Characterization of growth and chlamydospores production of a novel Colombian strain of nematophagous fungi Duddingtonia flagrans

Carlos Castillo-Saldarriaga 1,*, Johana Sanabria 1, Andrés Cubides 2, Dildo Márquez 2, Martha Gómez-Álvarez 1

1 Department of Bioproducts, Colombian Corporation of Agricultural Research – Corpoica, Km 14 vía Mosquera, 250047, Colombia
2 Nematophagous Fungi Laboratory, Nadalita Research Center, Colombian Corporation of Agricultural Research – Corpoica, Km 14 vía Mosquera, 250047, Colombia
*Email: crcastillo@corpoica.org.co

INTRODUCTION

Nematophagous fungi had been explored as an alternative to chemical products to control nematodes in grasslands. In CORPOICA, an isolated strain of Duddingtonia flagrans has shown a great potential controlling nematodes; nevertheless, there is no clear information about growth conditions and chlamydospores production system through fermentation. Therefore, the objective of this study was to evaluate the influence of two physicochemical conditions on radial growth rate of D. flagrans and chlamydospores production through solid fermentation.

MATERIALS AND METHODS

Physicochemical conditions effect on D. flagrans growth.

As is shown in Scheme 1, four pH levels (4, 5, 7, 8 and 9) and five temperatures (25 °C, 28 °C, 32 °C, 35 °C, 37 °C) were evaluated using a completely randomized experimental design on Wheat Flour Agar (WFA). Measurements of radial growth were conducted daily and radial growth rate was computed [1]. In vitro predatory capacity on free-living nematodes was determined under previously selected physicochemical conditions to guarantee the biological activity of D. flagrans according to Braga et al., 2009.

Fermentation assays.

Two-step fermentation was done: (1) inoculum production through liquid fermentation and (2) chlamydospores production in a solid-state fermentation, as is seen on Scheme 2. Two substrates were evaluated: rice and barley. Solid-state fermentations were conducted on aluminum pans at 20±2 °C during 21 days without moisture control. Each 7 days, chlamydospores concentration were evaluated: rice and barley. Solid-state fermentations were conducted on aluminum pans chlamydospores production in a solid-state fermentation, as is seen on Scheme 2. Two substrates were evaluated: rice and barley. Solid-state fermentations were conducted on aluminum pans at 20±2 °C during 21 days without moisture control. Each 7 days, chlamydospores concentration were evaluated: rice and barley.

RESULTS AND DISCUSSION

Radial growth rate of D. flagrans.

As is seen in Fig. 1, independently of pH values, the temperature that favored radial growth rate of D. flagrans was 28 °C. When the temperature increased to 32 °C, the radial growth rate was reduced in all pH values evaluated except for 9. However, at 35 °C and 37 °C, growth was not observed in any pH values. Based on radial growth rate value, the most favorable conditions to produce D. flagrans mycelia in WFA was 28 °C and pH 7 with a radial growth rate of 50 cm²/day.

Wheat Flour Agar (WFA) pH vs. T

(*) Average radial growth rate was computed
(*) Daily measurements

\[ \frac{dx}{dt} = \frac{\text{Area of a circle (cm²)}}{\text{Time (days)}} \]

Scheme 1. Protocol used to compute the radial growth rate of D. flagrans.

Two-step solid fermentation results.

Solid fermentation was inoculated with 20-mL of inoculum with a mycelial biomass concentration of 2.64 ± 0.19 g/L using an atomization system. As is shown in Fig. 2, for both substrates, the highest chlamydospores concentration was achieved after 14 days of incubation, 3.25x10⁷ CU/g and 2.33x10⁷ CU/g to barley and rice, respectively, with no significant increase in subsequent days.

Wheat Flour Agar (WFA)
After 7 days at 28 ± 2 °C

(*) Mycelial production 100 rpm x 25 °C x 7 d

Scheme 2. Two-step fermentation of D. flagrans.

The biological activity of the fungus under the selected conditions in terms of predatory capacity was 90 ± 4 % over free-living nematodes.

Figure 2. Chlamydospores and conidia concentration (CU/g) of D. flagrans in rice and barley after 21 days of fermentation.

CONCLUSION

The conditions established on Petri dishes used for inoculum production and solid fermentation applying barley or rice as a substrate could be used to produce D. flagrans chlamydospores, however, new substrates and different fermentation systems should be evaluated in order to select the most promising in terms of chlamydospores production and productivity.

REFERENCES


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