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Original Article

Streptomyces flaveus as a potential biocontrol agent due to its ability to degrade fungal biomass and colloidal chitin♣

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Abstract

Crop plant diseases caused by oomycetes and fungi have caused a lot of environmental, economic, and health damage to mankind. *Streptomyces flaveus* has been known to be able to suppress the growth of many oomycetes and fungal plant pathogens due to its ability to produce SW-B, an antibiotic. However, this antibiotic was reported not to be active enough to suppress the growth of a number of other fungal plant pathogens. In this paper, we demonstrate that *S. flaveus* RT1-1 was able to degrade fungal biomass and colloidal chitin due to producing chitinases. This promotes the potential of *S. flaveus* RT1-1 as a biocontrol agent that could help in achieving many sustainable development goals, to the benefit of mankind.

Keywords: *Streptomyces flaveus*, chitinase, antifungal, *Neoscytalidium dimidiatum*, *Colletotrichum truncatum*

1. Introduction

Vietnam has more than 80% of its employment in farming, so agricultural industries play an important role in the national economy. Besides improving the quantity and quality of agricultural products for domestic consumption, many agricultural products of high economic value are exported. However, in the recent years, crop diseases have greatly affected the quantity as well as quality of crops, making it difficult to export agricultural products. Aside from insect pests, plants often suffer from diseases caused by a wide range of pathogenic microorganisms that are fungi, bacteria, protozoa, and viruses. These diseases reduce the plants' ability to grow and can cause them to die. Plant diseases also appear around the world and are causing yield losses of 21.5% for wheat, 30% for rice, 22.5% for maize, 17.2% for potato, and 21.4% for soybean at the global level, being a significant threat to food security (Savary *et al.*, 2019). Among the microorganisms that cause plant diseases, fungi dominate (Doehlemann, Ökmen, Zhu, & Sharon, 2017).

Colletotrichum gloeosporioides, which belongs to the *Colletotrichum* genus, is able to cause anthracnose disease, and it causes a lot of damage to a large number of crops such as cereals, coffee, legumes, etc.; as well as to tropical and subtropical fruits such as chili, papaya, avocado, banana, mango, rose apple, citrus, strawberry, grape, longan, durian, etc. (Sharma, & Kulshrestha, 2015). Currently, the main method of preventing anthracnose disease in crops and valuable fruits in Vietnam still involves the use of fungicides such as Bavistin 50SL, Carbezim 50EC, or Benlate 50WG. The active substance in both Bavistin 50SL and Carbezim 50EC is carbendazim, and benomyl is the antifungal component of Benlate 50WG. After decomposition, benomyl forms carbendazim, which is known to cause embryotoxicity, cell death, teratogenicity, infertility, liver-cell dysfunction, endocrine dysfunction, as well as disruption of microbial communities in different ecosystems (Singh *et al.*, 2016).

Besides anthracnose, brown spot disease caused by *Neoscytalidium dimidiatum* in dragon fruit plants has also been of environmental and economic concerns. Discovered in Vietnam a decade ago, the disease has been prevented and controlled drastically in the localities where dragon fruit is

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grown according to a technical process of disease management, in which attention is paid to the use of *Trichoderma* combined with the spraying of copper-based active ingredients such as copper oxide, copper hydroxide, copper sulfate, etc. and synthetic chemical substances such as azoxystrobin, difenoconazole, hexaconazole, mancozed, metalaxyl, etc. *Trichoderma* has a good competition for living space against the fungus *N. dimidiatum* (Rusmarini, Shah, Abdullah, Mamat, & Hun, 2017). Copper-based substances, however, have the ability to damage fungal cells, thereby inhibiting the growth of pathogenic fungi (Oziengbe, & Osazee, 2015). Use of the chemical substances mentioned above has the potential risk of destroying the biological control in nature, polluting the living environment, and negatively affecting human health (Jørgensen, Kjær, Olsen, & Rosenbom, 2012; Moreira *et al.*, 2017). On the other hand, the fungus *N. dimidiatum* is also capable of causing dangerous respiratory diseases in humans (Dionne *et al.*, 2015) and of infecting other human-organs (Garinet, *et al.*, 2015; Razavyoon *et al.*, 2022; Shih-Jyun *et al.*, 2019). Nevertheless, the brown spot disease on dragon fruit plants has not yet been treated successfully. The disease is considered a problem for farmers in dragon fruit-growing areas across the country, especially in provinces that grow a lot of dragon fruits such as Binh Thuan, Tien Giang, and Long An.

The biological control of plant pathogens through the use of fungal antagonists is a promising alternative to fungicides (Wang, Sui, Li, Tian, & Wang, 2022). Biological control agents can be produced as dust, granules or liquid suspensions for direct application to plant roots or soil, or for spraying onto leaves, stems or fruits. Biological control agents can inhibit fungal growth in plants by competing for nutrients, or occupying vegetative space, or by producing compounds that compete for nutrients with plant pathogens such as siderophores competing for iron ions in the environment, or by creating compounds that help plants live well when infected with pathogens such as indole-3-acetic acid (IAA), which regulates plant growth and development. The biological control agent may also have the ability to solubilize the plant's phosphate, or the ability to produce antibiotics to inhibit or kill the plant pathogens, or to produce enzymes that are capable of hydrolyzing fungal cell structures such as chitinases, glucanases and proteases.

Streptomyces flaveus is an actinomycete known to have the ability to suppress the growth of several plant pathogens. *S. flaveus* strain A-11, which was reported to produce the antibiotic SW-B that was shown to have strong antifungal activity against plant harming oomycetes and fungi such as *Phytophthora capsici*, *Magnaporthe grisea*, *Cladosporium cucumerinum*, and *Alternaria mali* (Hwang, Lee, Kim, & Moon, 1996). Methanol culture extract from solid agar culturing of this strain also effectively inhibited the growth of many other crop-damaging fungi such as *Sclerotinia clerotiorum*, *Alternaria solani*, *Botryosphaeria dothidea*, *Cercospora capsici* and *Rhizoctonia solani*. However, the antibiotic produced by this strain was found not to effectively suppress the growth of *Colletotrichum gloeosporioides* (Yeop, Kim, & Hwang, 1995).

Our *S. flaveus* strain, RT1-1, originated from soil (Phan, Huynh, Phan, & Vo, 2015), and was able to well inhibit the growth of a *Colletotrichum* species isolated from the anthracnose diseased longan (Phan, Vo, & Do, 2021). The strain RT1-1 was also able to suppress the growth of *N. dimidiatum*, a fungus that causes the brown spot disease in dragon fruit plants (Phan, & Truong, 2018).

In this paper, we show that *S. flaveus* strain RT1-1 is capable of degrading fungal biomass and colloidal chitin.

2. Materials and Methods

2.1 Microorganisms

N. dimidiatum was isolated previously from the dragon fruit stems collected from Binh Thuan province, Vietnam (Phan & Truong, 2018). *C. truncatum* was also isolated in our laboratory from the anthracnose diseased chili peppers. *S. flaveus* strain RT1-1 was previously isolated from soil (Phan *et al.*, 2015).

2.2 Media and culture fermentation

Potato-dextrose broth (PDB) and Potato-dextrose agar (PDA) were used to grow the fungi and to test for the actinomycete's ability to suppress the fungal growth, respectively. An amount of 200 g of peeled and water-rinsed potato was sliced and placed in a pot, then one liter of distilled water was added. The potatoes were boiled for 30 minutes, then filtered through a cheesecloth, and the effluent was collected. To obtain the PDB, the effluent was added with glucose (20 g/L) and distilled water to reach 1 liter. pH of the medium was adjusted to 5.6 ± 0.2. For PDA, 15 g/L of purified agar was added. The medium was sterilized at 121◦C for 15 minutes. To obtain PDA plates, the medium after sterilizing was allowed to cool to 50 ◦C then 20 mL volumes were distributed to separate sterile Petri plates.

Starch-casein agar (SCA) contained soluble starch (10 g/L), casein (0.3 g/L), KNO_3 (2 g/L), NaCl (2 g/L), K_2HPO_4 (2 g/L), $MgSO_4.7H_2O$ (0.05 g/L), $CaCO_3$ (0.02 g/L), and $FeSO_4.7H_2O$ (0.01 g/L), and the pH was adjusted to 6.8 ± 0.2. The purified agar concentration was 15 g/L. The medium was autoclaved at 121ºC for 15 minutes. SCA plates were prepared as described above.

Colloidal chitin medium contained yeast extract (4 g/L), glucose 20 g/L and colloidal chitin (1%, v/v), with pH adjusted to 6.8 ± 0.2; it was used to culture *S. flaveus*. To 25 ml of liquid colloidal chitin medium in a 250 mL flask, the *S. flaveus* spores were inoculated. The flask was shaken on an orbital shaker at 120 rpm and 30 ºC for 48 h, then 2 mL of the culture was transferred to another 250 mL flask containing 25 mL of fresh colloidal chitin medium. The flask was shaken on an orbital shaker at 120 rmp and 30 ºC. The broth was collected every 24 h during the fermentation time course or after fermentation, and was centrifugated at 4000 rpm for 20 minutes to obtain the supernatant. The *S. flaveus* supernatant was directly used for *in vitro* chitinase test or was concentrated at 45 ºC using a rotary evaporator for 4 h for testing the ability to degrade fungal biomass. Colloidal chitin containing agar plates were prepared as described above.

Enhancement of chitinase production ability of *S. flaveus* by using chitin was examined by supplementing the colloidal chitin and fungal cell powder to the starch-casein broth (SCB) medium at the ratios of 1:3, 1:0 and 0:3 such that their total concentration in the medium was 10 g/L. *S. flaveus* spores were inoculated into 25 mL of SCB medium in a 250

mL flask. The flask was shaken at 30 ºC on an orbital shaker at 120 rpm for 30 h then 0.75 mL was transferred into another 250 mL flask containing 25 mL of fresh SCB supplemented with colloidal chitin and fungal cell powder. The flask was shaken on an orbital shaker at 120 rpm and 30 ºC. Culture broth was collected every 12 h during the fermentation time course. The collected samples were subjected to comparison of chitinase amounts at different time points during the fermentation.

2.3 Antifungal activity test

In testing the actinomycete's ability to suppress the fungal growth, fungal spores were inoculated onto the center of a PDA plate. At the same time, actinomycete spores were inoculated at three surrounding positions within the plate, about 2.5 cm from the center. The plate was inverted and incubated at 30 ◦C. Transparent areas around the actinomycete colonies were checked, if present, every day.

In testing the ability of the *S. flaveus* supernatant to degrade the fungal biomass, *C. truncatum* spores and mycelia from the PDA plate were inoculated into a 250 mL flask containing 50 mL of PDB. The flask was shaken at 30 ºC for 72 h on an orbital shaker at 120 rpm. The flask was shaken well and every 1 mL of the culture broth was taken and added to different sterile test tubes. Different volumes (0.5, 1, 2 and 3 mL) of the concentrated *S. flaveus* supernatant were then added into these tubes. The control tube contained fungal culture broth only. The tubes were covered with pieces of nylon and kept at 30 ºC for 3 days.

2.4 Colloidal chitin preparation

Chitin powder was first prepared by washing dried shrimp shells well with distilled water. The shells were then treated with 4% NaOH solution at 70 ºC for 4 hours and washed with distilled water to eliminate proteins, then with 8% HCl solution at room temperature for 16 hours to eliminate minerals, rinsed with distilled water, then dried at 70 ºC until constant weight and ground in a blender to obtain chitin powder. Colloidal chitin 1% (w/v) was prepared using the chitin powder as described previously (Dai, Hu, Huang, & Li, 2011).

2.5 *In vitro* chitinase test

The test was carried out on agar plates containing colloidal chitin to examine the presence of chitinases in the *S. flaveus* supernatant. 8-mm wells were created on colloidal chitin containing agar plates using a sterile hollow metal tube. 100 µL of the *S. flaveus* supernatant was added into each well. The plates were incubated at 30 ºC for 12 to 24 h, then the agar surfaces were covered with 1% Lugol solution and checked for a clear zone around the wells.

2.6 Fungal cell powder preparation

N. dimidiatum spores and mycelia from the PDA plate were inoculated into a 250 mL flask containing 150 mL of PDB. The fungus was cultured at 30 ºC for 72 h on an orbital shaker at 120 rpm, then sterilized at 121 ºC in 45 minutes, allowed to cool and filtered through Whatman filter paper No. 1 to collect mycelia. The mycelia were washed three times with distilled water, dried to constant weight in an oven at 70°C. The dried mycelia were then ground in a sterile stone mortar using a sterile stone pestle to obtain fungal powder.

2.7 Chitinase amount variability

Variability in the chitinase amount in the flask culture during the fermentation time course was evaluated using 3,5 dinitrosalicylic acid (DNS) as described previously (Hussin & Majid, 2020) with some modifications. One mL of sample containing chitinase in a tube was added with 1 mL of 1% colloidal chitin. The tube was boiled in a water bath at 40 ºC for 60 minutes, then 3 mL of DNS reagent was added. The tube was then boiled at 100 ºC in the water bath for 20 minutes, cooled and centrifuged at 4,000 rpm for 10 minutes**.** Supernatant was collected and optical density **(**OD**)** at 540 nm of the supernatant was spectrophotometrically measured**.** Each sample was carried out in triplicates, which resulted in OD_1, OD_2 and OD_3. A blank tube that contained no enzyme was done in parallel, which resulted in OD_0. The differences $\Delta OD_i = OD_i - OD_0$, (i = 1, 2, 3) were calculated. The ΔOD_{avr} of each sample was obtained by averaging these three ΔOD_i values.

3. Results and Discussion

Figure 1 below shows symptoms of the brown spot disease in dragon fruit stems and the anthracnose in chili, which were caused by *N. dimidiatum* and *C. truncatum*, respectively. Figure 2 shows morphology of *S. flaveus* strain RT1-1, which was demonstrated to suppress the growth of *N. dimidiatum* and *C. truncatum* on PDA (Figure 3). In Figure 3, in comparison with the controls, which freely grew outward from the center of the plate, the fungal growth in the plates on which the fungi were co-cultured with *S. flaveus* was suppressed, as shown by no mycelia around the *S. flaveus* colonies. This may be due to diffused substances produced by the *S. flaveus*, which negatively acted on the fungal cells and thus suppressed the fungal growth.

It was reported that the *S. flaveus* strain A11 could inhibit many plant pathogens including fungi and oomycetes (Yeop *et al.*, 1995). SW-B, a manumycin-type antibiotic that was produced by this strain, was found to be responsible for this suppression (Hwang et al, 1996). However, SW-B acted on *Colletotrichum* species very weakly (Yeop *et al.*, 1995). We asked how could *S. flaveus* strain RT1-1 act very well on *C. truncatum* (Figure 3B) and whether it could disrupt the fungal cell structures. We therefore obtained concentrated extracellular substances produced by *S. flaveus* strain RT1-1 and added these concentrated materials into the liquid culture of *C. truncatum*. We surprisingly found that the fungal biomass was degraded (Figure 4).

We suspected that the fungal cell wall could have been heavily decomposed by the materials in the supernatant. As the *C. truncatum* cell wall is mainly composed of chitin, we suspected chitinases could have acted on the cell wall of this fungus. Therefore, we tested for the presence of chitinases in the *S. flaveus* supernatant by inducing this microorganism with chitin. Colloidal chitin medium was used to culture *S. flaveus* RT1-1. After the fermentation, the culture broth was

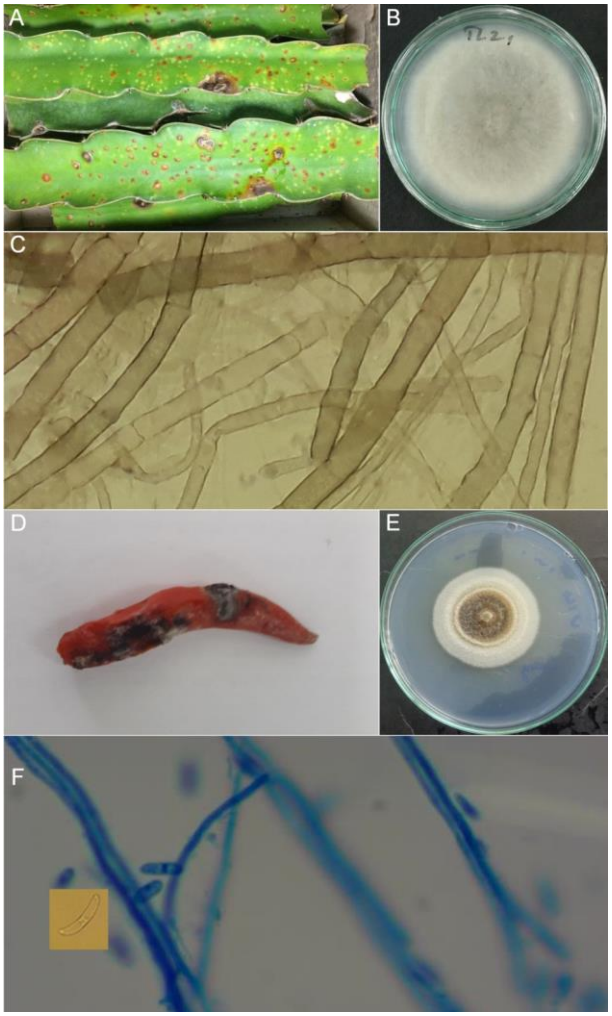

Figure 1. Brown spot disease in dragon fruit stem and anthracnose in chili. (A) Brown spot disease symptoms observed in the field. (B) The five-day-old *N. dimidiatum* isolated from the brown spot disease stem on PDA plate; (C) *N. dimidiatum* hyphae viewed under light microscope; (D) Symptoms of chili pepper anthracnose disease caused by *C. truncatum*; (E) The five-day-old *C. truncatum* isolated from the anthracnose diseased chili on PDA plate; (F) *C. truncatum* hyphae and spores stained with lactophenol cotton blue (Inset: a spore without staining under the light microscope)

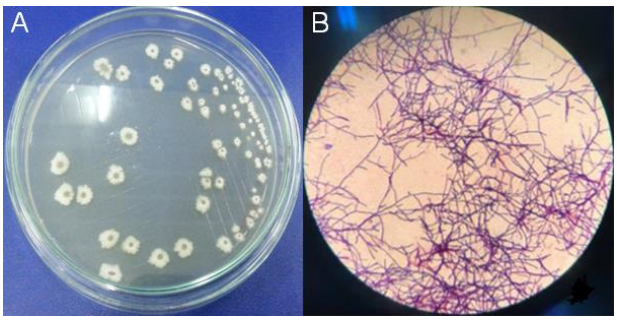

Figure 2. The actinomycete *S. flaveus*. (A) The seven-day-old colonies on the SCA plate; (B) Gram-stained hyphae observed under the light microscope

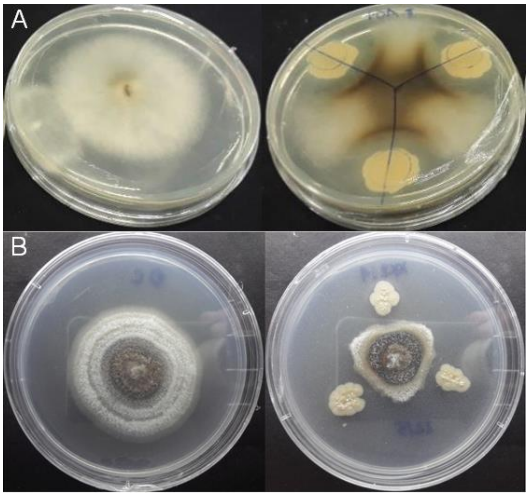

Figure 3. Fungal growth suppression by *S. flaveus*. (A, bottom view) The three-day-old *N. dimidiatum* on the PDA plate; (B, top view) The seven-day-old *C. truncatum* on the PDA plate. Left: control (only fungus); Right: fungus co-cultured with *S. flaveus*

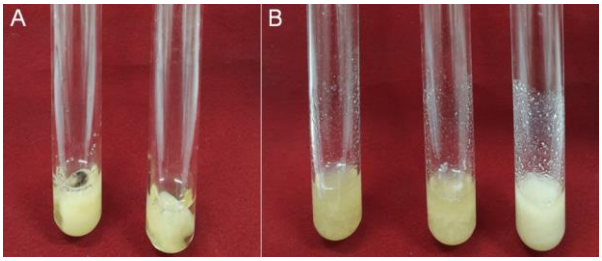

Figure 4. Fungal biomass degradation by *S. flaveus* concentrated supernatant. (A) Controls: *C. truncatum* mycelia only in liquid culture (in duplicates). (B) Fungal mycelia in liquid added with 3 mL of the *S. flaveus* concentrated supernatant (in triplicates)

centrifuged and the supernatant was obtained for an *in vitro* chitinase test. Figure 5 shows that, when *S. flaveus* was induced by colloidal chitin, chitinases were indeed produced extracellularly. In this *in vitro* test, the agar contained chitin substrate, and if chitinases were present, the chitin would be degraded so that there was no chelation forming between chitin and I_2/KI of Lugol solution. Thus, the region that contained chitinases was not stained in the purple-pink color of Lugol solution. Notably, chitinases were created very little at the beginning of the fermentation. Their amount increased in the late phases, as indicated by the bigger clear zones seen in Figure 5.

In order to determine if the *S. flaveus* secreted chitinases could be capable of utilizing fungal chitin, we grew the fungus *N. dimidiatum* in PDB medium (Figure 6A) to obtain fungal cell powder (Figure 6B). We supplemented this fungal cell powder into the SCB medium at different ratios with colloidal chitin to culture *S. flaveus* RT1-1. We found that the fungal powder indeed affected the production of chitinase by *S. flaveus* RT1-1.

Figure 7 shows that cultures supplemented with colloidal chitin and chitin powder at a ratio of 1:3 show better production of chitinases. On the other hand, the medium at

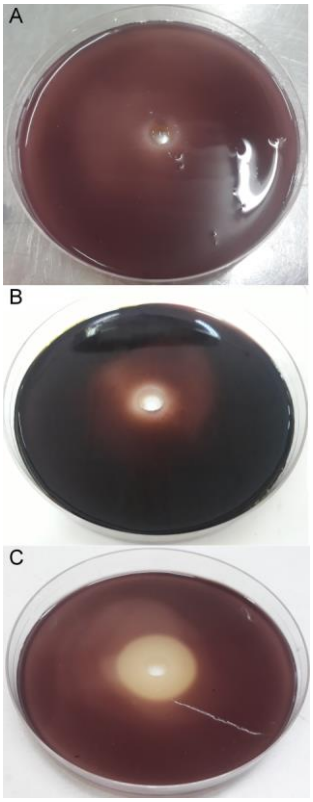

Figure 5. The *S. flaveus* fermentation broth supernatant degraded chitin *in vitro*. Agar medium containing colloidal chitin was in contact with the *S. flaveus* supernatant after (A) 48 h, (B) 72 h, and (C) 96 h of incubation

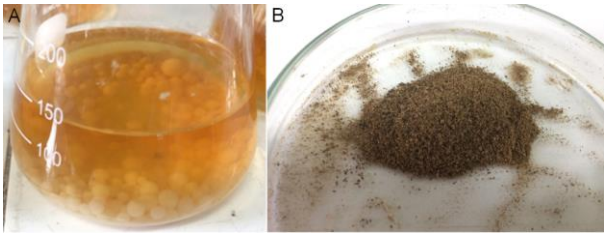

Figure 6. *Neoscytalidium dimidiatum*. (A) Broth after 72 h fermentation. (B) Fungal cell powder

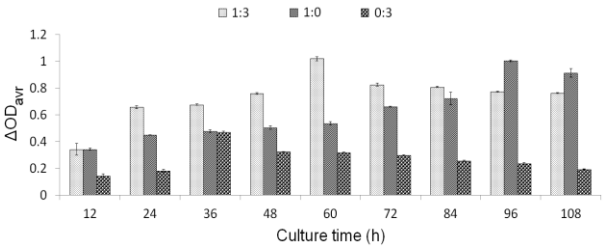

Figure 7. Variation in chitinase production by *S. flaveus* during the fermentation time course. ΔOD_{avr} represents the difference between OD values obtained from the sample and the blank without chitinase. 1:3, 1:0 and 0:3 are the ratios between colloidal chitin and fungal cell powder supplemented into the culture media.

this ratio resulted in earlier production of chitinase in comparison with that supplemented with colloidal chitin only (ratio 1:0). At ratio 0:3, the culture was observed to be very viscous, which might have made it difficult for oxygen to get access into the medium for the actinomycete to respirate. This explains why less chitinases were produced at this ratio.

Streptomyces capable of producing chitinases have been studied extensively, such as *Streptomyces viridificans* (Gupta, Saxena, Chaturvedi, & Virdi, 1995), *Streptomyces aureofaciens* CMUAc130 (Taechowisan, Peberdy, & Lumyong, 2003), and *Streptomyces* sp. ANU 6277 (Narayana, & Vijayalakshmi, 2009), but the ability of *S. flaveus* to produce chitinase is for the first time reported here.

4. Conclusions

We have presented that *S. flaveus* RT1-1 was able to degrade colloidal chitin and fungal biomass as the strain secreted chitinases, a property that has not been reported before for *S. flaveus*. This property contributed to make the *S. flaveus* RT1-1 an especially potent candidate for biocontrol agent. Developing biocontrol products based on this microorganism would help achieve many sustainable development goals, such as helping mankind have good health and well-being, clean water, and sanitation. The eventual product could also help in responsible material consumption and production, thereby protecting life on earth.

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References

Dai, D., Hu W., Huang G., & Li, W. (2011). Purification and characterization of a novel extracellular chitinase from thermophilic *Bacillus* sp. Hu1. *African Journal of Biotechnology*, *10*(13), 2476-2485. Retrieved from https://doi.org/10.5897/AJB10.1029

Dionne, B., Neff, L., Lee, S. A., Sutton, D. A., Wiederhold, N. P., Lindner, J., . . . Jakeman, B. (2015). Pulmonary fungal infection caused by *Neoscytalidium dimidiatum*. *Journal of Clinical Microbiology*, *53*(7), 2381-2384. Retrieved from doi:10.1128/JCM.00206-15

Doehlemann, G., Ökmen, B., Zhu, W., & Sharon, A. (2017). Plant pathogenic fungi. *Microbiology Spectrum*, *5*(1), 1-23. doi:10.1128/microbiolspec.FUNK-0023-2016

Garinet, S., Tourret, J., Barete S., Arzouk N., Meyer, I., Frances, C., . . . Fekkar, A. (2015). Invasive cutaneous *Neoscytalidium* infections in renal transplant recipients: a series of five cases. *BMC Infectious Diseases*, *15*, 535. doi:10.1186/s12879-015-1241-0

Gupta, R., Saxena, R. K., Chaturvedi, P., & Virdi, J. S. (1995). Chitinase production by *Streptomyces viridificans*: Its potential in fungal cell wall lysis. *Journal of Applied Bacteriology*, *78*(4), 370-383. doi:10.1111/j.1365-2672.1995.tb03421.x

Hussin, N. A., & Majid, A. H. A. (2020). Termiticidal activity of chitinase enzyme of *Bacillus licheniformis*, a symbiont isolated from the gut of *Globitermes sulphureus* worker. *Biocatalysis and Agricultural Biotechnology*, *24*, 101548. Retrieved from https://doi.org/10.1016/j.bcab.2020.101548

Hwang, B. K., Lee, J. Y., Kim, B. S., & Moon, S. S. (1996). Isolation, structure elucidation, and antifungal activity of a manumycin-type antibiotic from *Streptomyces flaveus*. *Journal of Agricultural and Food Chemistry*, *44*(11), 3653-3657. Retrieved from https://doi.org/10.1021/jf960084o

Jørgensen, L., F., Kjær, J., Olsen, P., & Rosenbom, A. E. (2012). Leaching of azoxystrobin and its degradation product R234886 from Danish agricultural field sites. *Chemosphere*, *88*(5), 554-562. doi:10.1016/j.chemosphere.2012.03.027.

Moreira, R. A., Daam, M., Vieira, M. H., Sanches, A. L. M., Vanderlei, M. A., Mansano, A., . . . Odete, R. (2017). Toxicity of abamectin and difenoconazole mixtures to a Neotropical cladoceran after simulated run-off and spray drift exposure. *Aquatic Toxicology*, *185*, 58-66. doi:10.1016/j.aquatox.2017.02.001.

Narayana, K. J. P. & Vijayalakshmi, M. (2009). Chitinase production by *Streptomyces* sp. ANU 6277. *Brazilian Journal of Microbiology*, *40*(4): 725-733. doi:10.1590/S1517-83822009000400002

Nawani, N. N., & Kapadnis, B. P. (2001). One-step purification of chitinase from *Serratia marcescens* NK1, a soil isolate. *Journal of Applied Micribiology*, *90*(5), 803-808. doi:10.1046/j.1365-2672.2001.01308.x

Oziengbe, E. O., & Osazee, J. O. (2015). Antifungal activity of copper sulphate against *Colletotrichum gloeosporioides*. *Journal of Asian Scientific Research*, *2*(12), 835-839. Retrieved from https://www.academia.edu/28844734/Antifungal_Activity_of_Copper_Sulphate_Against_Colletotrichum_Gloeosporioides

Phan, T. H., & Trương, T. B. (2018). Isolation of fungus causing brown spot disease on dragon fruit and determination of fungal susceptibility to *Streptomyces flaveus* fermentation broth. *Proceeding of National Biotechnology Conference 2018*. *Division of Microbiology Technology* (pp. 984-988). Hanoi, Vietnam

Phan, T. H., Huynh, T., Phan, N. U. P., & Vo, T.L.T. (2015). Actinomycetes isolated from soils collected in adjacent areas of Hochiminh city: Sources of potential antibiotics. *Science and Technololy Development Journal*, *18*(K3), 78-84. Retrieved from https://doi.org/10.32508/stdj.v18i2.1061

Phan, T. H., Vo, T. X. H., & Do, T. N. (2021). Isolation and potential biocontrol of the fungus causing anthracnose in longan in Vietnam. *Science and Technology Development Journal*, *24*(1), 894-901. doi:10.32508/stdj.v24i1.2500

Razavyoon, T., Hashemi, S. J., Ansari, S., Mansouri, P., Daie-Ghazvini, R., Khodavaisy, S., . . . Safaie, F. (2022). *Neoscytalidium dimidiatum* as onychomycosis causative agent in an Iranian patient: a case report and literature review. *New Microbes and New Infections*, *45*, 100952. doi:10.1016/j.nmni.2022.100952

Rusmarini, W., Shah, U. K. M., Abdullah, M. P., Mamat, S., & Hun, T. G. (2017). Identification of *Trichodema harzinum* T3.13 and its interaction with *Neoscytalidium dimidiatum*, a pathogenic fugus isolated from dragon fuit (*Hylocereus polyrhizus*) in Malaysia. *International Journal of Environmental Agriculture Research*, *3*, 3205-3228. Retrieved from https://1library.net/document/yd9vnx6z-identification-trichoderma-interaction-neoscytalidium-dimidiatum-pathogenic-hylocereus-polyrhizus.html

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., Nelson, A. (2019). The global burden of pathogens and pests on major food crops. *Nature Ecology and Evolution*, *3*(3), 430-439. doi:10.1038/s41559-018-0793-y

Sharma, M., & Kulshrestha S. (2015). *Colletotrichum gloeosporioides*: An anthracnose causing pathogen of fruits and vegetables. *Biosciences Biotechnology Research Asia*, *12*(2), 1233-1246. doi:10.13005/bbra/1776

Singh, S., Singh, N., Kumar, V., Datta, S., Wani, A. B., Singh, D., . . . Singh J. (2016). Toxicity, monitoring and biodegradation of the fungicide carbendazim. *Environmental Chemistry Letters*,*14*, 317-329. doi:10.1007/s10311-016-0566-2

Taechowisan, T., Peberdy, J. F., & Lumyong, S. (2003). Chitinase production by endophytic *Streptomyces aureofaciens* CMUAc130 and its antagonism against phytopathogenic fungi. *Annals of Microbiology*, *53*(4), 447-461. Retrieved from https://www.academia.edu/12632836/Chitinase_production_by_endophytic_Streptomyces_aureofaciens_CMUAc130_and_its_antagonism_against_phytopathogenic_fungi

Wang, Z., Sui, Y., Li, J., Tian, X., & Wang, Q. (2022). Biological control of postharvest fungal decays in citrus: A review. *Critical Reviews in Food Science and Nutrition*, *62*(4), 861-870. doi:10.1080/10408398.2020.1829542

Yang, S. J., Ng, C. Y., Wu, T. S., Huang, P. Y., Wu, Y. M., & Sun, P. L. (2019). Deep cutaneous *Neoscytalidium dimidiatum* infection: successful outcome with amphotericin B therapy. *Mycopathologia*, *184*(1), 169-176. doi:10.1007/s11046-018-0308-z

Yeop, L. J., Kim, B. S., & Hwang, B. K. (1995). Numerical identification of *Streptomyces flaveus* producing antibiotic substances inhibitory to plant pathogenic fungi. *Journal of Microbiology and Biotechnology*, *5*(6), 324-334. Retrieved from https://koreascience.kr/article/JAKO199511922234087.pdf