ARTICLE / INVESTIGACIÓN

Characterization of *Fusarium* species causing dry rot of potato mini tubers produced by biotechnological approaches

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Abstract: Soil-borne diseases affect potatoes and cause severe damage to tubers. Several Fusarium species have been associated as causal agents of potato dry rot. This research focused on characterizing fungal species causing dry rot in potato mini tubers produced using biotechnological approaches. Minitubers with typical symptoms of tuber dry rot were selected from freeze store chambers at Instituto de Biotecnología de las Plantas and processed in the applied microbiology laboratory. Potato Dextrose Agar (PDA, Fluka) with components reduced by 50% was used for fungal isolation, and Spezieller Nährstoffarmer Agar (SNA) was used for morphological characterization. Ten isolates were obtained from potato mini tubers. Mycelia growth was speedy in the culture media used, and CCIBP-Fp-1 had the greatest growth velocity. Cottony colonies were observed in isolates CCIBP-Fp-1, CCIBP-Fp-6, CCIBP-Fp-7 and CCIBP-Fp-9; felty texture was common in isolates CCIBP-Fp-2, CCIBP-Fp-3, CCIBP-Fp-4 and CCIBP-Fp-8, while subfelty texture was seen in isolates CCIBP-Fp-5 and CCIBP-Fp-10. CCIBP-Fp-2 and CCIBP-Fp-4 isolates showed characteristics similar to Fusarium solani, while CCIBP-Fp-3, CCIBP-Fp-5, CCIBP-Fp-6, CCIBP-Fp-7, CCIBP-Fp-8, CCIBP-Fp-9 and CCIBP-Fp-10 corresponded with *Fusarium oxysporum*. With the results of this work, potato tuber seeds may be protected with better conservation procedures and improve the health of Cuban Potato seeds produced by biotechnological approaches.

Key words: Biotechnology, fungi, post-harvest, *Solanum tuberosum*.

Introduction

Potato (*Solanum tuberosum* L.) is the fourth main food crop in the world after maize, rice and wheat[1]. Evidence suggests that this crop was domesticated 10,000 years ago close to Titicaca Lake (between Bolivia and Peru), where the most incredible diversity of wild species is found. Spain 1573 introduced potato in Europe, but only in the early seventeenth century, it became a critical food[2].

Approximately 40 soil-borne diseases affect potatoes Worldwide and cause severe damage to tubers, the economically most important part of the plant. The occurrence and development of soil-borne diseases depend on factors affecting the pathogen or the plant. Favorable conditions for potato disease development are frequently the same needed for potato growth[3]. Potato dry rot caused by the fungal pathogen is an essential disease causing postharvest rotting and loss of tuber seed quality. Potato seed infected with *Fusarium* species may reduce crop sprouting and vigor, affecting crop yield in field plantation[4].

Numerous *Fusarium* species have been identified as causal agents of potato dry rot worldwide. Potato dry rot may cause postharvest severe losses[6]. In Cuba some *Fusarium* spp. Pathogens have been identified as causing dry rot of tubers on imported seed from Canada and Holland (Baraka 57 %, Desirée 50 %, Atlantic 32.5 %, Chieftain 25 % and Red Pontiac 12.5 %)[7].

Dry rot is regulated by seed certification standards in several potato seed-producing countries[8]. Micropropagation using plant biotechnological approaches may help produce better quality potato seeds and facilitate large-scale production of disease-free planting material[9].

Minitubers are commonly used like seeds in potato production programs to increase seed tubers and as planting material in fields[10]. Cuban potato seed production program has incorporated micropropagation methods to improve the health of tuber seeds and efficiency in propagative systems[11]. However, none of the studies has focused on characterizing fungal species causing potato mini tubers dry rot. This work aimed to identify and characterize Fusarium species causing dry rot to Cuban Potato mini tubers produced using biotechnological approaches.

Materials and methods

Sampling sites

Based on the occurrence of dry rot associated with seed potato (mini tubers), affected mini tubers (Romano variety) from store chambers at Instituto de Biotecnología

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de las plantas (Cuba) were selected. Minitubers were produced using biotechnological approaches and preserved at long-term (6-8 months) conservation conditions (5-8 ºC). Selected mini tubers with typical symptoms of dry rot were further processed in the applied microbiology laboratory.

Cultivation and isolation of fungi

Potato Dextrose Agar (PDA, Fluka) with components reduced by 50% was used for fungal isolation to prevent saprophytic fungi and bacteria growth and facilitate recovery of the *Fusarium* species. Spezieller Nährstoffarmer Agar (SNA) medium composed of glucose, 0.2 g; $MgSO_4.7H_2O$, 0.5 g; KCl, 0.5 g; KNO_3, 1 g; KH_2PO_4, 1 g; Agar, 20 g; water, 1 L and pH was adjusted to pH 5.6[12]. Media were sterilized using an autoclave (model Sakura) at 121°C for 15. SNA culture media was used to stimulate macroconidia and microconidia formation.

Streptomycin stock solution was prepared by adding 5 g in 100 mL distilled and sterilized using sterile disposable filters (0.22 µm pore size), then 10 µL of suspension was used. The antibiotic solution was added before the medium was poured into Petri dishes (70 mm diameter).

Potato mini tubers from the Romano variety with dry rot symptomatology were washed under running tap water for 20 min and air dried in a laminar flow cabin (Model FASTER Bio 60). They were disinfected in 2 % (v/v) hypochlorite solution for 15 s and rinsed thrice in sterile distilled water. Minituber was placed in a wet chamber at 25°C for 7 days in the dark.

Mycelial growth from mini tuber was transferred using a metallic scalpel to Petri dishes containing 25 mL of PDA media. After 5 days of incubation at 25ºC temperature, fungal colonies using stereoscopic microscopy (Carl Zeiss, Stemi 100 model) were identified by microscopic observations and purified at least twice by serial transfers to PDA Petri dishes.

From purified colonies, 5 mycelia discs were introduced in Eppendorf tubes of 1.5 mL containing sterile glycerol solution at 25%(v/v). Ten tubes per fungal isolate were preserved at – 80 °C.

Cultural characterization

One disc from Eppendorf tubes, preserved at 4ºC, was used to inoculate Petri dishes (70 mm in diameter) containing 10 mL of PDA. Inoculated plates were incubated at 28 ºC in the dark for 10 days.

Growth velocity, the color of colonies, the color of the reverse of colonies, texture, presence of transpired liquid, and pigmentation of culture medium were evaluated as cultural characteristics.

Morphological characterization

The Spezieller Nährstoffarmer Agar (SNA) medium was poured into Petri dishes of 50 mm (diameter). Pieces of sterile filter paper (Whatman No. 1) of approximately 1 cm² were placed on the agar surface of each Petri dish to stimulate sporulation. Mycelia discs of 4 mm were placed in contact with sterile filter papers for each isolate and incubated for 10 days at 28ºC in dark conditions.

Microcultures for each isolate were mounted to facilitate sporulation and improve visualization of conidial morphology according to the method proposed by Riddel (1950)[12]. Characteristics of hyphae, microconidia, macroconidia, and chlamydospores were observed under a clinical microscope (model Olympus) with 400× magnification, and pictures were captured by a digital camera (Model CANON A-630).

Statistical analysis

Statistical analyses were performed using SPSS (Statistical Package for the Social Sciences Version 18.0) software (SPSS Inc., Chicago, IL, USA). The normal distribution was determined using the Shapiro-Wilk test, and variance homogeneity was determined using the Levene test. A nonparametric test of Kruskall-Wallis was used for variables without normal distribution or variance homogeneity.

Results

Isolation and cultural Characterization

Ten isolates were obtained from potato mini tubers (Romano variety) with dry rot symptomatology. The cultural characteristics of isolates were similar to those of *Fusarium* genera. According to the characterization of all isolates, mycelia growth was speedy in the culture media used, and CCIBP-Fp-1 had the most incredible growth velocity (16 mm day^{-1}, significant at $p < 0.05$). Potato dextrose agar (PDA) effectively isolated fungi without saprophytic fungi and bacterial growth.

Textures were different among isolates. Cottony colonies were observed in CCIBP-Fp-1, CCIBP-Fp-6, CCIBP-Fp-7, and CCIBP-Fp-9 isolates. The mat's texture was erect, with mycelium spreading in all directions. Felty texture with a mat composed of cottony or woolly mycelium forming a surface like felt was common in isolates CCIBP-Fp-2, CCIBP-Fp-3, CCIBP-Fp-4, and CCIBP-Fp-8, while poor aerial mycelium attached to agar surface contributing to subtlety texture was seen in isolates CCIBP-Fp-5 and CCIBP-Fp-10. The color of colonies' surfaces (white, pink, purple, orange, and violet) and reverses (white, yellow, purple, red, and violet) differed among isolates. Transpired liquid was observed only in CCIBP-Fp-1, CCIBP-Fp-6, and CCIBP-Fp-8 colonies. All isolates showed hyphal tissue with intricate texture types.

Morphological characterization

Morphological characteristics of asexual reproductive structure (macroconidia and microconidia), conidiophores, and chlamydospores, matched with *Fusarium*, excepting the isolate CCIBP-Fp-1. CCIBP-Fp-2 and CCIBP-Fp-4 showed characteristics similar to *Fusarium solani*, while CCIBP-Fp-3, CCIBP-Fp-5, CCIBP-Fp-6, CCIBP-Fp-7, CCIBP-Fp-8, CCIBP-Fp-9 and CCIBP-Fp-10 corresponded with *Fusarium oxysporum*.

SNA culture media was suitable to promote macroconidia and microconidia development in analyzed isolates. *F. solani* isolates showed macroconidia rounded in the basal cell, blunt and rounded in the apical cell, 0 to 3 septa, and 23.4-66.3 µm long. Abundant microconidia were observed in aerial mycelial, single-celled, 1–2 septa, and oval to kidney-shaped, associated in chain with 7.8-19.5 µm long. Conidiophores were short and long monophialides with apical spore mass. Chlamydospores were globose, lonely or forming pairs (Figure 1).

F. oxysporum showed macroconidia slightly curved, thin-walled, foot-shaped basal cell, curved apical cell, 0 to 5 septate, and 23.4-62.4 µm long. Abundant microconidia were observed in aerial mycelial with false heads, oval to kidney-shaped or elliptic, 0-4 septa, and 3.9-19.5 µm long. Few microsclerotia were observed. Conidiophores were short monophialides, hyaline, and simple with apical spores mass. Chlamydospores were globose and lonely.

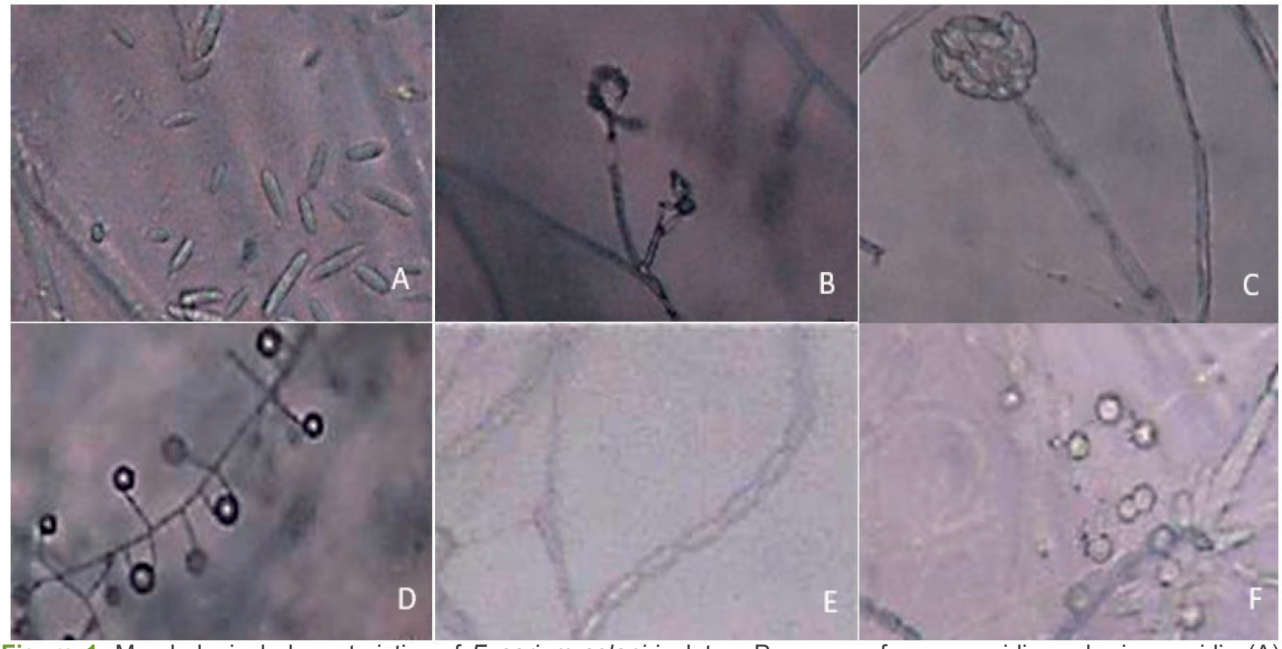

Figure 1. Morphological characteristics of *Fusarium solani* isolates. Presence of macroconidia and microconidia (A), Short and long conidiophore (B), Long mono phialides with apical spores mass (C), Short mono phialides with apical spores mass (D), Chain of microconidia (E), Chlamydospores lonely or forming pairs (F).

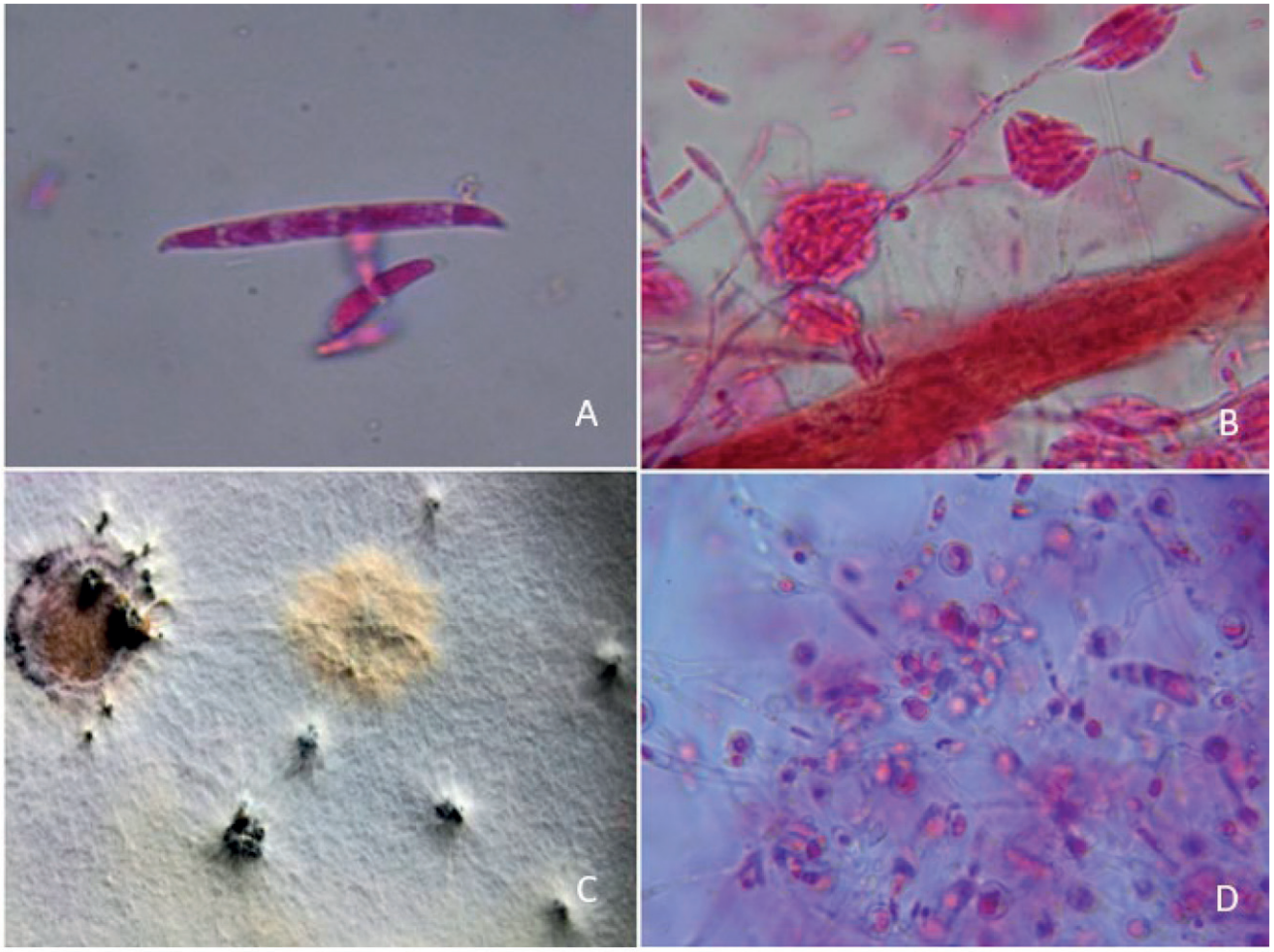

Figure 2. Morphological characteristic of *Fusarium oxysporum*. A- Macroconidia slightly curved. B- Microconidia in false heads at aerial mycelial. C- microsclerotia. D- Chlamydospores globose and lonely.

Discussion

Until now, no authors have reported fungal pathogens associated with potato seed mini tubers produced by biotechnological approaches, but fungi causing tuber dry rot have been very well documented in common potato tubers. A similar method for isolating *Fusarium* species associated with tuber dry rot was used to determine the diversity of *Fusarium* spp., affecting potato tubers in Upper Egypt[13].

García-Bayona (2011) described the macroscopic and microscopic morphology of two strains of *F. oxysporum* causing potato dry rot in Solanum tuberosum in Colombia[14] and observed similar characteristics with some of the obtained isolates from mini tubers. 2006 Leslie and Summerell suggested that PDA is unsuitable for *Fusarium* isolation. However, Peptone-PCNB Agar, Carnation Leaf-piece Agar, and SNA were the most effective mediums for the recovery of *Fusarium* species; nevertheless, in our study, PDA components were reduced at 50%.

Cultural characteristics observed in fungal isolates associated with potato mini tubers Dry Rot were similar to those described by Tiwari *et al.* (2021)[15]. The results presented in this paper were identical to some cultural features defined by this group.

Anand *et al.* (2018) described variability in cultural characteristics of *F. oxysporum* sp. chrysanthemi causing wilt to chrysanthemum plants in Kovilur village of Madurai, Tamil Nadu in India[16]. They detected significant differences among isolates concerning mycelia growth velocity in semisolid media and differences in mycelial dry weight using liquid culture media. Our results showed that cultural characteristics were variable among isolates of *F. oxysporum*. Similarly, Tiwari *et al.* (2020) have also found significant variability in cultural factors (color of surface colonies, texture and pigmentation) of colonies of *F. solani*, causing potato dry rot disease[17].

On the contrary, our results showed that cultural characteristics were similar among isolates of *F. solani*. In *Fusarium* genera, it is common to observe significant differences in cultural traits among isolates of different species and from the same species as well[18]. Several cultural characteristics of *Fusarium* isolates (growth velocity, abundance of aerial mycelia, pigment diffusion in agar, dimensions of conidia[19].

The preserved isolates of *F. solani* and *F. oxysporum* could be used as referenced strains in culture collection with fungal pathogens associated with Potato Dry Rot of mini tubers. According to morphological characteristics observed among fungal isolates, *F. oxysporum* and *F. solani* were the morphospecies associated with Potato Dry Rot of mini tubers. Other authors recorded similar morphology (dimension of macroconidia, microconidia, chlamydospores and conidiophores) in *Fusarium* strains from various substrates and geographic areas[20].

Similarly, Yli-Mattila *et al.* (2004) determined differences in morphological characteristics among *Fusarium* species (*F. avenaceum, F. arthrosporioides, F. sporotrichioides, F. culmorum, F. arthrosporioides, F. culmorum, F. sporotrichioides* and *F. graminearum*) and they observed correspondence with molecular identification[21]. Many *Fusarium* species have been associated with potato tubers dry rot (*F. sulphureum*[22]; *F. solani var. coeruleum*[23]; *F. oxysporum*[24] and *F. avenaceum*[25] Fr Sacc. Cullen *et al.* (2005) considered that the number of *Fusarium* species associated with this fungal disease may exceed[13], and they are worldwide distributed[26]. Contrary to these results, only two species of *Fusarium* were identified in the present study.

F. oxysporum has a cosmopolitan distribution, inhabiting soil fungus, causing vascular wilt, damping off, and rot on crops with economic importance[27]. Approximately 41 years ago, Correll (1991) reported that *F. oxysporum* has a high level of host specificity with over 120 described formae species and races that may cause vascular wilt diseases[28].

Some morphological characteristics of *F. oxysporum*, including the production of microconidia in false heads and short phialides, the presence of chlamydospores, and the shape of macroconidia and microconidia were defined by Leslie and Summerel (2008)[29]. These authors argued that *F. oxysporum* is similar to *F. solani* and *F. subglutinans*, but *F. solani* is distinguishable from *F. oxysporum* because the first form of microconidia in false heads and very long phialides inserted into hyphae. *F. subglutinans* is only distinct from *F. oxysporum* because of its microconidia form in poliphialides and the absence of chlamydospores.

On the other hand, *F. solani* is one of the most common species causing potato dry rot worldwide. It is associated with soil, causing wilt, root rot, basal rot, and canker in the stem. It is frequently associated with damages, as opportunistic or attacking weaken hosts[30]. Furthermore, *F. solani* is a soil inhabitant in tropical forests with high humidity. A wide range of plants of economic interest is attacked by this fungal species (potato, avocado, beans, citric, cocoyam, cowpea, orchid, passion fruit, pea, and pepper)[31].

Pathogenicity and virulence of *F. solani*-causing plant diseases may be explained by cutinase activity, modes of spore attachment, and penetration of the host's surface. Taxonomy and classification of *F. solani* is complex because this species has approximately 15 formae speciales, closely associated with different crops[32]. In Cuba, *F. solani* has been reported in Cuba[33] but not in potato mini tubers.

Therefore, this work made a valuable characterization of *Fusarium* species causing dry rot of Cuban microtubes and may facilitate better conservation procedures and health of Cuban Potato seeds produced by biotechnological approaches.

Conclusions

The present study concluded that 10 isolates from *Fusarium* were recovered from potato mini tubers. *F. solani* and *F. oxysporum* were the species causing dry rot of potato mini tubers. Better conservation procedures may be developed to improve the health of Cuban Potato seeds produced by biotechnological approaches.

Author Contributions

Conceptualización, Michel Leiva Mora, Metodología, Michel Leiva Mora y Mayra Acosta Suárez, Software, Michel Leiva Mora y Mayrebi Herrera-Capote, Validación, Michel Leiva Mora y Mayra Acosta Suárez, Análisis formal, Michel Leiva Mora, Mayra Acosta Suárez ; Investigación, Catherine Silva Agurto y Natalys Solis; Recursos, Michel Leiva Mora, Catherine Silva Agurto, Walter Oswaldo Veloz Naranjo, Curado de datos, Michel Leiva Mora y Rodrigo Núñez; Redacción Mayrebi Herrera-Capote redacción borrador original y la corrección realizada por Michel Leiva Mora, Revisión y edición, Michel Leiva Mora, Miguel y Angel Osejos Merino; supervisión, Catherine Silva Agurto; administración del proyecto, Michel Leiva Mora, adquisición del financiamiento, Michel Leiva Mora y Catherine Silva Agurto;

Todos los autores han leído y están de acuerdo con la versión publicada del manuscrito.

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Conflicts of Interest

Los autores declaran no tener conflicto de interés.

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