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RESEARCH ARTICLE

Strains of cellulose-degrading *Trichoderma* spp. were isolated and identified from acid sulfate soil for pineapple cultivation in Vi Thanh, Hau Giang Province

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Abstract

Pineapple cultivation in Vietnam results in many byproducts that are costly to chemically decompose, while acid sulfate soil for pineapple is deficient in nutrients. *Trichoderma* spp. fungi are a more significant means of bio-decomposers in agriculture and can degrade agricultural byproducts to produce organic fertilizers for crops, which is one of the trends of sustainable agriculture. Therefore, the current study aimed to isolate *Trichoderma* spp. strains that could degrade cellulose in byproducts after pineapple harvest in Vi Thanh City, Hau Giang Province. Forty-eight soil samples for *Trichoderma* spp. Isolation was collected at 5-20 cm depth in the rhizosphere of pineapple farms in Vi Thanh City, Hau Giang province, Vietnam. The isolation was based on the *Trichoderma* Specific Medium. The isolated strains were investigated for growth rate and production of cellulose-degrading enzymes under acidic conditions (pH 4.0) and finally identified based on their Internal Transcribed Spacer regions. The results revealed that 90 *Trichoderma* spp. strains were morphologically described and found to degrade cellulose under pH 4.0. Their growth was roughly 1.50 -2.90 cm in 24 h. The key mechanism for cellulose degradation was enzymes produced by the selected fungi, in which TCD-VT-02, TCD-VT-85 and TCD-VT-88 strains had significant endo-β-1,3-glucanase, endo-β-1,4-glucanase and exo-β-1,3-glucanase productions, with 853.4, 438.7 and 320.8 UI/h, respectively. These fungal strains were identified as *Trichoderma hamatum* TCD-VT-02, *T. asperellum* TCD-VT-85 and *T. asperellum* TCD-VT-88 with 99 % similarity. These strains should be further investigated for making bio-compost from the local pineapple waste.

Keywords

by-product; cellulose; enzymes; pineapple; *Trichoderma* spp.

Introduction

Pineapple (*Ananas comosus* L.), belonging to the Bromeliaceae family, is the third most important tropical fruit plant (1), which features a short stem, long hard leaf and above-average fruit size and is usually used as food or refined as different products (2). The pineapple has flowers on a terminal spicate inflorescence, forming edible fruits (3). There are two fruiting types of pineapple: plant and ratoon pineapple (4). Its global production was 27816403 t in 2020 (5) and significantly affects the economy of many Asian countries, with an average production of 2500000 tons in 437571 ha (5, 6). In

Vietnam, pineapple is usually farmed in regions with acid sulfate soil and plays an essential role in the economy of these regions (7). These pineapple farming regions in Vietnam, mainly in Hau Giang (8), possessed 38554 ha of area and 617944 t of pineapple production (5). However, annually, pineapple cultivation results in 188.0 t/ha of dry stem and 270.6 t/ha of dry leaf (9).

Agriculture production has been making a large quantity of waste after harvesting. The unused waste, including straw, pineapple leaves and maize core, is borne by farmers, creating smoke that can severely affect human health and the environment (10). Thus, these byproducts are essential to organic cultivation and environment conservation to ensure sustainable agriculture (11). Hence, the pineapple byproducts can be recycled into organic fertilizers (12). The main component of those agricultural wastes is cellulose, which can be hydrolyzed under acidic or alkaline conditions (13, 14). Industrially, pyrolysis can also decompose cellulosic components (15). Many studies have investigated different models to improve cellulose degrading processes (16, 17). However, cellulose hydrolysis by physical or chemical approaches is complicated, costly and can harm the environment (18). On the other hand, micro-fungi are fungi that can strongly degrade cellulose by their secretion of enzymes. These enzymes belong to the O-glycoside hydrolase family that can cleave glycosidic bonds (19). These cellulases can be synthesized by mesophilic, thermophilic and extremophilic microbes, including fungi and bacteria, with or without oxygen (20).

Therein, fungi that can remarkably degrade cellulose belong to *Trichoderma* spp. (21). Most of the *Trichoderma* spp. chemotropically in soils and can degrade cellulose compounds. *Trichoderma* spp. can degrade plant residues in soils and contribute to organic metabolism (22). Particularly *Trichoderma* spp. secrete cellulase as an enzymatic complex responsible for hydrolyzing β-1,4-glucoside linkage in cellulose (23). Moreover, endoglucanase, also called endo-β-1,4-glucanase or carboxymethyl cellulase, is an enzyme that cuts the β-1,4-glucoside linkage in cellulose and some other polysaccharides into oligosaccharides (24). Thus, many *Trichoderma* spp. strains are greatly potent in degrading plant waste (25, 26). Some well-known *Trichoderma* spp. can be called *T. reesei*, *T. harzianum*, etc. (27, 28). However, soil pH can affect the abundance of soil fungi and cellulase activity (29). The optimal cellulase activity pH is slightly acidic to neutral (6.0–8.0) (30). At the same time, in the Vietnamese Mekong Delta, pineapple production is carried out on acid-sulfate soil, whose pH fluctuated from 3.5 to 5.0, particularly in Hau Giang province (31). Thus, there is a need to isolate indigenous acid-tolerance *Trichoderma* spp. strains to decompose pineapple production waste in this locality. Therefore, the study aimed at isolating, selecting and identifying cellulose-degrading *Trichoderma* spp. strains in soils for sustainable pineapple farming in Vi Thanh City, Hau Giang province.

Materials and Methods

Materials

Soil samples in healthy pineapple rhizosphere were collected in Hoa Tien and Tan Tien communes, Vi Thanh City, Hau Giang province. These acid sulfate soil samples were measured for acidity, presented in the supplementary data Table S1.

The research duration began in November 2021 and ended in June 2022 in the Microbiology Laboratory, the Experiment-Practice Section, An Giang University, Vietnam National University Ho Chi Minh City. The TSM (*Trichoderma* Specific Medium) was used to isolate *Trichoderma* spp. fungi and was composed of $MgSO_4.7H_2O$ 0.2 g, KH_2PO_4 1.18 g, KCl 0.15 g, NH_4NO_3 1.0 g, glucose 0.5 g, agar 20.0 g and distilled water for 1.0 L of medium. The PDA (Potato Dextrose Agar) consisted of: potato extract 200.0 g, sucrose 10.0 g, agar 20.0 g and distilled water in 1.0 L of medium. Both media had their pH adjusted to 6.5-6.8.

Soil sampling

Soil samples for *Trichoderma* spp. during the non-flowering stage, isolation was collected at a 5-20 cm depth (300 g/ sample) around healthy pineapple plants' root system (humid soils). Each soil sample was combined from 13 spots on a pineapple field. Twenty-four pineapple fields were investigated in each commune. The collected soil samples were stored in plastic bags, labelled and returned to the laboratory. In the laboratory, the soil was stored at 4 °C until isolation.

Trichoderma spp. isolation

Trichoderma spp. fungi were isolated according to the method of Kumar *et al.* (32). In particular, each soil sample was diluted at 95 water: 5 soil and left sediment for 24 h. Subsequently, 20.0 µL of the diluted soil solution was dropped on the surface of TSM. A sell spreader was used to scatter the solution until the surface dried. The inoculated dishes were wrapped, turned upside down and incubated at 28 ± 2 °C for 96 h. Then, mycelia with common traits of *Trichoderma* spp. were collected and purified on PDA. Strains of *Trichoderma* spp. were named based on their cellulose-degrading ability (TCD-*Trichoderma* Cellulose Degradation), isolation site (Vi Thanh - VT) and sampling order.

Investigation of Trichoderma spp. growth

The growth of the *Trichoderma* spp. strains was investigated on PDA (pH 4.0). Fungal dishes of *Trichoderma* spp. were cultured at 28±2 °C for 7 days (33). Their mycelium diameters were measured at 24, 48, 72 and 96 hours of culture.

Selection of Trichoderma spp. strains that can degrade CMC (Carboxymethyl Cellulose) under pH 4.0

Strains of the selected fungi were further experimented with for their cellulose-degrading capacity. The fungi were propagated in liquid TSM (pH 4.0) for 120 h. Then, 20.0 µL of the fungal solution was placed in wells on a petri dish containing TSM + 0.5 % CMC and cultured at 28 ± 2 °C for 120 h. After 5 days of culture, 5.0 mL of Lugol was used to dye the petri dish, at which the diameter of a CMC-degrading zone was measured (34).

Investigation of the exo-β-1,3 glucanase-producing capacity of Trichoderma spp. at pH 4.0

0.5 mL of the fungal culture was combined with 0.5 mL cellobiose 0.5 % and incubated at 50° for 2 h. Samples after incubation were added with 1.0 mL of DNS and 3.0 mL of distilled water, shaken well and boiled for 30 min. The enzymatic activity of exo-β-1,3 glucanase was measured by a spectrophotometer of UV-VIS 1900 Shimadzu at 535.0 nm wavelength (34).

Investigation of the endo-β-1,4 glucanase-producing capacity of Trichoderma spp. at pH 4.0

After propagation in liquid TSM, the fungal culture was mixed with CMC 0.5 % in a total 1 mL solution (1:1) and incubated at 50 °C for 2 h. According to the above investigation, the sample was measured for enzymatic activity (34).

Investigation of the endo-β-1,3 glucanase-producing capacity of Trichoderma spp. at pH 4.0

The fungal culture at the volume of 0.5 mL per sample was mixed with 0.5 mL of cellulose 0.5 % and incubated at 50 ° for 2 h. According to the above investigation, the sample was measured for enzymatic activity (34).

Identification of the selected fungal strains

The ITS identification of the selected *Trichoderma* spp. was from the DNA extracted from the fungal hyphae. The fungal spores were cultured for 7 days on PDA. Subsequently, the hyphae were collected into a 2.2 mL Eppendorf and incubated at room temperature for 10 min. It was then centrifuged at 13,000 rpm for 5 min to collect the cell pellet, which was later rinsed with 500.0 µL ethanol 70 %. Centrifugation at 13,000 rpm for 5 min was made again before vacuum drying. The extracted DNA was dissolved in 100.0 µL TE 0.1X. Then, the PCR was conducted with the primers ITS 1: 5'-TCCGTAGGTGAACCTGCGG-3' and ITS 4: 5'-TCCTCCGCTTATTGATATGC-3' (35). The total volume of PCR was 50 µL and went through denaturation at 95 °C for 5 min, 30 cycles (95 °C for 90 s, 52 °C for 60 s and 72 °C for 90 s) and termination at room temperature. The PCR amplicons were purified and sequenced by an automatic sequencing machine. The results were compared to those in the GenBank database by BLASTN in NCBI.

Statistical analysis

The data was processed by the Microsoft Office Excel 2019. The SPSS 13.0 was used to compare means of the Duncan test.

Results

Isolation of Trichoderma spp. fungi from acid sulfate soil for pineapple cultivation in Vi Thanh City, Hau Giang Province

***Morphology of Trichoderma spp*:** According to Table 1, 90 *Trichoderma* spp. strains were isolated from pineapple farming soils in Tan Tien and Hoa Tien communes, Vi Thanh City, Hau Giang Province. The *Trichoderma* spp. were described as follows: the fungi had septa, the conidiophores branched and were round or pyramid-shaped, the sporangia formed on the top of the conidiophores, the conidia appeared at the tip of the sporangia (Fig. 1).

Table 1. Growth diameter of the *Trichoderma* spp. fungi at 72 h of incubation

Growth diameter (cm)	Number of strains (strains)	Percentage (%)
0-8.00	9	10
8.01-8.50	41	45.5
>8.50	40	44.4

Growth of Trichoderma spp. under acidic conditions

At 24 h of culture, the fungal strains showed growth diameters from 1.50 to 2.90 cm. The strains TCD-VT-19, TCD-VT-32, TCD-VT-79, TCD-VT-82 and TCD-VT-83 had equivalent growth diameters, roughly 2.83-2.90 cm and were more significant than the others. Furthermore, the TCD-VT-02 strain had the smallest growth diameter at 1.50 cm. Likewise, at 48 h of culture, the strains TCD-VT-32 and TCD-VT-83 had the most significant growth diameters, with 7.57 and 7.47 cm, respectively. On the contrary, the strains TCD-VT-50 and TCD-VT-77 had the smallest growth diameters, with 5.60 cm. At 72 h of culture, most fungal strains shared equivalent growth diameters ranging from 8.80-9.00 cm. However, the TCD-VT-02, TCD-VT-18, TCD-VT-34, TCD-VT-44 and TCD-VT-77 had smaller growth diameters than the others, ranging from 8.50–8.58 cm. Furthermore, at 96 h of culture, fungal strains covered the surface of the Petri dishes. Based on the results at 72 h of culture, strains with growth diameters greater than 8.50 cm were chosen for the following experiment (Table 2).

Characteristics of Trichoderma spp. fungi isolated from acid sulfate soils for pineapple cultivation in Vi Thanh City, Hau Giang Province

Cellulose-degrading capacity of the Trichoderma spp. fungi : All selected 40 *Trichoderma* spp. strains can degrade cellulose, according to the degradation of CMC by the fungi, resulting in clear zones of approximately 0.90-1.90 cm. Therein, the TCD-VT-10 strain showed the greatest clear zone (1.90 cm), while the TCD-VT-53 strain showed the smallest one (0.90 cm) (Table 3).

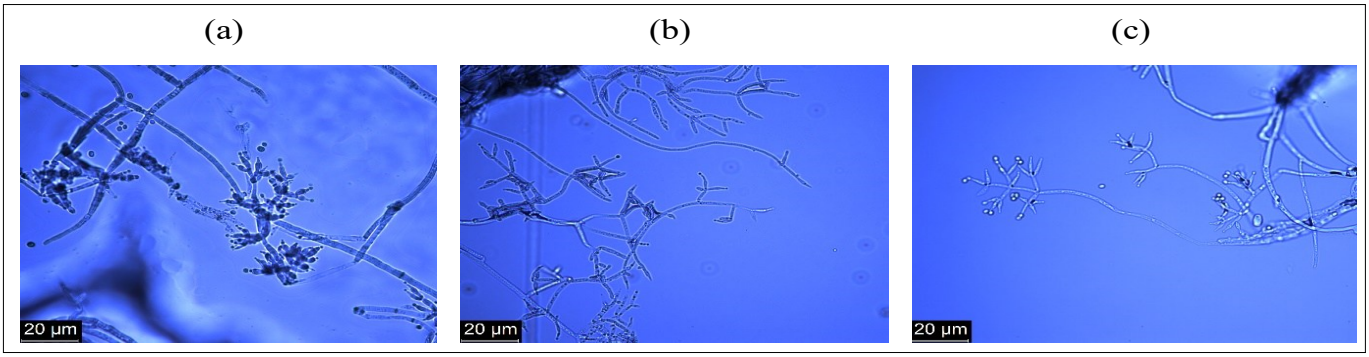

Fig. 1. Spores and conidiophores of *Trichoderma* spp. a. TCD-VT-02; b. TCD-VT-85; c. TCD-VT-88.

Table 2. Growth diameter of the *Trichoderma* spp. fungi

Strain	Growth diameter (cm)			
		48 h	72 h	96 h
TCD-VT-02	1.50^{lm}	5.85^{nop}	8.53^{c}	9.00
TCD-VT-04	2.40^{d-g}	6.20^{i-m}	8.80^{b}	9.00
TCD-VT-07	2.23^{e-i}	6.53^{e-i}	8.95^{ab}	9.00
TCD-VT-08	2.27^{e-h}	6.05^{k-o}	8.97^{ab}	9.00
TCD-VT-10	1.77^{j-l}	6.07^{j-n}	8.97^{ab}	9.00
TCD-VT-11	2.20^{f-i}	5.93^{m-p}	8.97^{ab}	9.00
TCD-VT-12	1.90^{ijk}	5.80^{nop}	8.97^{ab}	9.00
TCD-VT-14	2.40^{d-g}	6.73^{def}	8.97^{ab}	9.00
TCD-VT-15	2.10^{jkl}	6.05^{k-o}	9.00^{a}	9.00
TCD-VT-16	2.20^{f-i}	5.80^{nop}	8.95^{ab}	9.00
TCD-VT-18	2.35^{d-g}	6.40^{f-j}	8.50^{c}	9.00
TCD-VT-19	2.90^{a}	6.80^{de}	8.80^{ab}	9.00
TCD-VT-20	2.30^{e-h}	6.40^{f-j}	9.00^{a}	9.00
TCD-VT-21	1.70^{klm}	5.90^{m-p}	8.90^{ab}	9.00
TCD-VT-23	1.85^{jk}	5.70^{op}	8.97^{ab}	9.00
TCD-VT-24	2.20^{g-i}	6.33^{g-k}	8.97^{ab}	9.00
TCD-VT-25	2.33^{d-h}	6.73^{def}	8.97^{ab}	9.00
TCD-VT-27	2.47^{c-f}	6.97^{cd}	8.97^{ab}	9.00
TCD-VT-32	2.83^{ab}	7.57^{a}	8.97^{ab}	9.00
TCD-VT-34	1.80^{jkl}	5.70^{op}	8.57^{c}	9.00
TCD-VT-35	1.77^{jkl}	6.28^{h-l}	8.97^{ab}	9.00
TCD-VT-38	2.43^{c-g}	6.40^{f-j}	8.97^{ab}	9.00
TCD-VT-40	2.43^{c-g}	6.07^{j-n}	8.97^{ab}	9.00
TCD-VT-44	1.80^{jkl}	5.83^{nop}	8.58^{c}	9.00
TCD-VT-48	1.40^{m}	5.80^{nop}	8.90^{ab}	9.00
TCD-VT-50	2.20^{f-i}	5.60^{p}	8.97^{ab}	9.00
TCD-VT-53	1.90^{ijk}	5.90^{m-p}	9.00^{a}	9.00
TCD-VT-73	2.67^{a-d}	6.80^{de}	8.97^{ab}	9.00
TCD-VT-75	2.57^{b-e}	7.30^{ab}	9.00^{a}	9.00
TCD-VT-76	1.90^{ijk}	5.90^{m-p}	8.90^{ab}	9.00
TCD-VT-77	2.00^{h-k}	5.60^{p}	8.57^{c}	9.00
TCD-VT-79	2.87^{ab}	7.23^{bc}	8.97^{ab}	9.00
TCD-VT-81	2.43^{c-g}	6.63^{d-g}	8.97^{ab}	9.00
TCD-VT-82	2.90^{a}	6.80^{de}	8.90^{ab}	9.00
TCD-VT-83	2.90^{a}	7.47^{ab}	8.97^{ab}	9.00
TCD-VT-85	2.00^{h-k}	5.97^{l-o}	8.97^{ab}	9.00
TCD-VT-87	2.30^{e-h}	5.80^{nop}	8.97^{ab}	9.00
TCD-VT-88	2.20^{f-i}	6.50^{e-i}	8.97^{ab}	9.00
TCD-VT-89	2.75^{abc}	7.20^{bc}	8.97^{ab}	9.00
TCD-VT-90	2.10^{g-j}	6.60^{e-h}	8.97^{ab}	9.00
Level of significance	*	*	*	-
CV (%)	7.89	2.83	0.94	-

Note: In the same column, numbers with identical letters are not different according to Duncan test. *: different at 5 % significance; ns: no significance.

Production of cellulose-degrading enzymes by the Trichoderma spp. fungi

Exo-β-1,3-glucanase: Table 3 shows that all of the *Trichoderma* spp. stains can produce exo-β-1,3-glucanases at rates from 8.20 to 320.8 UI/h. In particular, the TCD-VT-88 strain can produce the most significant amount of enzyme. Furthermore, the TCD-VT-40 strain produced the enzyme the least. Moreover, the growth of the TCD-VT-88 strain was recorded in Fig. 2.

***Enzyme endo-β-1,4-glucanase*:** All of the *Trichoderma* spp. can produce endo-β-1,4-glucanase from 1.82-438.7 UI/h. In particular, the TCD-VT-85 had the most significant enzyme production. However, although the TCD-VT-10 strain showed the greatest clear zone, it produced the least enzyme (Table 3). Besides, the TCD-VT-85 strain had a growth stage in Fig. 3.

Endo-β-1,3- glucanase : Table 3 revealed that endo-β-1,3-glucanase production among *Trichoderma* spp. strains significantly differed. Notably, the TCD-VT-02 strain showed the most significant result, while the lowest one was the

Table 3. The cellulose-degrading capacity based on the clear zone and enzyme production by the *Trichoderma* spp. fungi

Strain	Growth diameter (cm)			
	24 h	48 h	72 h	96 h
TCD-VT-02	1.50^{lm}	5.85^{nop}	8.53^{c}	9.00
TCD-VT-04	2.40^{d-g}	6.20^{i-m}	8.80^{b}	9.00
TCD-VT-07	2.23^{e-i}	6.53^{e-i}	8.95^{ab}	9.00
TCD-VT-08	2.27^{e-h}	6.05^{k-o}	8.97^{ab}	9.00
TCD-VT-10	1.77^{j-l}	6.07^{j-n}	8.97^{ab}	9.00
TCD-VT-11	2.20^{f-i}	5.93^{m-p}	8.97^{ab}	9.00
TCD-VT-12	1.90^{ijk}	5.80^{nop}	8.97^{ab}	9.00
TCD-VT-14	2.40^{d-g}	6.73^{def}	8.97^{ab}	9.00
TCD-VT-15	2.10^{jkl}	6.05^{k-o}	9.00^{a}	9.00
TCD-VT-16	2.20^{f-i}	5.80^{nop}	8.95^{ab}	9.00
TCD-VT-18	2.35^{d-g}	6.40^{f-j}	8.50^{c}	9.00
TCD-VT-19	2.90^{a}	6.80^{de}	8.80^{ab}	9.00
TCD-VT-20	2.30^{e-h}	6.40^{f-j}	9.00^{a}	9.00
TCD-VT-21	1.70^{klm}	5.90^{m-p}	8.90^{ab}	9.00
TCD-VT-23	1.85^{jk}	5.70^{op}	8.97^{ab}	9.00
TCD-VT-24	2.20^{g-i}	6.33^{g-k}	8.97^{ab}	9.00
TCD-VT-25	2.33^{d-h}	6.73^{def}	8.97^{ab}	9.00
TCD-VT-27	2.47^{c-f}	6.97^{cd}	8.97^{ab}	9.00
TCD-VT-32	2.83^{ab}	7.57^{a}	8.97^{ab}	9.00
TCD-VT-34	1.80^{jkl}	5.70^{op}	8.57^{c}	9.00
TCD-VT-35	1.77^{jkl}	6.28^{h-l}	8.97^{ab}	9.00
TCD-VT-38	2.43^{c-g}	6.40^{f-j}	8.97^{ab}	9.00
TCD-VT-40	2.43^{c-g}	6.07^{j-n}	8.97^{ab}	9.00
TCD-VT-44	1.80^{jkl}	5.83^{nop}	8.58^{c}	9.00
TCD-VT-48	1.40^{m}	5.80^{nop}	8.90^{ab}	9.00
TCD-VT-50	2.20^{f-i}	5.60^{p}	8.97^{ab}	9.00
TCD-VT-53	1.90^{ijk}	5.90^{m-p}	9.00^{a}	9.00
TCD-VT-73	2.67^{a-d}	6.80^{de}	8.97^{ab}	9.00
TCD-VT-75	2.57^{b-e}	7.30^{ab}	9.00^{a}	9.00
TCD-VT-76	1.90^{ijk}	5.90^{m-p}	8.90^{ab}	9.00
TCD-VT-77	2.00^{h-k}	5.60^{p}	8.57^{c}	9.00
TCD-VT-79	2.87^{ab}	7.23^{bc}	8.97^{ab}	9.00
TCD-VT-81	2.43^{c-g}	6.63^{d-g}	8.97^{ab}	9.00
TCD-VT-82	2.90^{a}	6.80^{de}	8.90^{ab}	9.00
TCD-VT-83	2.90^{a}	7.47^{ab}	8.97^{ab}	9.00
TCD-VT-85	2.00^{h-k}	5.97^{l-o}	8.97^{ab}	9.00
TCD-VT-87	2.30^{e-h}	5.80^{nop}	8.97^{ab}	9.00
TCD-VT-88	2.20^{f-i}	6.50^{e-i}	8.97^{ab}	9.00
TCD-VT-89	2.75^{abc}	7.20^{bc}	8.97^{ab}	9.00
TCD-VT-90	2.10^{g-j}	6.60^{e-h}	8.97^{ab}	9.00
Level of significance	*	*	*	-
CV (%)	7.89	2.83	0.94	-

Note: In the same column, numbers with identical letters are not different according to Duncan test. *: different at 5 % significance; ns: no significance

TCD-VT-38 strain. In addition, the TCD-VT-88 strain was also found to have great endo-β-1,3- glucanase production, with 316.6 UI/h.

Identification of the selected cellulose-degrading Trichoderma spp. strains

All the selected fungal strains were TCD-VT-02, TCD-VT-85 and TCD-VT-88 based on their ITS regions. They were *T. hamatum* TCD-VT-02, *T. asperellum* TCD-VT-85 and *T. asperellum* TCD-VT-88, with 99 % similarity, with their accession numbers of PP574629, PP574630 and PP574631, respectively (Fig. 4).

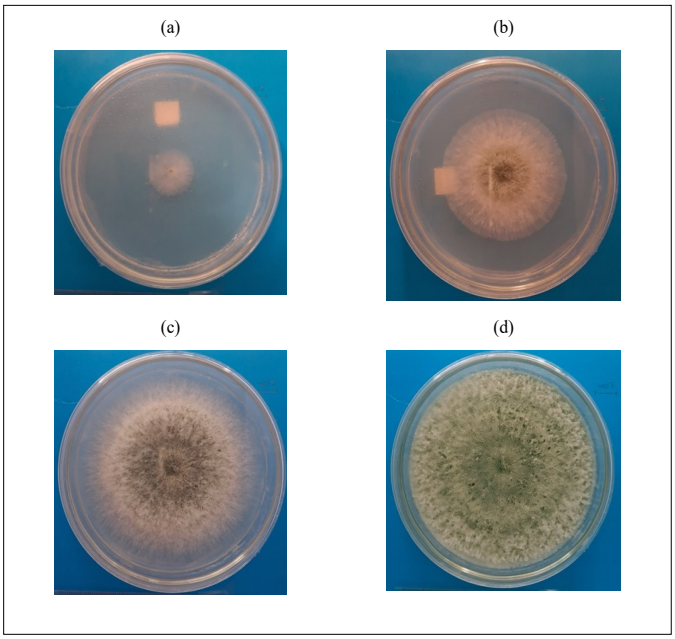

Fig. 2. Growth of *Trichoderma* sp. TCD-VT-85 at a. 24; b 48; c. 72; d. 96 h of incubation.

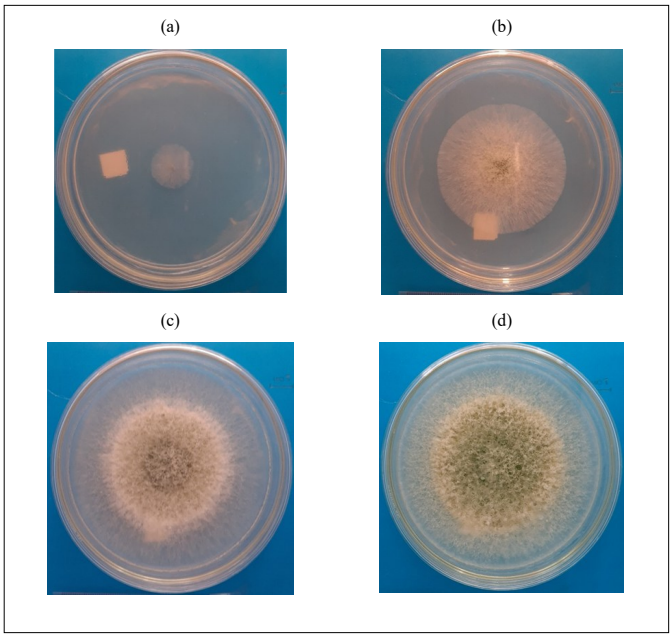

Fig. 3. Growth of *Trichoderma* sp. TCD-VT-88 at a. 24; b 48; c. 72; d. 96 h of incubation.

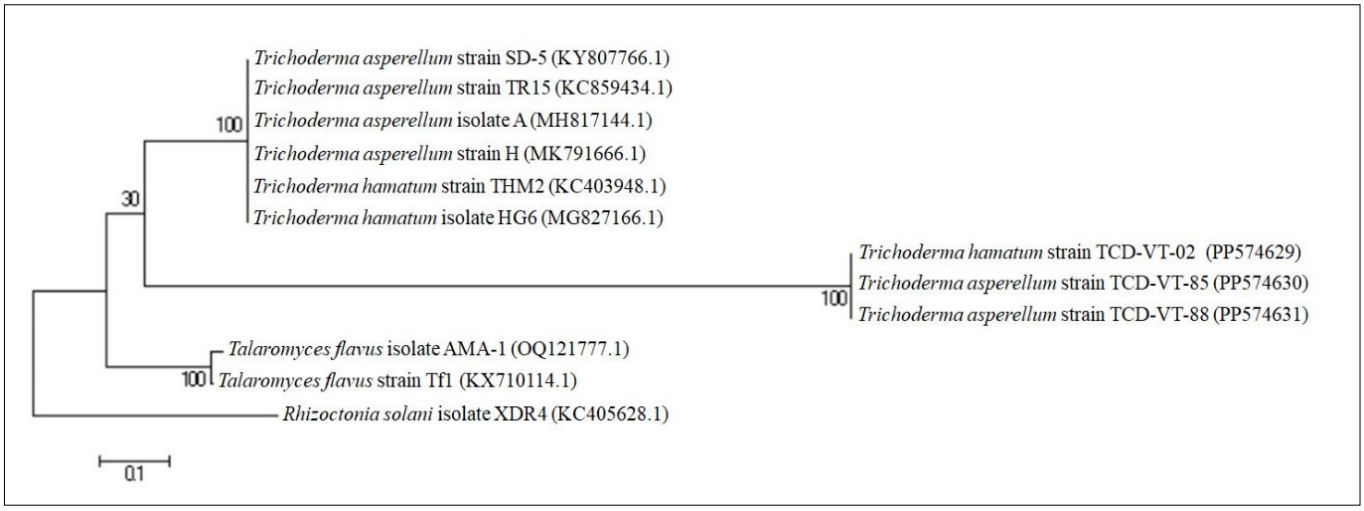

Fig. 4. Neighbor-joining phylogenetic trees based on ITS sequences of the three selected *Trichoderma* spp. TCD-VT-02, TCD-VT-85 and TCD-VT-88 compared to the closely related strains in the GenBank database. The percentage levels of bootstrap analysis of 1,000 replicates are indicated at each node. Bar, 0.1 substitutions per nucleotide position. *Rhizoctonia solani* isolate XDR4 was used as the outgroup strain. Access numbers of GenBank sequences are implied in brackets.

Discussion

In Tables 1 and 2, 90 isolated *Trichoderma* spp. strains can survive and grow on acidic PDA. The strains growth fast on acid PDA reaching 1.50-2.90 cm at 24h, 5.60-7.57 cm at 48h, 8.80–9.00 cm at 72h. This means that these 90 isolated *Trichoderma* spp. strains adapted to the acidic conditions. Among them, 40 strains with significant growth were chosen. Research indicates that *Trichoderma* spp. strongly grow when the conditions are 25 °C and low pH (4.0-5.5) (36). Likewise, the *Trichoderma* spp. strains isolated in Vi Thanh, Hau Giang grew well under an acidic condition (pH 4.0). However, unlike the current study, some previous studies tested *Trichoderma* spp. strains under greater pH. For example, research showed that the optimal hydrolysis pH was 5.0 and the most significant enzyme activity was 6.0 (37). Research indicates that the mediums' pH was above 6.0 (38). Furthermore, greater pH shows greater cellulases' activities under applying *T. guizhouense*, while some cellulases show more significant activity under acidic conditions (39). This raises a need for a specific cellulose degrader for a particular pH condition. In other words, the pH of the soil remarkably influences soil characteristics, e.g. nutrient availability and microbial composition, leading to direct or indirect effects on the cellulose degradation process in the soil by microbes (40).

In the current study, selected strains of *Trichoderma* spp. can produce cellulase that can degrade CMC substrate and form clear zones. The TCD-VT-10 strain exhibited the most significant result with 1.90 cm (Table 3). *T. reesei* strains can degrade CMC because *T. reesei* can produce cellulase to hydrolyze cellulose (41). This shows the potential of *Trichoderma* spp. in degrading cellulose from plant residues and reusing it in agriculture. Moreover, apart from cellulose degradation, *Trichoderma* spp. can also be a plant growth promoter and a biocontrol agent (42). Besides acidic conditions, *Trichoderma* spp. can also live under different adverse conditions, such as saline, drought, high temperature, low temperature, toxic, etc. (42). Therefore, they can stimulate plant growth under such conditions. Therefore, the select *Trichoderma* spp. strains are promising cellulose-degrading agents for pineapple wastes in acid-sulfate soil.

All 40 *Trichoderma* spp. strains can produce exo-β-1,3-glucanase, endo-β-1,4-glucanase and endo-β-1,3-glucanase, with the rates ranging from 8.20–320.8, 1.82–438.7 and 7.57-853.4 UI/h, respectively. Therein, the TCD-VT-88 strain produced endo-β-1,4-glucanase the greatest, the TCD-VT-85 strain produced endo-β-1,4-glucanase the best and the TCD-VT-02 strain produced endo-β-1,3-glucanase the best (Table 3). A previous study showed that the endo-1,4-β -xylanase and endo-1,4-β-glucanase can be produced by *T. citrinoviride* (43). Cellulase is a multifunctional enzyme made of cellobiohydrolase, endoglucanase and β-glucosidase and participates in the degradation of organic matter. For instance, *T. reesei* can produce the above enzymes to degrade cellulose (44). Moreover, *T. reesei* hyphae contain many enzymes, such as cellulase, hemicellulase and proteins, to degrade cellulose and other biomass matter, leading to its wide use in industrial cellulase production (45). Cellobiohydrolase and endoglucanase cooperate to degrade cellulose into cellobiose and then β-glucosidase degrades the oligosaccharide into glucose (46). *T. guizhouense* isolated from the substrate for fungi cultivation showed the most significant cellulase activity after 72 hours of incubation on wheat straw (roughly 0.70 UI/mL) (32). After 7 days of culture, *T. reesei* QPE36 had an enzyme content of 5.8 UI/mL (47). *T. harzianum* IOC3844 can strongly degrade because of the high gene expression related to cellulose and hemicellulose degradation, enzyme activity and enzyme production (48, 49). *Trichoderma* spp. can improve crop yield, induce resistance against non-biotic stresses, enhance uptake, ameliorate nutrient availability and promote plant growth and root development (50). Moreover, these cell well degrading enzymes can be a biocontrol mechanism of *Trichoderma* spp. against plant pathogens (42).

Conclusion

Ninety *Trichoderma* spp. strains that can live under pH 4.0 were isolated from 48 soil samples in Tan Tien and Hoa Tien communes of Vi Thanh City, Hau Giang Province. Among them, the selection resulted in the TCD-VT-02 strain with the greatest endo-β-1,3- glucanase production (853.4 UI/h), the TCD-VT-85 strain with the greatest endo-β-1,4-glucanase production (438.7 UI/h) and the TCD-VT-88 strain with the β-1,3-glucanases production (320.8 UI/h). The selected *Trichoderma T. hamatum* TCD-VT-02, *T. asperellum* TCD-VT-85 and *T. asperellum* TCD-VT-88 were identified according to their ITS regions with a 99 % similarity. The selected *Trichoderma* spp. should be tested to degrade plant residues and provide nutrients for pineapple fields.

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Authors' contributions

NDT conducted the sampling, participated in the sequence alignment and drafted the manuscript. TCN carried out the sampling and fungi culture. LNTX participated in the sequence alignment. NTHN participated in the study design and performed the statistical analysis. NXD conceived of the study and participated in its design and coordination. LTMT carried out the sampling and biochemical tests. LHMT carried out the sampling and biochemical tests. VMT carried out the sampling and the fungi culture. LTQ carried out the fungi culture and manuscript revision and editing. NQK participated in the study's design, performed the statistical analysis and revised the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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