

Biochemical characterization of *Pochonia chlamydosporia* Q30 as biocontrol agent and plant growth promoter

Caracterización bioquímica de *Pochonia chlamydosporia* Q30 como agente de biocontrol y promotor del crecimiento vegetal

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Abstract

Pochonia chlamydosporia is a natural soil fungi, which in recent years has gained importance for its ability to nematodes biocontrol and its beneficial plant interaction. The biochemical characterization of new isolates is important to determine their potential as biocontrol agent and plant growth promoter. In the present work, through the use of specific culture media, it was found that *Pochonia chlamydosporia* Q30 possesses chitinolytic activity, which could involve in the degradation of the egg wall and the cuticle of the juvenile nematode stages, amilolytic and pectinolytic activity related to root colonization as endophyte was also found. Finally, phosphate solubilization (242.6 ± 27.1 mg/L) and indoleacetic acid production (10.0 ± 1.2 mg/L) were detected as plant growth promoting properties, which were later verified in watermelon seedlings, whose inoculation with 10^5 chlamydospores, increased the height and the stem diameter in a 13 and 6 %, respectively. In conclusion, *P. chlamydosporia* Q30 has potential to be tested as biocontrol agent for nematodes and plant growth promoter.

Resumen

Pochonia chlamydosporia es un hongo natural del suelo, que en años recientes ha cobrado importancia por su capacidad en el biocontrol de nematodos y su interacción benéfica con las plantas. La caracterización bioquímica de nuevos aislados es fundamental para determinar su potencial como agente de biocontrol y como promotor del crecimiento de plantas. En el presente trabajo, mediante el empleo de medios de cultivos específicos, se encontró que *Pochonia chlamydosporia* Q30 posee actividad quitinolítica, la cual podría estar involucrada en la degradación de la pared de los huevos y la cutícula de las fases juveniles de los nematodos, también se registraron actividades amilolítica y pectinolítica, relacionadas con la colonización de la raíz como endófito. Finalmente, se detectó la solubilización de fosfatos (242.6 ± 27.1 mg/L) y la producción de ácido indolacético (10.0 ± 1.2 mg/L) como propiedades promotoras del crecimiento vegetal, lo cual posteriormente fue comprobado en plántulas de sandía, cuya inoculación con 10^5 clamidiosporas, incremento la altura y el diámetro del tallo en un 13 y 6 %, respectivamente. En conclusión, *P. chlamydosporia* Q30 posee potencial para ser ensayado como agente de biocontrol de nematodos y como promotor de crecimiento vegetal.

Indoleacetic acid, Nematodes, Endophyte

Ácido indolacético, Nematodos, Endófito

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Introduction

Once the germination of the seeds in the soil begins, the root is quickly colonized by microorganisms, attracted by the exudate radicals, from here begin the plant-microorganism relationships that have been studied in recent decades, these relationships can be neutral, beneficial or pathogenic (Nelson et al., 2018).

Among the beneficial relationships are those where the microorganisms protect the plant against parasitism and infections produced by bacteria, fungi and nematodes, the latter penetrate the roots, using them as niches where they complete their development and sexual cycle, producing tumors and galls, which affect the development of the host plant. Nematodes are non-segmented worms that are part of the soil microfauna and, that come to affect crops of agricultural importance such as chili, cucumber, tomato and eggplant among others (Carrillo et al., 2000).

Among the antagonistic agents, it has been identified *Pochonia chlamydosporia*, which is a natural soil fungus that has shown biocontrol effect against nematodes of the genus *Globodera*, *Heterodera*, *Meloidogyne*, *Nacobbus* and *Rotylenchulus*. This fungus penetrates the roots of the plants in order to parasitize the eggs of the nematodes (Manzanilla et al., 2013).

However, the ability of *P. chlamydosporia* to promote host plant growth has also been reported, making it a versatile biocontrol agent (Dalle-Mole et al., 2015). Biochemical characterization of new isolates of this species is necessary to exploit its full agronomic potential. In the present trial, the *Pochonia chlamydosporia* Q30 strain provided with SINQUIMICA SA de CV was characterized through biochemical tests in order to determine the presence of activities related to biological control and plant growth promotion.

Methodology to be developed

Phosphate solubilization

In order to determine the capacity to solubilize insoluble calcium phosphates, the liquid medium of Pikovskaya containing insoluble tricalcium phosphate was used as the only source of phosphorus (Nautiyal, 1999), in which active mycelium of the fungus was inoculated, being left to grow for 7 days at 25 °C with constant agitation at 150 rpm.

Then, the mycelium was filtered, and in the supernatant the soluble phosphorus was determined by spectrophotometry at 882 nm according to the molybdenum blue method (Ben et al., 2009).

Production of indolacetic acid (AIA)

The determination of AIA production was performed by growing *P. chlamydosporia* in Czapek Dox liquid medium with tryptophan (2 mM) for 6 days at 25 °C with constant agitation at 150 rpm. Then, cultures were centrifuged at 4000 rpm for 20 min and, the supernatant was subjected to fractionated extractions with ethyl acetate to recover the AIA produced, the solvent was then recovered using a rotavapor, and the residue was resuspended in methanol, being then mixed with Salwoski's reagent to quantify the indolent compounds at 530 nm by spectrophotometry (Luna et al., 2013; Patel et al., 2018).

Chitin degradation

To determine chitinolytic activity, a disk of active mycelium of *P. chlamydosporia* was inoculated in triplicate into a modified liquid chitin medium containing (g/L): colloidal chitin 2.0, (NH₄)₂SO₄ 1.0, K₂HPO₄ 0.1, MgSO₄·7H₂O 0.01 and, TSB 0.05. Cultures were kept at 25 °C for 7 days with constant agitation at 150 rpm. Mycelial growth of the fungus was considered positive for this activity (Halimahtussadiyah et al., 2017).

Starch and pectin degradation

Amylolytic and pectinolytic activities were tested by sowing a disk of active mycelium in the center of plates containing 1% starch agar and 1% pectin agar.

Then, they were incubated for 7 days at 28 °C. At the end, the plates were developed using a 0.5% lugol solution. Clarification of the area surrounding mycelial growth was considered positive for each of the activities (Guzmán et al., 2014).

Chlamydospore production

To obtain the spores that were used for the plant growth promotion trial, solid culture medium was prepared in 250 mL flasks, in which 20 g of washed, sifted (1 mm) and sterilized sand were placed, together with 20 g of sifted rice flour (mesh no. 70). The mixture was sterilized and cooled to room temperature, where 40 mL of sterile water and 10 mL of PDB medium where *P. chlamydosporia* had been previously grown for 7 days were added. Cultures were incubated at 18 °C for three weeks. At the end, 50 mL of a sterile 0.05% Tween solution were added to each flask to remove chlamydospores, the mixture was filtered through sieves with several openings to eliminate the substrates, and finally the chlamydospores were quantified in a microscope using a Neubauer camera (Silva et al., 2017).

Plant growth promotion trials

To evaluate the plant growth promoting effect of *P. chlamydosporia*, inoculation trials were carried out in watermelon seedlings, as a model plant. For this purpose, seeds were germinated in polystyrene trays that contained peat moss as substrate, after germination, plants were fertilized with a Steiner solution, and after 21 days, they were inoculated with a chlamydospore solution (10^5 chlamydospores/mL). After 14 days, the seedlings were recovered to evaluate the effect of the inoculation on the height, diameter, stem and root weight. Five seedlings constituted an experimental unit with six replicates, as control plants were used without inoculation (Luna et al., 2013).

Análisis Estadístico

The results obtained from the plant growth promotion trial were subjected to analysis of variance and Tukey mean comparison test (0.05).

Results

Biochemical properties related to the promotion of plant growth

In the liquid medium Pikovskaya, *P. chlamydosporia* solubilized the insoluble tricalcium phosphate, producing average soluble phosphorus values of 242.56 ± 27.16 mg/L. These values are high compared to those reported by De Souza et al. (2019), who tested this capacity in three field isolates, which produced values in the range of 36.6 to 47.5 mg/L. This property is related to the fungal production of organic acids such as acetic, citric and propionic acids. Soil applied inoculants with this property make phosphates more bioavailable to the plant, thus improving its development. As for the production of indol-acetic acid (AIA), average values of 10.0 ± 1.24 mg/L were recorded, which are low compared to those reported by Zavala et al. (2015), who found in nine environmental isolates, values in the range of 158 - 267 mg/L. AIA is a phytohormone that is closely related to physiological processes such as induction of seed germination, root formation and plant growth.

Biochemical properties related to root colonization and biological control

P. chlamydosporia needs to penetrate the root tissues in order to gain access to and parasitize the nematodes. To do this, it first needs to produce pectinases and amylases that allow it to colonize the internal root tissues (Sunitha et al., 2013), Fig 1, shows the production of these enzymes by the Q30 strain. Afterwards, the production of chitinases is essential to carry out parasitism, since this enzyme carries out the degradation of the nematodes' eggshell and the integument of the juvenile stages (Olivares et al., 2002).



Figure 1 Pectinolytic and amylolytic activities (left and right, respectively), detected in Q30

Chitinolytic activity was recorded by mycelial growth observed in liquid media, which contained mineral salts whose only source of carbon was colloidal chitin.

Watermelon seedling growth promotion

In the plant growth promotion trial, the inoculation of chlamydospores in watermelon seedlings had a significant effect on height and diameter, where an increase of 13% and 6%, respectively, was registered (Table 1), although no differences were observed in the total biomass with respect to the control.

Treatment	Height (cm)	Diameter (mm)	Stem weight (g)	Root weight (mg)
Witness	6.77A	4.28A	0.741A	75.33A
Q30	7.65B	4.52B	0.724A	62.28A

Table 1 Effect of the inoculation of chlamydospores of *P. chlamydosporia* Q30 on the growth of watermelon seedlings. Equal letters in each column are not statistically different (Tukey, 0.05)

These increases could be due to the action of the AIA, which although in vitro low amounts of production were detected, could have an effect on the growth of seedlings. Dallemole et al. (2015) report that both mycelium and chlamydospore discs inoculation can be used as inoculants, since their application to tomato and lettuce sprouts significantly increased height and biomass. Zavala et al. (2015), report that *P. chlamydosporia* inoculation can also reduce tomato flowering and fruiting time, which may be due to AIA production and phosphate solubilization. This shows that *P. chlamydosporia*, is a versatile microorganism with great potential for use in agriculture.

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Conclusions

P. chlamydosporia Q30 produces AIA and solubilizes insoluble calcium phosphates, properties related to plant growth promotion.

Biochemical tests detected amylolytic, pectinolytic and chitinolytic enzyme activities in *P. chlamydosporia* Q30, probably involved with root colonization and biocontrol effect towards nematodes.

P. chlamydosporia Q30 could be used to improve the quality of watermelon seedling production, due to the effect it has as a plant growth promoter.

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