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In Vitro Evaluation of Phosphate Adaptability and Solubilization Capacity of *Trichoderma* Sp Strains at Various Temperatures

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Abstract

The aim of this study was to evaluate in vitro the ability of the isolate Trichoderma viride to grow and solubilize phosphate. Sampling was carried out in avocado soil in the municipality of Chalán in the Mountain of María. Twelve strains of Trichoderma spp. were isolated, which by morphological and structural characteristics identified Trichoderma viride as the predominant strain. The results show that T. viride grew at temperatures of 30, 35 and 40 °C. The spore concentration found at 30°C was 4.5 x 10⁶ spores/mL and at 40°C showed a spore concentration of 1.5 x 10⁶ spores/mL. The phosphate solubilisation capacity of T. viride at 40°C was also demonstrated. The capacity of Trichoderma is variable and depends directly on the strain and its capacity for action. The study predicts the ability of T. viride to adapt to 40°C and retain the ability to solubilize phosphate, which may be an alternative to climate change with increasing temperatures in agricultural systems.

Keywords: Strains, Fungus, Growth, Phosphate, Temperature.

Introduction

The fungal genus *Trichoderma* spp. is a cosmopolitan micro-organism, naturally inhabiting soils, especially those containing organic matter or decaying plant debris (Harman and Lumsden, 1990). *Trichoderma* species have been studied for more than 70 years as antagonists of plant pathogenic fungi. However, it was not until the beginning of this century that they began to be commercialized as agricultural bio-controllers, due to the change in the agricultural model that increasingly demands healthy food with less fungicide traces.

As stated by Petra et al., (2023), there is ample scientific evidence related to the general characteristics of the filamentous fungus *Trichoderma* spp. that demonstrates its wide range of interrelationships in ecosystems and its beneficial activity for the agricultural sector and agro-industry. Its importance in the preservation and restoration of soil microbiota has also been documented. The biological and ecological benefits of *Trichoderma* are due to its reproductive capacity as well as its efficiency in the use of soil nutrients.

As described by Djonovic et al (2007); Hohmann et al, (2011), the increasing environmental damage generated by the use of chemical substances to control plant diseases has motivated the use of biological alternatives, such as *Trichoderma*, which is a fungal genus of the rhizosphere considered an opportunistic plant symbiont, capable of producing elicitors that induce plant defense against pathogens and insects, help to solubilize phosphorus, and promote the synthesis

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of substances that promote plant growth. The fungus genus *Trichoderma*, as stated by (Diorio et al., (2003); Marques et al., (2017); Idris et al., (2017), has not only been characterized by its use as a bioinoculant and as a biocontrol agent, but also by its ability to generate enzymes capable of degrading solid organic waste, and can therefore contribute to the mineralization and reuse of waste.

As indicated by Harman, (2011), the mechanisms by which *Trichoderma* promotes plant growth are very varied, one of them is to increase the synthesis of phytohormones by plants, in addition to its own synthesis. Hoyos et al. (2009) state that it also stimulates the production of vitamins, increases the translocation and absorption of nutrients and increases root development, carbohydrate metabolism and photosynthesis⁴¹. Some *Trichoderma* isolates are able to produce phytohormones, such as auxins and auxin-like secondary metabolites, as well as gibberellic acid and ethylene (Contreras, 2009).

Often, *Trichoderma* species also increase nutrient availability due to their ability to solubilize inorganic phosphates and convert iron to Fe²⁺ through the production of hydroxamate side-phosphors into Fe²⁺ through the production of hydroxamate-type side-phosphors⁴⁶. The use of phosphate solubilizing microorganisms, which assist the plant in phosphorus uptake, is a sustainable strategy to manage phosphorus deficiencies in agricultural soils and increase the efficiency of fertilizer uptake (Khan et al., 2010). As expressed by Chávez-Díaz et al., (2020); Martínez-Medina et al., (2020), the ability of fungi such as *Trichoderma* to occupy a wider space in the soil and plants, as well as their capacity to produce a variety of organic acids, highlights their role as a plant growth promoter

The use of *Trichoderma* in agriculture can be a useful strategy to cope with the effects of climate change. *Trichoderma*, a beneficial soil fungus, can help improve plant resistance to water stress and high temperatures, as well as promote plant growth and soil health. This may be especially relevant in areas where climate change has affected water availability and soil quality.

Based on the above considerations, the aim was to evaluate *in vitro* growth and phosphate solubilisation of *Trichoderma viride* at different temperatures.

Materials and Methods

The test was carried out in two stages: field and laboratory. In the field stage, the following steps were carried out:

- Trap crops for the capture of *Trichoderma* spp. The samplings were carried out in the productive unit of avocado crops in the municipalities of Chalán (Vereda el Limón) and Ovejas (Vereda Salitral), for them were used trap crops built with plastic containers, in which, they were placed with a portion of pre-cooked rice and then covered with a gauze mesh. These traps were randomly placed below ground level next to the avocado plants, all over the crop plot. Those places with the highest humidity and presence of decomposing organic matter were selected, and plant debris from the soil (pieces of leaves and decomposing stems) were placed on top of the traps. A total of 10 traps were placed per sampling site, which were left for a period of 7 to 14 days to give the fungus time to colonize the rice.

The Laboratory Phase Comprised

- Morphological identification of *Trichoderma* spp. isolates. Petri dishes showing the cultural characteristics of *Trichoderma* spp. were isolated from pure cultures. The sticky tape technique was used, which consists of placing the tape on the surface of the *Trichoderma* spp.

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colony, rubbing it gently and removing it, and then placing it on a slide with a drop of lactophenol, and observing it under a 4X to 40X microscope. For each isolate, macroscopic (mycelial density, odor and color) and microscopic (conidiophores, conidia, phialides and chlamyospore shape) structures were observed. Taxonomic identification to genus level was carried out using the keys proposed by (Barnett and Hunter, 1998).

- Evaluation of growth at different temperatures. The culture medium suitable for the growth of *Trichoderma* sp, is PDA agar. Pure isolates of the *Trichoderma viride* strain were inoculated onto the culture medium and incubated at different temperatures (30°C, 35°C 40 °C). The growth of the *Trichoderma viride* strains by measuring the biomass was carried out 7 days after inoculation of the isolates at each of the temperatures evaluated. After this time, growth characteristics and phosphate solubilization capacity were evaluated.
- Phosphate solubilization. The phosphate solubilizing capacity of each strain was determined using SRS culture medium. The color change from purple to yellow in the medium is considered positive for phosphate solubilization (Sundara & Sinha, 1963).

Results and Discussion

The strain isolated from the municipality of Chalán corresponds to the fungal species *Trichoderma viride*, which showed microscopic characteristics such as conidiophores with paired lateral branches forming a 90° angle with respect to the axis from which they arise. The phialides were elongated in shape, somewhat broad in the center and with an elongated, hyaline-colored neck. Sometimes individual phialides were observed while in other cases they were found in groups of 2 to 3 (Figure 1). The conidia observed were ovoid and sometimes globose in shape, with light green coloring and smooth edges. The conidia were embedded in the phialides in the form of a globose cluster of well-defined coconut-scented umbels. The coloring of the mycelium is white, turning green after four days with a slight yellow tinge (Figure 1).

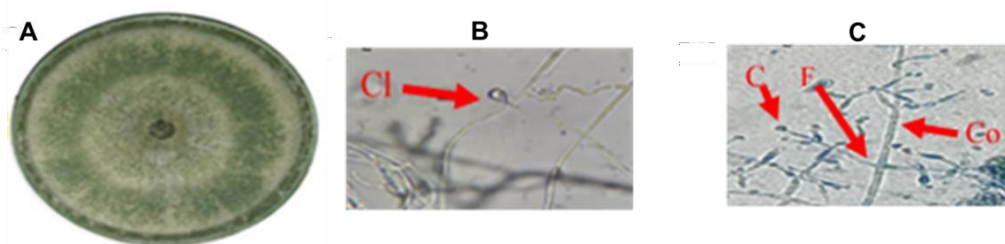


Figure 1.

Identification of *Trichoderma viride*, A) Mycelial growth on PDA medium day 7, B) Chlamyospores (Cl), C) Conidiophore (Co), Phialides (F), Conidium (C). Source: Microbiological Research Laboratory, Faculty of Agricultural Sciences, University of Sucre, 2024.

Table 1 shows the growth and phosphate solubilisation behaviour of the *Trichoderma viride* strain after being subjected to temperatures of 30, 35 and 40°C. As can be seen under temperatures of 40°C the *Trichoderma viride* species had excellent growth and phosphate solubilization.

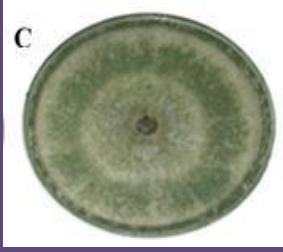
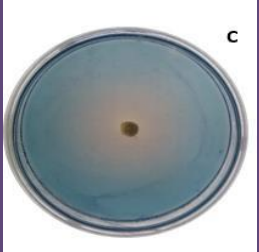


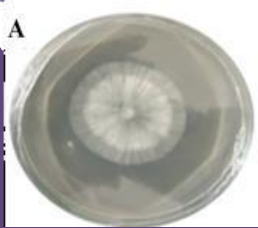
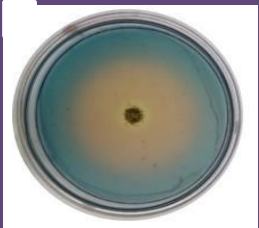
Species	Temperature	Growth	Phosphate solubilization
<i>Trichoderma viride</i>	30 °C		
<i>Trichoderma viride</i>	35 °C		
<i>Trichoderma viride</i>	40 °C		

Table 1. Growth And Phosphate Solubilisation of *Trichoderma Viride* At Different Temperatures

In figure 2, the growth of *Trichoderma viride* is depicted as the number of spores after being subjected to temperatures of 30, 35 and 40°C. *Trichoderma viride* is a filamentous fungus that can grow in a variety of environmental conditions, including high temperatures. Mechanisms of adaptation to high-temperature growth include: *Trichoderma viride* can produce heat shock proteins that help protect cells against heat stress and may make adjustments to cell membrane composition to maintain its integrity and function at high temperatures.

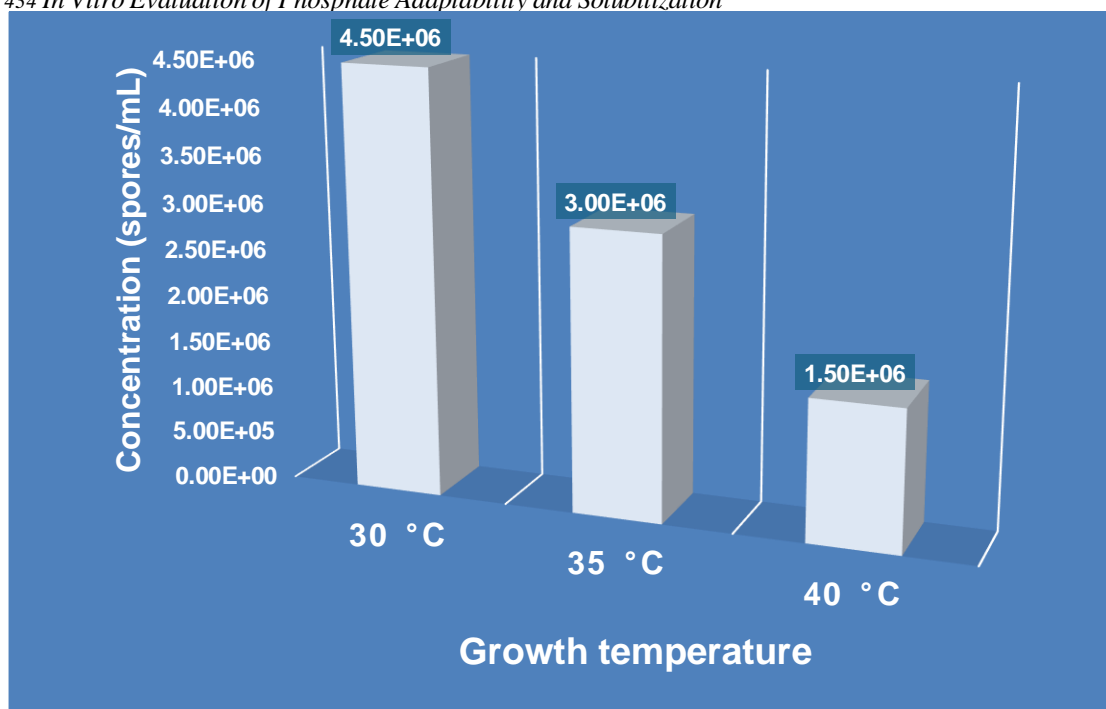


Figure 2. Spore Concentration/MI of *Trichoderma Viride* Subjected to Different Temperature Ranges.

The implications that *Trichoderma viride* may have are defined in the sense of: *Trichoderma viride*'s ability to grow at high temperatures makes it a valuable agent for applications in hot environments, such as agriculture in tropical regions and why *Trichoderma viride*'s ability to grow at extremely high temperatures may be limited, which may affect its efficacy in certain applications. *Trichoderma harzianum*, *Trichoderma viride*, *Trichoderma koningii* y *Trichoderma hamatum* are among the most prominent species in this genus with beneficial effects in the control of phytopathogenic fungi and the most widely applied in the development of modern sustainable agriculture. These species are characterized by rapid growth and a wide capacity for sporulation and adaptation to a wide range of agricultural soils. Their high adaptability allows them to survive naturally in different environments.

Moreover, phosphate solubilisation by microorganisms is considered one of the most important benefits, which are being used as a biofertiliser in crops. Several studies have demonstrated the application of *Trichoderma* sp. fungi to solubilize phosphates, including in areas contaminated with heavy metals (López-Bucio, 2015; Rawat, 2011).

Conclusion

In conclusion, this protocol allowed isolation of *Trichoderma* sp strains from soil and in vitro evaluation of their phosphate solubilizing activity and growth at different temperatures, which can be useful for various biotechnological applications. *Trichoderma viride* strains with phosphate solubilizing activity and optimal growth at different temperatures can be used as biofertilisers to improve soil fertility and promote plant growth. *Trichoderma viride* strains can be used for bioremediation of soils contaminated with heavy metals or other pollutants.

Como posibles mecanismos de adaptación de *Trichoderma viride* a 40°C en el presente estudio

estaría sustentado por:

Trichoderma viride is a filamentous fungus that can grow in a variety of environmental conditions, including high temperatures. Some characteristics of the adaptability of *Trichoderma viride* to high temperatures are presented below:

Temperature Range

1. Optimal temperature: The optimal temperature for *Trichoderma viride* growth is between 25-30°C.
2. Temperature range: *Trichoderma viride* can grow in a temperature range of 10-40°C.

High temperature tolerance

1. Tolerance to high temperatures: *Trichoderma viride* can tolerate high temperatures, but growth may be affected at temperatures above 35°C.
2. Tolerance variability: Tolerance to high temperatures may vary depending on the specific strain of *Trichoderma viride*.

Adaptation Mechanisms

1. Production of heat shock proteins: *Trichoderma viride* can produce heat shock proteins that help protect cells against heat stress.
2. Cell membrane adjustments: *Trichoderma viride* can make adjustments to the composition of the cell membrane to maintain its integrity and function at high temperatures.

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Author Contribution.

Alexander Pérez Cordero: experiment execution, data analysis. Donicer Montes V and Yelitza Aguas M, conceptualization, writing - revision and editing. All authors have read and approved the manuscript.

Conflict of Interest.

All the authors of the manuscript declare that they have no conflict of interest.

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