T2	4 min 12 sec	30 min
T3	8 min 25 sec	1 h
T4	12 min 37 sec	1.5 h
T5	16 min 50 sec	2 h

*UVB lamp with 302nm wavelength, radiation 0.28kW/m²
** 10% max. global solar radiation Orinoquia (Col.) = 0.45kWh/m² (IDEAM, 2018)

pH 10^8 conidia/mL

Growth temperature 10^7 conidia/mL

Treatment	
pH:	4, 5, 6, 7, 8
Temperature:	20°C, 25°C, 30°C, 35°C

2. Hydrophobicity and enzymatic activities

Conidia production:
- YM and SMAY
- 25±2°C; 7 days

2.1. Hydrophobicity

3mL Aqueous phase: Conidia + KNO_3 0.1M

1mL Organic phase: Toluene 98%

$$H = \frac{A_0 - A_f}{A_f} 100$$

H: hydrophobicity
A_0: aqueous phase absorbance (600nm)
A_f: mixture absorbance

2.2. Enzymatic activities

Conidial suspension → Supernatant recovery → Analytical analysis

Enzymes	Substrates
NAGase (Santos, 2014)	p-nitrofenil-N acetylglucosamine
Protease (Cupp-Enyard, 2008)	Casein
Lipase (Pignede *et al*. 2000)	Olive oil

3. Response variables

Ecophysiological characterization:
Conidial vigor and germination (%)
Sporulation (conidia/cm²)

Hydrophobicity:
Relative hydrofobicity (%)

Enzymatic Activities: Enzyme unit per dry conidia (IU/g)
Absorbance: NAGase (400nm) and protease (660nm)
Titration with NaOH: Lipase

Completely randomized experimental designs with three replications. Mean Comparisons (Tukey or Kruskal-Wallis test, 95%) used for statistical analysis

Results and discussion

1. Ecophysiological characterization

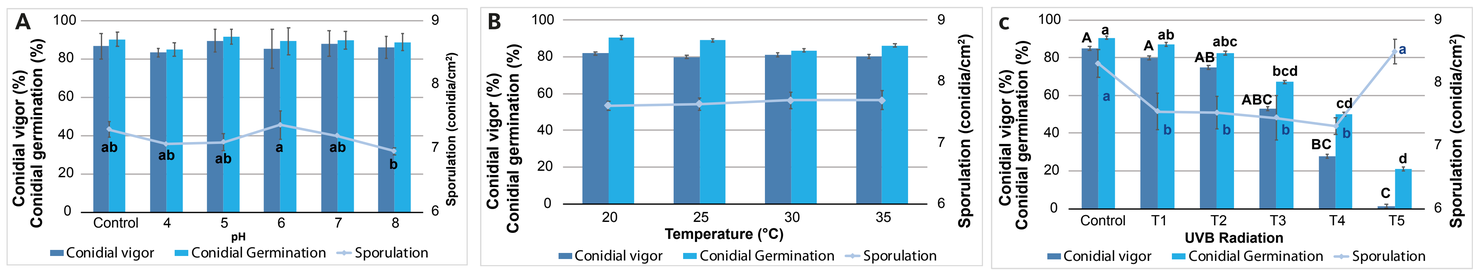

Fig. 1. Response variables: A pH; B Growth temperature; C UVB radiation. NOTE: Response variables with equivalent or without letters did not show statistical differences (Tukey/Kruskall-Wallis test, p>0.05)

- No significative variations in conidial vigor and germination were observed at several pH and temperatures. Germination was strongly affected by UVB radiation.
- The sporulation values did not present variation respect to the control treatment at different pH and temperatures. Instead, UVB radiation exposure caused wide deviations of the sporulation values between treatments. Nonetheless, treatment T6 (the highest exposure time) showed a similar value as control, apparently the exposure stimulates the fungus sporulation. High sensibility to UVB radiation in entomopathogenic fungi is a remarkable disadvantage for biopesticides development.
- This could be overcome with the formulation process of conidia by improving the tolerance to UVB with biocompatible substances (Wenzel *et al*. 2016; Rangel *et al*. 2018)

2. Hydrophobycity and enzymatic activities

Table 1. Enzymatic characterization of M. rileyi Nm017. NOTE: Equivalent letters within columns did not show statistical differences (Tukey/Kruskall-Wallis test, p>0.05).

Production medium	Hidrophobicity (%)	NAGase (IU/g conidia)	Protease (IU/g conidia)	Lipase (IU/g conidia)
YM	92.8 ± 1.2 (a)	3.47 ± 0.15 (b)	17.75 ± 3.29 (b)	8.33 ± 2.08 (a)
SMAY	83.7 ± 2.0 (b)	13.18 ± 0.94 (a)	43.92 ± 5.96 (a)	2.78 ± 1.20 (b)

- In all the response variables evaluated, significative differences were observed between the two-production medium.
- The conidia showed an elevated hydrophobicity in both cases.
- The higher NAGase and total protease activities were obtained from conidia harvested in SMAY. For instance, lipase activity was by 200% greater in YM.
- These variables are signs of the fungi virulence mechanism. Hydrophobicity is an indicator of hydrophobins synthesis, which are amphipathic proteins involved in the adhesion of conidia to insect's cuticle. These enzymes are implicated in the cuticle hydrolysis (Villamizar *et al*. 2001; Ortiz 2013; Mondal *et al* 2016).
- Hydrophobins and enzymes production are influenced by fermentation conditions, especially by nutritional conditions such as C:N ratio (Shah *et al*. 2005; Holder *et al*. 2007; Safavi *et al*. 2007).

Conclusions

- *M. rileyi* Nm017 easily tolerate growth temperature (20 to 35°C), and a pH (4 to 8). However, the fungus was susceptible to UVB radiation greater than 4min or a field equivalent time of 30min. Formulation strategies could be applied to improve its resistance to UVB radiation.
- The composition of the production medium had a notable influence on the hydrophobicity, enzymatic activities, and hence, virulence

Acknowledgements

The authors would like to thank the Ministerio de Agricultura y Desarrollo Rural of Colombia for the financial support to this project

Bibliography

-Angulo A, *et al*. 2008. Universidad de Concepción, Concepción, 154pp
-Cupp-Enyard C. 2008. JoVE. 19. -Loera-Gallardo J, López-Arroyo, J, Reyes-Rosas M. 2008 Casos de control biológico en México, 57-74pp
-Holder D, *et al*. 2007. Microbiology, 153, 3448-3457pp
-IDEAM. 2018. Atlas de radiación
-Mondal S, *et al*. 2016. Pacific Sci Rev, 18(2), 85-99pp
-Oliveira M, *et al*. 2016. Arquivos do Instituto Biológico, 83: 1-7pp
-Ortiz A, Keyhani N. 2013. Insects, 4(3), 357-374pp
-Pignède G, *et al*. 2000. Journal of Bacteriology 182 (10):2802–2810pp
-Rangel D. 2006. PhD Thesis, Utah State University, 282pp
-Rangel D, *et al*. 2008. Mycological Research 112 (11).: 1362–1372pp
-Safavi S, *et al*. 2007. FEMS Microbiology Letters, 270(1), 116-123pp
-Santos A. 2014. Master Thesis. Universidad Nacional de Colombia, 137pp
-Shah F, *et al*. 2007. FEMS Microbiology Letters 276 (1):60–66pp
-Wenzel L, *et al*. 2016. Adv Entomol, 4: 151-162pp