Endophytic mycobiota associated to plants of *Vaccinium corymbosum* L. in Cañete valley- Perú

Micobiota endófita asociada a plantas de *Vaccinium corymbosum* L. en el valle Cañete- Perú

Micobiota endofítica associada a plantas de *Vaccinium corymbosum* L. no vale do Cañete - Perú

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Abstract

Endophytic fungi (EF) are microorganisms that live asymptomatically within plant tissues. They can confer benefits to the plant as inducers of resistance to pests and diseases, tolerance to abiotic factors, plant growth promoters and mycoparasites of pathogens. This study aimed to isolate, identify, and determine occurrence frequency of endophytic fungi in stems and leaves of blueberry plants collected from ten farms in Lima and Ica Region, Peru. Productive branches of 39 healthy plants were sampled. For the isolation of EF, 702 tissue sections were disinfected and transferred to Petri dishes with potato dextrose agar (PDA) medium, and reproductive structures were induced for identification. A total of 134 EF strains were isolated successfully. Nine genera were identified (*Trichoderma, Aspergillus, Alternaria, Ulocladium, Cladosporium, Fusarium, Nigrospora, Lasiodiplodia* and *Stemphylium*). EF occurrence frequency varied greatly between organs, being in leaves more frequent than the stems; likewise, the genera *Alternaria* and *Cladosporium* were the most frequent and with most colonized in plant tissues, while *Aspergillus, Ulocladium* and *Lasiodiplodia* were the least frequent. These strains could be useful to control plant diseases and, therefore, of great interest in sustainable agriculture.

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Resumen

Los hongos endófitos (HE) son microorganismos que viven de manera asintomática dentro de los tejidos vegetales. Pueden conferir beneficios a la planta, comoinductores de resistencia a plagas y enfermedades, tolerancia a factores abióticos, promotores de crecimiento vegetal y micoparásitos de patógenos. El objetivo del presente trabajo fue aislar e identificar los hongos endófitos,para determinar su frecuencia de aparición en tallos y hojas de plantas de arándano,colectadas en 10 fundosde la región Lima e Ica, Perú. Se muestrearon ramas productivas de 39 plantas sanas, y fueron llevadas al laboratorio de Sanidad Vegetal de la Universidad Nacional de Cañete. Para el aislamiento de HE, 702 secciones de tejidos fueron desinfestados y transferidos a placas Petri con medio de cultivo papa dextrosa agar (PDA) y se indujo estructuras de reproducción para la identificación. Un total de 134 cepas de HE fueron aisladas, de los cuales se identificaron nueve géneros (*Trichoderma, Aspergillus, Alternaria, Ulocladium, Cladosporium, Fusarium, Nigrospora, Lasiodiplodia*y *Stemphylium*). La frecuencia de aparición de los HE fue muy variada según los órganos. Las hojas tuvieron mayor frecuencia que los tallos; así mismo, los géneros *Alternaria y Cladosporium* resultaron ser los más frecuentes y con mayor colonización en los tejidos de las plantas mientras que *Aspergillus, Ulocladium* y *Lasiodiplodia* fueron los menos frecuentes. Estas cepas podrían ser útiles para el control de enfermedades vegetales y, por consiguiente, de gran interés en la agricultura sostenible.

Palabras clave: Hongo, arándano, colonización, identificación, cepas.

Resumo

Os fungos endofíticos (FE) são microrganismos que vivem de forma assintomática nos tecidos vegetais. Eles podem conferir benefícios à planta, como indutores de resistência a pragas e doenças, tolerância a fatores abióticos, promotores de crescimento vegetal e micoparasitas de patógenos. O objetivo deste trabalho foi isolar e identificar fungos endofíticos, para determinar sua frequência de aparecimento em caules e folhas de plantas de mirtilo, coletados em 10 fazendas na região de Lima e Ica, Peru. Ramos produtivos de 39 plantas sadias foram amostrados e levados ao laboratório de Fitossanidade da Universidade Nacional de Cañete. Para o isolamento de FE, 702 cortes de tecido vegetal foram desinfetados e transferidos para placas de Petri contendo como meio de cultura batata dextrose ágar (PDA), e as estruturas de reprodução foram induzidas para identificação. Um total de 134 cepas de FE foram isoladas, das quais nove gêneros foram identificados (*Trichoderma, Aspergillus, Alternaria, Ulocladium, Cladosporium, Fusarium, Nigrospora, Lasiodiplodia e Stemphylium*). A frequência de aparecimento de FE foi muito variada de acordo com os órgãos. As folhas tiveram maior frequência do que os caules; da mesma forma, os gêneros *Alternaria* e *Cladosporium* foram os mais frequentes e com maior colonização nos tecidos vegetais, enquanto *Aspergillus, Ulocladium* e *Lasiodiplodia*, foram os menos frequentes. Essas cepas podem ser úteis para o controle de doenças de plantas e,portanto, de grande interesse uma agricultura sustentável.

Palavras-chave: Fungo, mirtilo, colonização, cepas, identificados.

Introduction

The blueberry is the second most exported product of the Peruvian fruit and vegetable basket. The United States of America is one of the main destinations for Peruvian exports with a 55% share during the 2019-2020 agricultural campaign, because they are the largest consumer of this fruit worldwide (Redagricola, 2020). The requirements of the global blueberry market require organic production of this fruit, with the biological control of diseases being one of the key factors in this practice. A study of its endophytic mycobiota would allow the identification of microorganisms that could help in this process, contributing to the current demands of international markets.

Endophytic fungi are symbiotic organisms that live in association with plants for most or all of their life cycle. These microorganisms cover different orders, they are found inside the tissues, in the intercellular spaces and sometimes, intracellularly in leaves, stems and flowers, absorbing nutrients from the plant without producing symptoms of any disease, on the contrary they provide protection against biotic agents and abiotic (Busby *et al.*, 2016; Carroll, 1988; Lugtenberg *et al.*, 2016; Mane *et al.*, 2018). In the process of biocontrol of diseases, these microorganisms protect the host plant mainly through antibiosis, parasitism and competition mechanisms, which by inhibiting the growth of plant pathogens, result in better plant performance (Segaran and Sathiavelu, 2019).

Currently, endophytic fungi are investigated in many crops, due to their importance in plants (Busby *et al.*, 2016; Mane *et al.*, 2018). They are found in some species of *Vaccinium* and are divided into endophytic, pathogenic and ectotrophic (Miao *et al.*, 2013). In leaves of *Vaccinium dunalianum*, the existence of a high diversity of these fungi is reported, managing to identify taxa with potential antimicrobial activity, being a source of natural bioactive compounds for future agroindustrial applications (Fan *et al.*, 2020; Li *et al.*, 2016). However, in *Vaccinium corymbosum* there are no studies on the presence of these microorganisms. The report of associated endophytic fungi in blueberry production will be very useful for the search for strains with potential for biocontrol of diseases in this crop. With the isolation of endophytic fungi from plants, biologically active agents can be produced on a large commercial scale, without affecting ecological biodiversity, since they are easily cultivated in the laboratory (Mane *et al.*, 2018). For these reasons, the objective of this research is to isolate, identify and determine the frequency of appearance of endophytic fungi in stems and leaves of blueberries (*Vaccinium corymbosum* L.) produced in the Cañete valley in Lima, Peru.

Materials and methods

Sample Collection

During the months of January, February and March 2020, 39 healthy blueberry plants of the Biloxi variety, three years old, were sampled in 13 production lots belonging to ten farms in four districts of the province of Cañete, Lima Region (Nuevo Imperial, Imperial, Roma and Quilmaná) and on a farm in the district and province of Pisco, Ica Region under a definitive soil production system (Table 1). The climate of the province of Cañete is temperate with an average temperature of 13 °C in winter and 28 °C in summer and an average annual rainfall of 11.4 mm. The crop production system is carried out in black polyethylene bags (instead of pots) containing a substrate composed of peat and rice husks, all plantations are

irrigated and fertilized through the system called "drip irrigation". A non-probabilistic - intentional or convenience sampling method was used (Arias-Gómez *et al.*, 2016), three blueberry plants with the best characteristics in terms of growth and health were selected from each production lot. For each plant, productive branches were extracted from the lower, middle and upper third, the samples were labeled, deposited in polypropylene bags and taken to the Plant Health Laboratory of the Universidad Nacional de Cañete (UNDC) being kept at 4 °C for 24 to 48 hours until processing.

Isolation of endophytic fungi

To obtain plant tissue sections, three leaves and three stem pieces were taken from each third of the blueberry plant; For each leaf, a section of 1 cm^2 was obtained between the central rib and the edge of the leaf, and 1 cm lenth for each piece of stem, having 18 sections per plant and 54 sections per lot (three plants). For the isolation of endophytic fungi, the superficial disinfestation of the sections of stems and leaves was carried out inside a biosafety cabinet, by means of immersion; previously, the different sections were washed with sterile distilled water (ADE), followed by 3 min in 2% sodium hypochlorite, 1 min in 70% (v/v) alcohol, and three washes with ADE (De Sousa Leite *et al.*, 2013). Sterilized tissue sections were placed on sterile paper towels to dry. The material was then cut into small fragments (approximately 5 mm^2), and a total of 702 tissue sections (from 39 plants) were aseptically transferred to Petri dishes (6 sections per plate) containing potato dextrose agar (PDA) culture medium with chloramphenicol (500 $mg.L^{-1}$) to rule out the presence of bacteria (Lima and Cavalcanti, 2014; Mane *et al.*, 2018). The seeded Petri dishes were incubated in the dark at 25 °C for seven days, after this time, the strains were purified by successive picking of the youngest fungal mycelial edges until an axenic culture was obtained, then they were placed under artificial light for seven days to induce the production of sexual and asexual reproductive structures for identification. Strains that failed to form reproductive structures were left under light for up to 30 days and were observed daily for structures. The presence of a fungus was recorded as positive if it was detected in an organ segment. The colonization percentage was determined for each plant as the total number of sections colonized by the fungus in relation to the total number of fragments per 100 (Russo *et al.*, 2016).

Morphological identification of endophytic fungi

The identification of the strains of endophytic fungi was carried out at the genus level, using the conventional method based on their morphological characteristics (Aharwal and Kumar, 2016). From the axenic cultures of the fungi in PDA, the cultural characteristics of the colony were observed, such as appearance, diameter, texture and coloration, and the microscopic characteristics: type and size of the vegetative and reproductive structures. Fragments of mycelia were extracted from the cultures and placed on slides containing lactophenol, the structures were visualized with an Olympus brand binocular optical microscope, model CX23, series 7L88230 and with the use of taxonomic keys of Barnett and Hunter (1998), Barron (1968) and Watanabe (2002) endophytic fungal strains were identified to the genus level.

Analysis of data

To analyze the relationship between the variables presence or absence of each of the genera of endophytic fungi detected by organs (leaves and stems) and production lots, the non-parametric Pearson Chi-square test was used with the statistical program Infostatversión 2008 (Di Rienzo *et al.*, 2008).

Results and discussion

Isolation and identification of endophytic fungi from leaves and stems

In this study, a total of 133 strains of endophytic fungi were isolated from healthy plant tissues of *Vaccinium corymbosum* L., 82 strains from leaves and 51 from stems. According to the cultural and morphological characteristics of the fungi, nine genera belonging to the Ascomycota division were identified: *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Lasiodiplodia*, *Nigrospora*, *Stemphylium*, *Trichoderma* and *Ulocladium* (figure 1); There was also a group of endophytic fungi (21 strains) that did not form reproductive structures, which is why they were called sterile mycelium.

Table 1. Sampling locations for blueberry plants in the province of Cañete and Pisco, 2020, Peru.

Zone	Lot	Province	District	Farm	South Latitude	West Longitude	Altitude (m a.s.l)
Zone 1	Lot 1 y 2	Cañete	Quilmaná	Blueagro	12°57'17''	76°21'39''	169
Zone 2	Lot 3	Cañete	Quevo Imperial	Agropec	13°00'35''	76°21'09''	153
Zone 3	Lot 4	Cañete	Quilmaná	Agroberries	12°58'08''	76°21'54''	138
Zone 4	Lot 5	Cañete	Imperial	Peruvian Prime (Huacachivato)	13°03'59''	76°20'24''	98
Zone 5	Lot 6 y 7	Cañete	Nuevo Imperial	Fundo a3f- Caltopia	13°03'49''	76°14'30''	333
Zone 6	Lot 8	Cañete	Quilmaná	Los Ángeles	12°57'16''	76°21'28''	159
Zone 7	Lot 9	Cañete	Imperial	Arándanos del Sur Cerro Alegre	13°02'02''	76°21'05''	137
Zone 8	Lot 10	Cañete	Imperial	Malvinas	13°02'02''	76°21'05''	137
Zone 9	Lot 11	Cañete	Roma	Agroconta	13°01'20''	76°20'47''	162
Zone 10	Lot 12	Cañete	Quilmaná	Agroconta – Victoria	12°59'13''	76°24'12''	110
Zone 11	Lot 13	Pisco	Pisco	Agroinversiones Valle y Pampa	13°48'19''	76°03'19''	279

Note. The selection of number of plots for each sampling zone is in accordance with availability of plots in the estates with plantations of *V. corymbosum* var. Biloxy of the same age and crop management system.

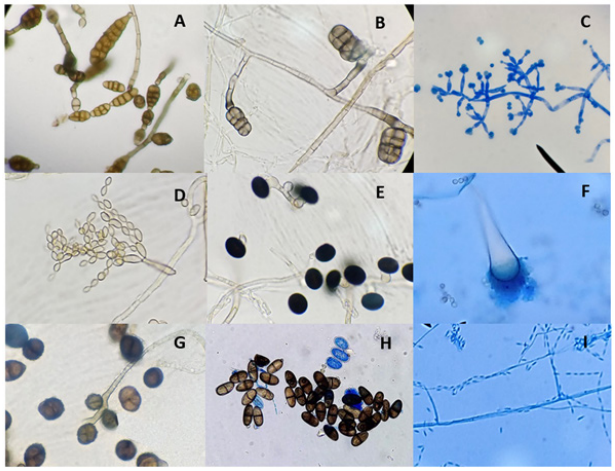

Figure 1. Genera of endophytic fungi identified in *Vaccinium corymbosum* L. from the province of Cañete. Lima Peru. A. *Alternaria* sp. B. *Stemphylium* sp. C. *Trichoderma* sp. D. *Cladosporium* sp. E. *Nigrospora* sp. F. *Aspergillus* sp. G. *Ulocladium* sp. H. *Lasiodiplodia* sp. I. *Fusarium* sp. at 100X.

Genus frequencies of endophytic fungi on leaves and stems of blueberry plants

The genera of the endophytic fungi and the plant organs where they are found vary according to the type of plant organ and the production lot (p=0.0054) and are associated with the production lots (p<0 .0001).

Of the total strains isolated from blueberry plants, 61.65% were from leaves and 38.35% from stems. Being the genera *Alternaria*, *Cladosporium*, *Fusarium*, *Nigrospora*, *Stemphylium* and *Ulocladium* the most frequent in leaves; on the contrary, *Aspergillus*, *Cladosporium*, *Lasiodiplodia* and *Trichoderma* appeared more frequently in stems. Likewise, of the total strains isolated and identified in stems and leaves, *Alternaria* and *Cladosporium*were the most frequent genera with 44% and 23% respectively, followed by *Stemphylium* and *Nigrospora* with 5%, *Trichoderma* with 4% and *Fusarium* with 3 %; while the genera *Aspergillus*, *Lasiodiplodia* and *Ulocladium* were the least frequent with 1 %. In the production plots, the relative frequency varied from 3.01 to 14.29%, in plot 2 it was observed with the highest presence of strains of endophytic fungi compared to plot 7, which was the one with the lowest frequency (figure 2 and table 2).

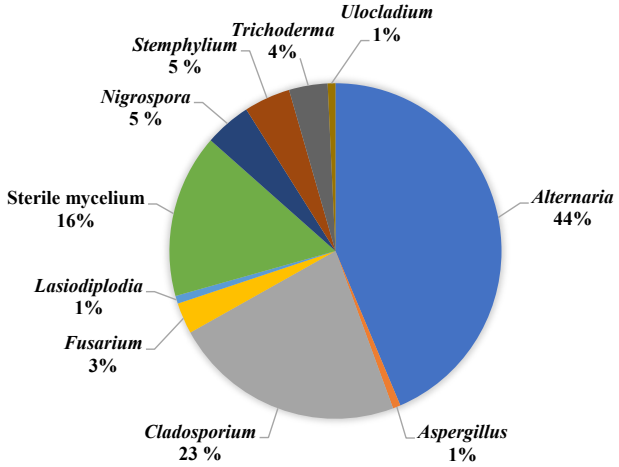

Figure 2. Percentage relative frequency of genera of endophytic fungi isolated from blueberry plants (*Vaccinium corymbosum* L.) from production plots in the province of Cañete, Peru, 2020.

Table 2. Absolute and relative frequencies with the variables production plots, genera of endophytic fungi, leaves and stems of blueberry plants.

Production plots	Genera of endophytic fungi associated with blueberry leaves (H) and stems (T).																					
	Alternaria		*Aspergillus*		*Cladosporium*		*Fusarium*		*Lasiodiplodia*		Sterile mycellium		*Nigrospora*		*Stemphylium*		*Trichoderma*		*Ulocladium*		Total	
	H	T	H	T	H	T	H	T	H	T	H	T	H	T	H	T	H	T	H	T	FA	FR (%)
Plot 1	3	1	0	0	2	0	0	0	0	0	3	0	0	0	2	0	0	0	0	0	11	8.27
Plot 2	6	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	14.29
Plot 3	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	4	11.28
Plot 4	2	0	0	0	0	2	0	0	0	0	1	0	0	0	0	0	1	0	0	0	6	12.03
Plot 5	4	1	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	8	5.26
Plot 6	0	2	0	0	2	1	0	0	0	0	3	0	0	0	0	1	0	0	0	0	9	7.52
Plot 7	3	0	0	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	3.01
Plot 8	6	5	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	14	4.51
Plot 9	4	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	6.02
Plot 10	4	4	0	0	1	2	3	0	0	0	1	0	1	0	2	0	0	0	1	0	19	6.77
Plot 11	4	0	0	0	0	1	0	0	0	1	2	3	1	0	1	0	0	2	0	0	15	5.26
Plot 12	8	0	0	0	1	2	0	1	0	0	2	2	0	0	0	0	0	0	0	0	16	10.53
Plot 13	0	0	0	1	0	3	0	0	0	0	0	2	0	0	0	0	0	1	0	0	7	5.26
Total FA	45	13	0	1	11	19	3	1	0	1	12	9	4	2	5	1	1	4	1	0	133	
Total FR (%)	33.8	9.77	0	0.75	8.27	14.3	2.26	0.75	0	0.75	9.02	6.77	3.01	1.5	3.76	0.75	0.75	3.01	0.75	0		100

FA: Absolute frequency RF: Relative frequency expressed as a percentage.

Of the total isolated strains and genera identified in blueberry plants, *Aspergillus* and *Lasiodiplodia* were only found in stems; Similarly, *Ulocladium* only in leaves compared to the other genera that were isolated in both organs. This is how, in leaves, *Alternaria* was the most frequent genus with 54.88% followed by *Cladosporium* with 13.41%, the opposite resulted in stems, the genus *Cladosporium* was the most frequent with 37.25% followed by *Alternaria* with 25.49%; while the least frequent were *Ulocladium* and *Trichoderma* with 1.22% in leaves, and *Aspergillus*, *Stemphylium* and *Lasiodiplodia* with 1.96% in stems (figure 3).

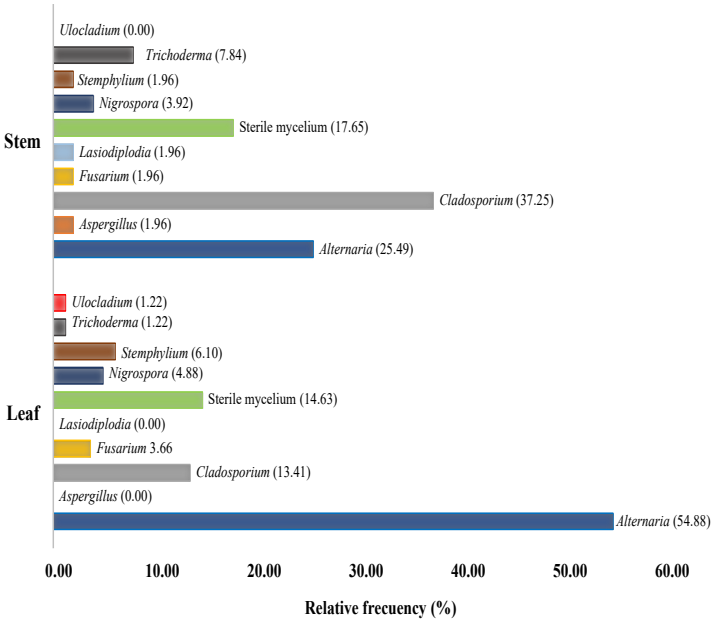

Figure 3. Percentage relative frequency of genera of endophytic fungi at the level of leaves and stems of blueberry plants (*Vaccinium corymbosum* L.) in the province of Cañete, Peru, 2020.

The colonization of endophytic fungi in the tissues of three-year-old blueberry plants, under a production system in polyethylene bags, varies from 5.56% to 19.91%, with the *Alternaria* genus having the highest colonization, followed by *Nigrospora*; while the genera *Aspergillus*, *Lasiodiplodia* and *Ulocladium* presented the lowest colonization (table 3).

Table 3. Percentage of colonization of genera of endophytic fungi isolated from stems and leaves of blueberry plants in the province of Cañete, Peru, 2020.

Genus	Endophytic colonization (%)		
	Leaves	Stems	Plant
Alternaria	10.88	9.03	19.91
Aspergillus	0	5.56	5.56
Cladosporium	6.12	7.04	13.16
Fusarium	8.34	5.56	13.9
Lasiodiplodia	0	5.56	5.56
Sterile mycelium	6.02	6.26	12.28
Nigrospora	5.56	11.11	16.67
Stemphylium	6.95	5.56	12.51
Trichoderma	5.56	7.41	12.97
Ulocladium	5.56	0	5.56

Of the identified genera, *Alternaria* and *Cladosporium* were the most frequent endophytic fungi followed by sterile mycelium (microorganisms did not form reproductive structures); likewise, the greatest diversity was in blueberry leaves. Similar studies indicate that these genera were also the most frequent endophytes in soybeans (Impullitti and Malvick, 2013). On the other hand, Larrain *et al.* (2000) reported that the highest number of endophytic fungal taxa isolated were from the leaves of healthy wheat plants. Likewise, *Cladosporium* was one of the genera most frequently isolated from *Glycine max* leaves (Fernandes *et al.*, 2015). Instead, *Cladosporium cladosporioides* was sporadically reported on healthy leaves of *Caesalpinia echinata* Lam. (Lima and Cavalcanti, 2014). They were also reported on the leaves of *Vaccinium dunalianum* var. *Urophyllum* the greatest diversity of endophytic fungi and the genus *Cladosporium* was found in branches (Li *et al.*, 2016). In contrast, a higher number of endophytes were isolated from stem tissues than from leaves in soybean and corn varieties (Russo *et al.*, 2016).

Species of *Fusarium*, *Aspergillus*, *Alternaria* and *Nigrospora* were reported as stem and leaf endophytes in medicinal plants with a colonization frequency of less than 6.66% (Anitha *et al.*, 2013). Likewise, the endophytic genera *Cladosporium*, *Nigrospora* and *Trichoderma* were isolated from leaves of *Caesalpinia echinata* Lam. less frequently (Lima and Cavalcanti, 2014). Similarly, in this investigation, these genera appeared less frequently in the tissues of blueberry plants, as well as *Ulocladium*, *Lasiodiplodia* and *Stemphilium*, except for *Alternaria* and *Cladosporium*, which were the ones that presented the highest frequency. On the contrary, Piontelli *et al.* (2002) reported the genus *Ulocladium* as the most dominant endophyte in all steps and plant substrates. Likewise, the most frequently isolated species in soybean was *Fusarium graminearum* and *Aspergillus terreus* in corn (Russo *et al.*, 2016). Likewise, *Ulocladium*, *Fusarium* and *Aspergillus* sp. were identified as endophytes of palm hearts (*Euterpe precatoria*Mart.) (Batista *et al.*, 2018). On the other hand, Aharwal and Kumar (2016) reported *Aspergillus*, *Alternaria* and *Fusarium* among the isolated genera of *Ricinus communis*. Also, the *Nigrospora* genus was isolated from leaves of healthy *Vinca rosea* plants (Metwaly *et al.*, 2014). Regarding the genus *Lasiodiplodia*, there are reports indicating that it is an endophytic fungus of *Caesalpinia echinata* Lam. (Lima and Cavalcanti, 2014); Similarly, strains of *Lasiodiplodia theobromae* and *Nigrospora sphaerica* were identified as endophytes of *Theobroma cacao* (Vásquez *et al.*, 2018). Therefore, it would be confirmed that the genera identified in this research are endophytic fungi.

These microorganisms colonize inter and/or intracellularly the plant tissue asymptomatically; however, very little is known about the diversity and function of these fungi in species of economic importance (Russo *et al.*, 2016). Thus, Rothen (2015) showed that not all endophytic fungi isolated from *G. max* behave in this way, only 26% behave as endophytes in the strict sense, that is, they do not cause symptoms, and the rest as endophytes in broad sense (17%), biotrophic pathogens (40%) and necrotrophic pathogens (17%) that present symptoms. Therefore, future research is required for the molecular identification at the species level of the isolated strains of *V. corymbosum* and confirm their endophytic capacity. Thus, it would allow the selection of endophytic and pathogenic strains, since there are species of the genera isolated in this study that behave as pathogens of plant species, except for the genus

Trichoderma, which is used as a biocontrol agent against many phytopathogenic fungi. (Citrus, 2005). In addition, in *V. corymbosum*, species of *Lasiodiplodia* (Rodríguez-Gálvez *et al.*, 2020), *Nigrospora sphaerica* (Wright *et al.*, 2007), *Alternaria alternata* (Nadziakiewicz *et al.*, 2018), *Cladosporium tenuiussimum* were identified as pathogens. (Lei *et al.*, 2019), *Stemphylium* sp. (Flores and Medina, 2012) and *Fusarium oxysporum* (Moya-Elizondo *et al.*, 2019) with the exception of the *Aspergillus*, *Ulocladium* and *Trichoderma* genera that do not cause disease in blueberry plants. On the contrary, these genera have antifungal activity against phytopathogens (XiaoXue *et al.*, 2018). There are reports indicating that strains of the same species have a great diversity of responses to their host. Thus, some pathogens, before showing disease symptoms, are in a latent phase within the host tissue (Photita et al, 2004), such is the case of *L. theobromae* that behaves as a latent pathogen (Mohali *et al.*, 2005) and as an endophytic antagonist (Vásquez *et al.*, 2018); likewise, certain endophytes can become pathogens when the host plant is stressed, due to excessive humidity or nutrient deficiency, which induce the transition from one way of life to another (Fisher and Petrini, 1992).

In addition, in this research it was found that endophytic fungi colonized less than 20% of blueberry plant tissues, because the plants are grown in non-sterile conditions, which means that there is competition for other microorganisms (De Souza *et al.* ,2008). Likewise, the presence of species of endophytic fungi in plants can be affected by many factors, such as the type of culture, type of tissue sampled, age of the plants, climate and location in which they were grown (Impullitti and Malvick, 2013; Russo *et al.*, 2016).

The species of the genus *Trichoderma* are a group of microorganisms most investigated for the control of fungal plant diseases, they colonize the root surface or live as endophytes within the tissues and have the ability to reduce diseases through their mechanisms of action (mycoparasitism, antibiosis, competition and induced systemic resistance), promote plant growth and improve plant productivity (Bailey and Melnick, 2013). Similarly, *Aspergillus versicolor*, an endophyte of *Vaccinium dunalianum*, has inhibitory effects on phytopathogenic fungi (XiaoXue *et al.*, 2018) and has insecticidal activity (Senthilkumar *et al.*, 2014). Also, *Alternaria* species as a potential biocontrol agent for plant pathogens (Lou *et al.*, 2013) and against some agricultural pests (Sharma and Sharma, 2014). On the other hand, *Cladosporium omanense* has recently been described as a new endophytic fungus that has the ability to suppress *Pythium aphanidermatum* (Halo *et al.*, 2021). Therefore, the isolated strains of *V. corymbosum* in the Cañete valley could be possible biological control agents for pests and diseases of blueberries and other crops.

Conclusions

For the first time, nine genera of endophytic fungi (*Trichoderma*, *Aspergillus*, *Alternaria*, *Ulocladium*, *Cladosporium*, *Fusarium*, *Nigrospora*, *Lasiodiplodia* and *Stemphylium*) and a group of fungi called sterile mycelium were isolated and identified by taxonomic classification in leaves and stems of healthy plants of blueberry variety biloxi from the Cañete valley in Lima, Peru, the leaves being the ones that obtained the highest frequency of strains. The genera *Alternaria* and *Cladosporium* turned out to be the most frequent and with the highest colonization in plant tissues, while *Aspergillus*, *Ulocladium* and *Lasiodiplodia* were the least frequent.

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