Cellulase and Polygalacturonase Activity by *Trichoderma viride*

Anatolii ANATOLIIOVYCH PAVLENKO (✉)
Evgeniy PAVLOVYCH KOPILOV
Olena VOLODYMYRIVNA NADKERNYCHNA
Hanna VIKTORIVNA TSEKHMISTER
Anna SERHIIVNA KYSLYNSKA

Summary

In this study the ability of *Trichoderma viride* IMB F-100076 to synthesize cellulase and pectinase enzymes was investigated. Clear zones in agar medium with Na-CMC, cellobiose and pectin as carbon sources were identified at different pH values. It was found that the mentioned fungus had cleaved Na-CMC, cellobiose and pectin, forming clear zones. The optimal pH value for cellulase and pectinase synthesis amounted to 5.0. At this value the clear zone and radial growth rate of *T. viride* was the highest. The ability of *T. viride* IMB F-100076 to synthesize cellulase enzymes and polygalacturonase was quantitatively proven. The maximum cellulase activity in static culture was reported on day 15 of cultivation. The total cellulase activity was 0.88 U mL^{-1}, exoglucanase – 0.76 U mL^{-1}, endoglucanase – 1.25 U mL^{-1} and β-glucosidase – 1.27 U mL^{-1}. The highest value of polygalacturonase activity was 0.59 ± 0.01 U g^{-1} IDS reported at day 6 of cultivation.

Key words

Trichoderma viride, cellulase activity, endoglucanase; exoglucanase, β-glucosidase, pectinolytic enzymes, polygalacturonase

Institute of Agricultural Microbiology and Agroindustrial Manufacture, NAAS, Chernihiv, Ukraine

✉ Corresponding author: tolik.pavlenko@gmail.com

Received: January 13, 2021 | Accepted: July 26, 2021

Introduction

A rising concern in agriculture due to usage of agrochemicals, including pesticides, has led to the necessity of enzyme-based biocontrol agents produced by microorganisms or direct application of live bacterial and fungal cultures (Costa et al., 2001; Zhihui et al., 2008).

The members of *Trichoderma* genus are the most promising biological agents for the control of a wide range of plant pathogens. *Trichoderma* strains inhibit the growth of plant pathogens by competing for nutrients, antibiosis and ability to synthesize cell wall degradation enzymes (Limon et al., 2004). *Trichoderma* fungi synthesize a number of extracellular enzymes that are important in the inhibition of phytopathogens: chitinase and β-glucanase, which can hydrolyse the cell of the fungal pathogen (Markovich and Kononova, 2003) and pectinase, which can induce plant resistance to disease (Bai et al. , 2004). In addition, *Trichoderma* metabolites could also improve plant growth and yield (Kolombet et al., 2001).

Trichoderma fungi seem to be one of the most promising bioagents of microbial preparations not only for plant protection but also for the degradation of plant residues (Nava-Cruz et al., 2016; Kaur et al., 2020). The cell wall of plants consists of polysaccharides such as cellulose, hemicellulose, pectin and lignin (Antoniak et al., 2013). As far as its main component is cellulose (Antoniak et al., 2013), a significant number of reasearch is aimed at studying cellulase (Califano et al., 2020; Toscano et al., 2007). Fungi of the genus *Trichoderma* synthesize several types of cellulase enzymes separately or in the form of a complex. For example, they include such natural cellulases as endo-1,4-β-D-glucanase (hydrolyzes β-1,4-bonds in polymers of cellulose or other β-glucans), exo-1,4-β -D-glucosidase (hydrolyzes β-1,4-bonds in 1,4-β-D-glucans with sequential cleavage of glucose residues) and β-glucosidase (cleaves the final residues of β-D-glucose by hydrolytic method and can hydrolyze molecules of cellobiose and β-D-glucosides) (Baldrian and Valaskova, 2008; Bhat and Bhat, 1997).

It has been shown that *T. viride* synthesizes cellulases, ligninases and hemicellulase into complexes (Neethu et al., 2012). The synthesis of hydrolytic enzymes was influenced by the substrate, being a source of carbon. Juhasz et al. (2005) studying cellulases and hemicellulases of *T. reesei*, showed that the synthesis of enzymes differed depending on plant residues (corn straw or other substrates).

Pectic substances are heteropolysaccharides consisting of the main range of partially methyl esterified α-1-1-D-galacturon (Agrios, 2005), their biological role being limited to preserve the structural integrity of plant cell components (Kurchenko, 2013). Enzymatic hydrolysis of pectin has advantages over chemical hydrolysis since enzymes target specific bonds of pectin molecules, while the chemical method is less specific (de Vries et al., 2002). Pectolytic enzymes of fungal origin attract the attention of researchers since they have significant potential for both industry and agriculture. Pectinases are decomposed glycosidic bonds containing long chains of galacturonic acid (De-Gregorio et al., 2002; Nazia et al., 2003; Anisa and Girish, 2014). By their action on substrate, they are divided into polygalacturonase, pectinesterase, pectin lyase (Alkorta et al., 1998; Mohamed et al., 2009). The sequential action of these enzymes achieves complete decomposition of pectin molecule. Micromycetes' pectolytic enzymes are widely used in industry during plant residues processing (for flax and vegetable fibres, depectinization and clarification of fruit juices, extraction of oils from vegetables and citrus peels, paper production and pre-treatment of pectin wastewater) (Moyo et al., 2003; Saito et al., 2004; Jayani et al., 2005).

Widespread *Trichoderma* micromycetes usage causes an urgent need to find and research a new promising fungi strain with high cellulosolytic and antagonistic activity. The strain of *T. viride* IMB F-100076 which was isolated in this study had high antagonistic activity against phytopathogenic fungi - the causative agents of agricultural crops root diseases.

The objective of this work was to investigate cellulase and polygalacturonase activity of *T. viride* IMB F-100076 for its further usage in agricultural production as a strain with multifunctional properties.

Material And Methods

Fungal Strain

T. viride IMB F-100076 was obtained from the collection of beneficial soil microorganisms of the Institute of Agricultural Microbiology and Agroindustrial Manufacters of the National Academy of Agrarian Science (Volkogon et al., 2015).

Qualitative Measurement of Cellulolytic and Pectolytic Activity

The linear growth rate and preliminary verification on the ability of *T. viride* IMB F-100076 to synthesize cellulases and pectinases were measured by a qualitative method.

The studied fungus was grown in Petri dishes at 26 ± 2°C for 10 days on Czapek medium containing carboxymethylcellulose sodium salt (Na-CMC, 0.5%) (Moroz et al., 2013), cellobiose (0.2%) (Osadcha et al., 2009) and pectin (0.05 %) (Kurchenko, 2013) as sources of carbon and substrate for enzymes. Congo red was used as an indicator (0.5%, injected into agar medium). The range of the hydrogen index was 4.0 to 9.5 with a step of 0.5.

The diameter of the fungus colony and the clear zone was measured in three directions twice daily. Based on the obtained data, the radial growth rate was determined (Perth, 1978)

Cellulase and pectinase activity were assessed by the size of the clear zones, which was defined as the ratio between the average radius of the clear zone and the average radius of the fungal colony.

Inoculum Preparation

For quantitative tests, *T. viride* IMB F-100076 was superficially cultured in the test tubes on barley meal agar (BMA) for 14 days at 26°C before conidias were isolated. To obtain a conidia suspension, the fungus was carefully removed from the BMA surface with a sterile loop and suspended in sterile water. The concentration of conidia was determined using a Goryaev chamber and a light microscope. Then spores suspension with a concentration of 1×10^6 CFU was prepared and inoculated with culture medium in an amount of 5% by volume.

Cultural Conditions

To study the cellulase activity, *T. viride* IMB F-100076 was superficially cultured in the test tubes containing 5 ml of liquid modified Czapek medium (g L^{-1}): 2.5 $(NH_4)_2HPO_4$, 1.0 K_2HPO_4, 0.5 $MgSO_4$, 0.5 KCl, 0.01 $FeSO_4$.Then pH of medium was reduced to 5.0 by 10% HCl. The only source of carbon was a 50 mg (1×6 cm) strip of filter paper (Filtrak), which was placed in each test tube. The fungus was incubated in the dark at 26°C for 8 days. The culture fluid was obtained by filtering the fungal mycelium through a glass filter. The supernatant was used as extracellular enzyme.

Cellulase Assay

Total cellulase activity was assayed by mixing 1 ml of cultural fluid with 50 mg filter paper in 1 mL 0.05 M citratephosphate buffer with diferent pH (5.5, 7.0, 8.5) and incubating it in a water bath at 40°C for 60 min.

Exoglucanase activity was assayed by mixing 1 mL of cultural fluid with 50 mg avicel "Evalar" in 1 mL 0.05 M citratephosphate buffer with different pH (5.5, 7.0, 8.5) and incubating it in a water bath at 40 °C for 60 min.

Endoglucanase activity was assayed by mixing 1 mL of cultural fluid with 1 ml 0.5 % Na-CMC in 0.05 M citratephosphate buffer with diferent pH (5.5, 7.0, 8.5) and incubating it in a water bath at 40°C for 30 min.

β–glucosidase activity was assayed by mixing 1 ml of cultural fluid with 1 ml substrate 0.025% celobiose "Merck" in 0.05 M citratephosphate buffer with different pH (5.5, 7.0, 8.5) and incubating it in a water bath at 40°C for 30 min.

One unit (U) of enzyme activity was defined as the amount of enzyme required to liberate 1 μmol min^{-1} of reducing sugar expressed as glucose equivalents.

Reducing sugars released were assayed by the Somogyi method (Somogyi, 1952) modified from Nelson procedure (Nelson, 1944) with glucose as standard. 1 mL of Somogyi reagent was added to 2 ml of the reaction mixture and incubated at 100°C for 15 min. The mixture was then rapidly cooled in an ice bath and 1 mL of Nelson reagent was added. The volume was adjusted to 25 mL with distilled water. The solution was mixed thoroughly. Determination of reducing sugars was performed on a photoelectrocolorimeter at 560 nm.

One unit (U) of enzyme activity was defined as the amount of enzyme required to liberate 1 μmol min^{-1} ml^{-1} of reducing sugar expressed as glucose equivalents.

Cellulase activity was measured every 3 days. This experiment was repeated in triplicate.

Pectinase Assay

Determination of the activity of pectolytic enzymes was based on determining the rate of the enzymatic reaction of pectin hydrolysis, which was measured by the amount of formed products that were not precipitated with zinc sulphate. The total amount of these products was determined by colorimetric anthrone method equivalent to monogalacturonic acid. The activity was calculated by the equation of the amount of pectin converted during the reaction into galacturonic acid and the number of enzyme units introduced into this reaction (Sysoeva et al., 2010).

One unit of polygalacturonase activity was defined as the amount of enzyme that catalyses the conversion of 1 g of pectin to galacturonic acid in 1 minute at 30 °C and pH 4.1. The enzyme yield was expressed as units/g of initial dry substrate (U g^{-1} IDS).

Polygalacturonase activity was measured every 3 days. The experiment was repeated in triplicate.

Statistical Methods

Data obtained were analysed using STATISTICA 12 software. The distribution of data was checked for normality using the Shapiro-Wilk's W-test. The nonparametric Mann–Whitney *U* test was applied to compare variable between groups. Differences were significant from each other, considering their P-value of ≤ 0.05.

Results

We have studied the ability of *T. viride* IMB F-100076 to synthesize cellulase and pectinase enzymes and to form clear zones on agar medium with the addition of Na-CMC, cellobiose and pectin at different pH. It was established that pH 5.0 was the most optimal pH for the synthesis of both cellulase and pectinase enzymes (Table 1). For example, the clarification zones formed by *T. viride* IMB F-100076 on the medium with the addition of CMC (Fig. 1) and cellobiose (Fig. 2) were 1.32 ± 0.16 and 2.53 ± 0.10, respectively. The radial growth rate of the fungus was 0.18 mm h^{-1} and 0.05 mm h^{-1}, respectively. The clear zones on the medium with the addition of pectin (Fig. 3) were 1.94 ± 0.07, the radial growths rate of 0.019 mm h^{-1}.

The next step was to determine *T. viride* IMB F-100076 cellulase activity. Maximum total (Fig. 4), exoglucanase (Fig. 5), endoglucanase (Fig. 6) and β-glucosidase (Fig. 7) activities were recorded on day 15 of cultivation. Upon further cultivation of *T. viride* IMB F-100076, enzymatic activities were reduced. The highest amount of total cellulase activities was 0.88 U ml^{-1}, exoglucanase – 0.76 U mL^{-1}, endoglucanase – 1.25 U mL^{-1} and β-glucosidase – 1.27 U mL^{-1}.

Further work has quantitatively confirmed the ability of *T. viride* IMB F-100076 to synthesize pectolytic enzymes, namely polygalacturonase (Fig. 8). The highest parameter of enzymatic activity was 0.59 ± 0.01 U g^{-1} IDS on day 6 of cultivation.

Table 1. Clear zone and radial growth rate due to pectinase and cellulose activity of the *T. viride* IMB F-100076 on pectin agar, cellobiose agar and Na-CMC agar culture media, respectively

pH	Clear zone			Radial growth rate (mm h^{-1})		
	Pectinase on pectin agar media	Cellulase on Na-CMC agar media	Cellulase on cellobiose media	Pectinase on pectin agar media	Cellulase on Na-CMC agar media	Cellulase on ellobiose media
4.0	1.10 ± 0.05	1.11 ± 0.08	2.36 ± 0.04	0.16	0.14	0.03
4.5	1.90 ± 0.07	1.22 ± 0.03	2.41 ± 0.03	0.18	0.16	0.04
5.0	1.94 ± 0.07	1.32 ± 0.16	2.53 ± 0.10	0.19	0.18	0.05
5.5	1.37 ± 0.09	1.23 ± 0.10	1.47 ± 0.06	0.18	0.16	0.06
6.0	1.23 ± 0.01	1.06 ± 0.01*	1.39 ± 0.07*	0.17	0.14	0.06*
6.5	1.21 ± 0.11	0.97 ± 0.07	1.31 ± 0.02	0.16	0.15	0.08
7.0	1.16 ± 0.13	0.81 ± 0.07	1.29 ± 0.01	0.16	0.13	0.09
7.5	1.14 ± 0.09	0.77 ± 0.11	1.24 ± 0.03	0.15	0.12	0.08
8.0	1.08 ± 0.03*	0.52 ± 0.06	1.10 ± 0.05*	0.15*	0.10	0.06*
8.5	0.94 ± 0.08	0.47 ± 0.09	1.00 ± 0.03	0.14	0.08	0.06
9.0	0.86 ± 0.05*	0.31 ± 0.07	0.88 ± 0.04*	0.13*	0.06	0.05*
9.5	0.81 ± 0.07	0.20 ± 0.06	0.69 ± 0.08	0.12	0.04	0.03

* significant increase or decrease in the indicator in relation to the previous value with a significance level of $P \leq 0.05$ according to Mann–Whitney U test

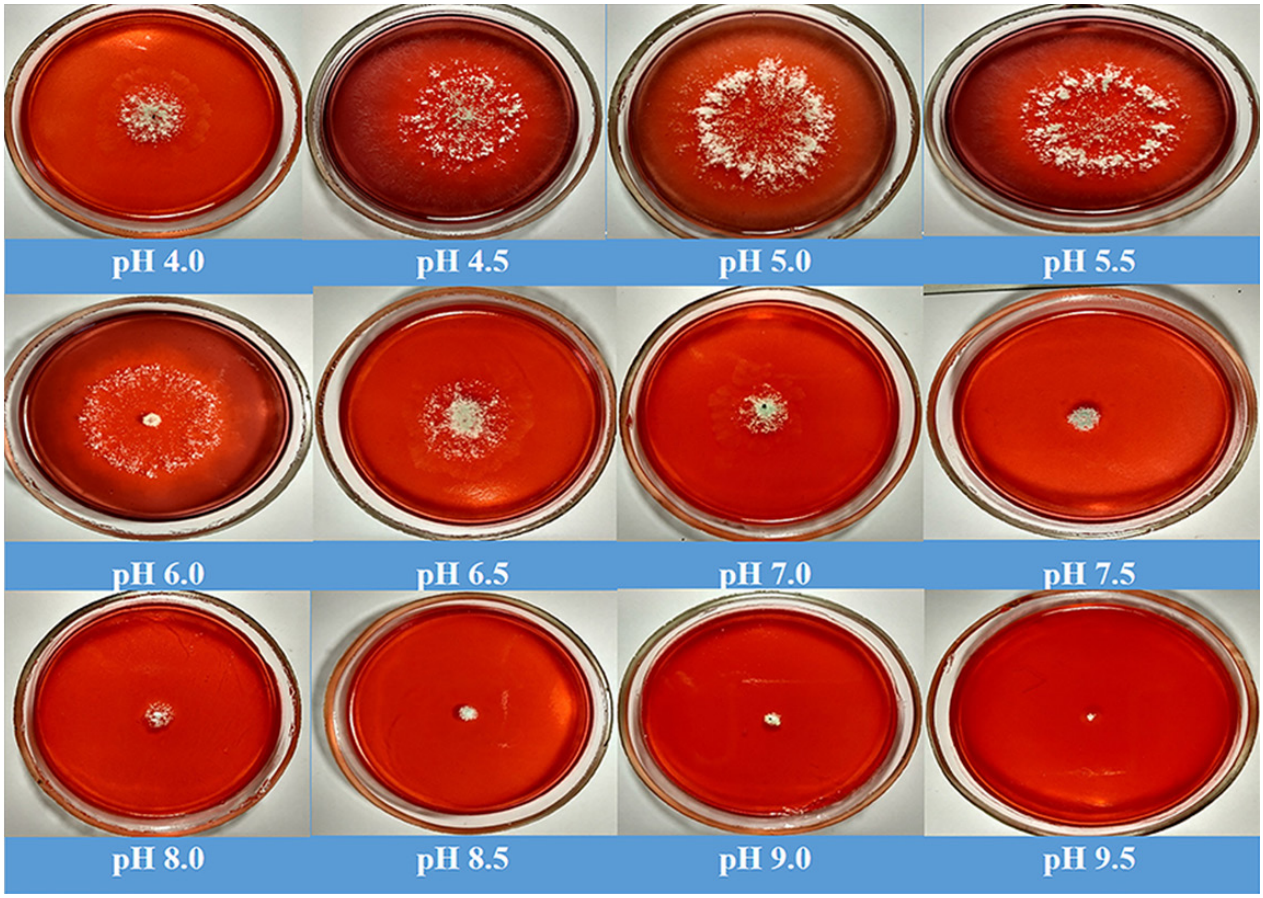

Figure 1. Na-CMC hydrolysis zones induced by *T. viride* IMB F-100076 under different pH (Day 3 of cultivation)

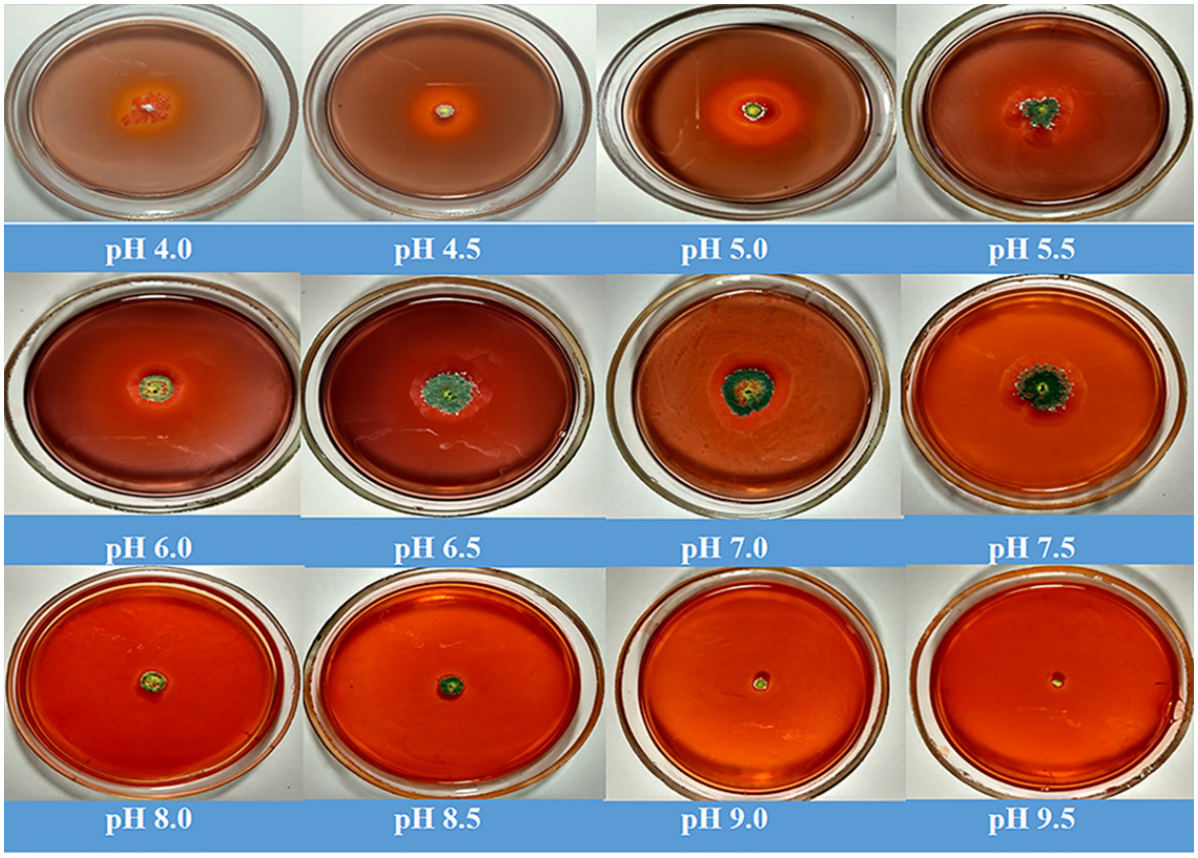

Figure 2. Celobiose hydrolysis zones induced by *T. viride* IMB F-100076 under different pH (Day 5 of cultivation)

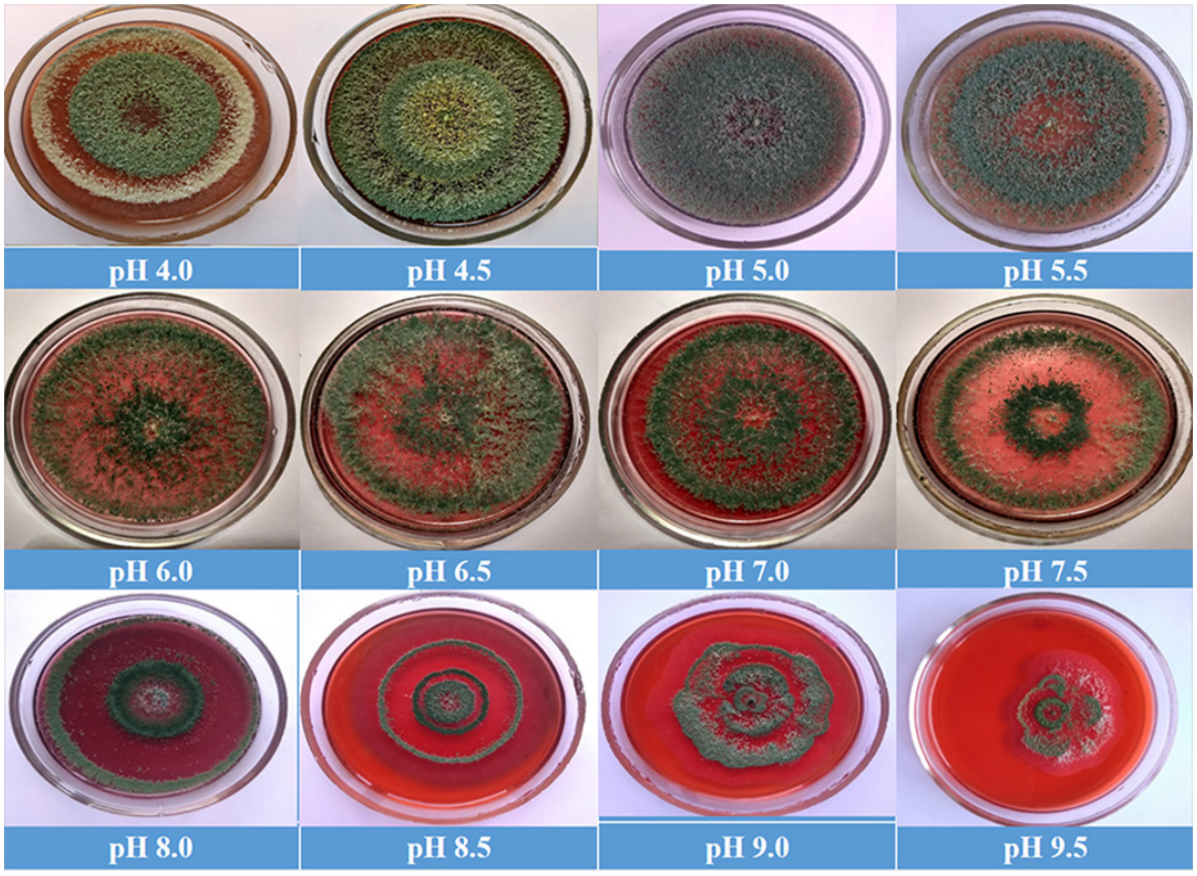

Figure 3. Pectin hydrolysis zones induced by *T. viride* IMB F-100076 under different pH (Day 5 of cultivation)

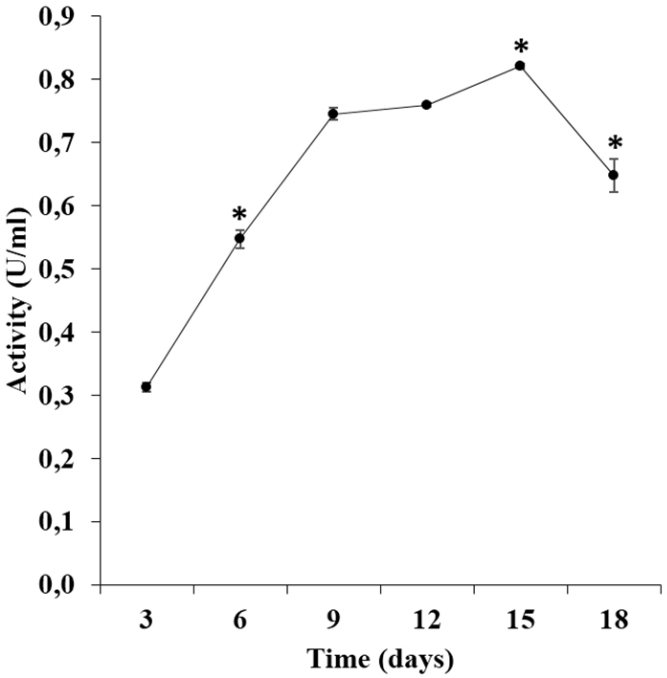

Figure 4. Total cellulase activity of *T. viride* IMB F-100076

* significant increase or decrease in the indicator in relation to the previous value with a significance level of P ≤ 0.05 according to Mann–Whitney U test

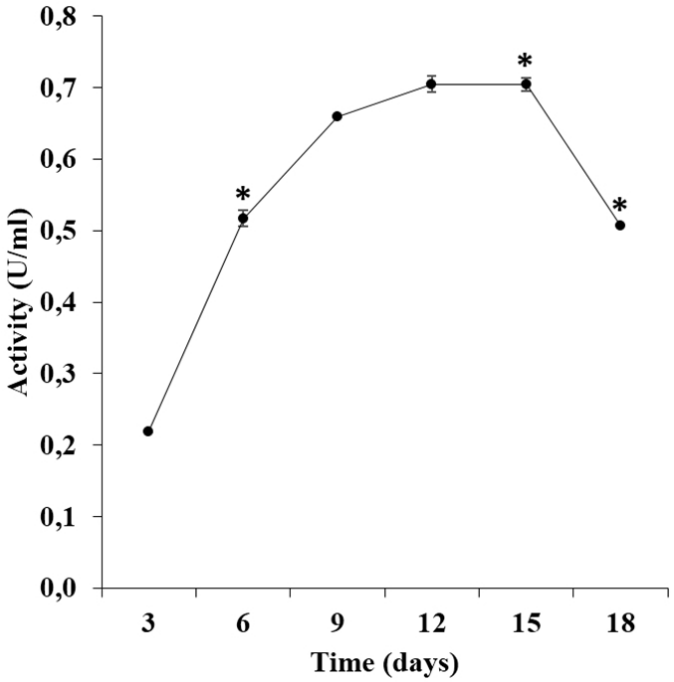

Figure 5. Exoglucanase activity of *T. viride* IMB F-100076

* significant increase or decrease in the indicator in relation to the previous value with a significance level of P ≤ 0.05 according to Mann–Whitney U test

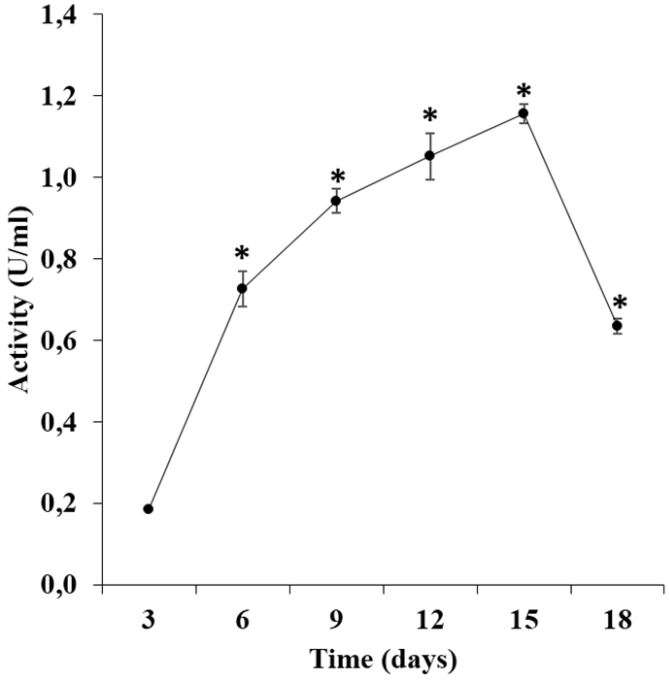

Figure 6. Endoglucanase activity of *T. viride* IMB F-100076

* significant increase or decrease in the indicator in relation to the previous value with a significance level of P ≤ 0.05 according to Mann–Whitney U test

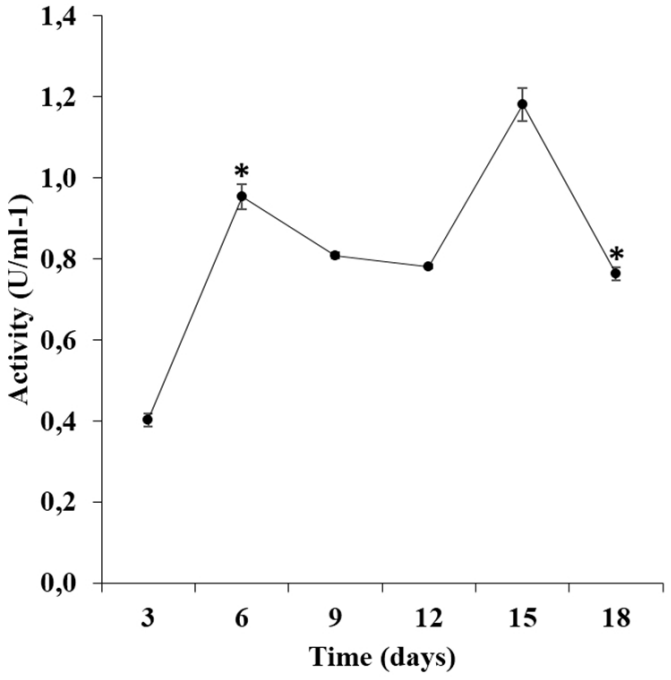

Figure 7. β-glucosidase activity of *T. viride* IMB F-100076

* significant increase or decrease in the indicator in relation to the previous value with a significance level of P ≤ 0.05 according to Mann–Whitney U test

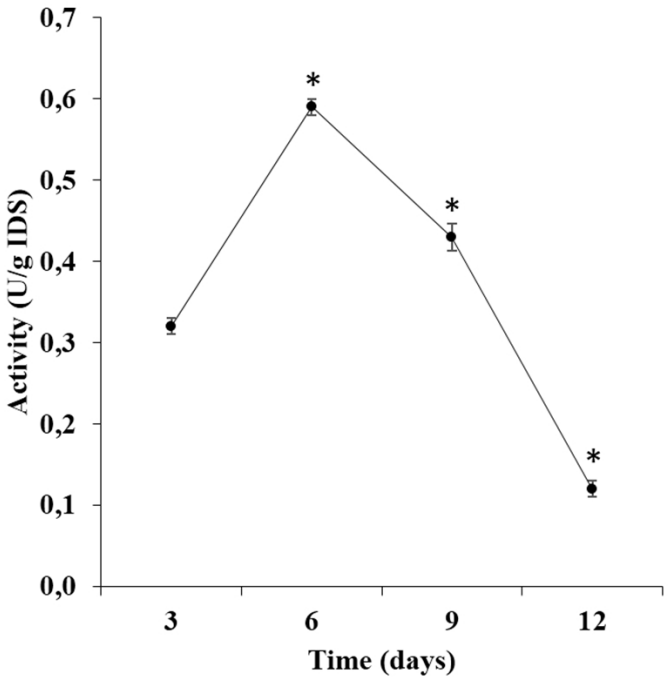

Figure 8. Polygalacturonase activity of *T. viride* IMB F-100076

* significant increase or decrease in the indicator in relation to the previous value with a significance level of $P \leq 0.05$ according to Mann–Whitney U test

Discussion

The fungi *Trichoderma* and *Chaetomium* are known as active antagonists of phytopathogenic fungi, producers of cellulase enzymes and often used as protective agents in microbiological preparations (Kolombet et al., 2001; Kopilov at al. 2020). However, pH is an important factor which regulates not only the growth and development of microorganisms, but also their synthesis of enzymes. Studying fungal cellulase and polygalacturonase activity with qualitative method, we noticed that colonies' clear zones diameter and radial growth rate had been different depending on pH level. The optimal level of pH for *T. viride* IMB F-100076 was 5.0. Neethu et al. (2012), examining the cellulase activity of *T. viride*, showed that the pH optimum of the medium was 4.0. Sinha et al. (2018) had found that the optimal pH for *T. harzianum* and *T. viride* were 6.0 and 5.0, respectively, consistent with data in this study (pH optimum for *T. viride* IMB F-100076 was 5.0). According to data from the study by Cherkupally et al. (2017), the isolates of *T. harzianum*, *T. reesei*, *T. atroviride* formed larger clear zones than isolates of *T. viride* and *T. virens*. In another study of the cellulase activity of *T. harzianum* and *T. viride*, the clear zone was independent of species, with the optimal pH for the MUSH strain with the largest clear zone being 7.0 (Gauchan et al., 2020).

Baer and Gudmestad (1995) grew the cellulosolytic fungus *C. olivacerum* on a medium with Na-CMC and cellobiose (as a carbon source), They showed that the clear zone was the highest at pH 7.0. For the phytopathogenic fungus *Fusarium oxysporum*, cellulase activity was the highest at the optimum pH for the microorganism - 6.0 (Ramanathan, 2010).

Analyzing the literature data we can conclude that fungi can be characterized by variability in the optimal pH value for the synthesis of cellulase and pectinase enzymes.

Gautam et al., (2012) studying the biodegradation of cellulose, showed that the maximum cellulosolytic activity of *T. viride*, *Penicillium digitatum*, *Aspergillus niger* and *Chaetomium* sp., had been noted on day 6 of cultivation. The maximum exoglucanase activity for *T. viride* was 2.22 U mL^{-1}, for *P. digitatum* - 1.19 U mL^{-1}, for *A. niger* - 2.05 U mL^{-1} and for *Chaetomium* sp. - 1.54 U mL^{-1}. The maximum endoglucanase activity of *T. viride* was recorded and was 2.03 U mL^{-1}, for *P. digitatum* - 1.37 U mL^{-1}, for *A. niger* - 1.76 U mL^{-1} and for *Chaetomium* sp. - 1.19 U mL^{-1}. β-glucosidase was also characterized by high activity in strains: *T. viride*, *P. digitatum*, *A. niger*, *Chaetomium* sp., and was - 1.98 U mL^{-1}, 1.53 U mL^{-1}, 1.96 U mL^{-1} and 1.34 U mL^{-1}, respectively.

Gautam et al., (2012) show that *Trichoderma* sp. has much higher exoglucanase (0.76 U mL^{-1}) and endoglucanase (0.78 U mL^{-1}), but low β-glucosidase (0.69 U mL^{-1}) activities in comparison with the data received in this study.

Gomes et al., (1992) showed that when growing *T. viride* on a substrate with an optimally selected carbon source for maximum production of cellulosolytic enzymes, the total cellulosolytic activity was low and was 0.56 U/ml, and high β-glucosidase activity - 2.92 U mL^{-1}.

Gomes et al., (1989) in order to obtain the strain with high cellulosolytic activity, conducted a screening among micromycetes *T. reesei*, *T. viride*, *T. harzianum* and others. Maximum endoglucanase activity was detected on day 6 in the following micromycetes: *T. reesei* - 4.20 U mL^{-1}, *T. viride* - 2.85 U mL^{-1} and *T. harzianum* - 2.47 U mL^{-1}. The maximum activity of β-glucosidase was also detected on day 6 and was for *T. reesei* - 0.94 U mL^{-1}, for *T. viride* - 0.81 U mL^{-1}, *T. harzianum* - 0.58 U mL^{-1} and was slightly lower in comparison with the data received in this study.

Improving the efficiency of enzymatic hydrolysis can be achieved in various ways, one of which is the use of drugs (based on fungi-antagonists *Trichoderma*) with a mixture of cellulases of two or more bioagents that will act synergistically and can give a better result compared to their separate use (Kshirsagar et al., 2020).

Khokhar et al., (2012) studying the cellulase activity of fungi of the genera *Trichoderma*, *Aspergillus* and *Penicillium* on nutrient medium with the addition of CMC showed that the fungi *T. harzianum*, *T. viride*, *T. koningii* were characterized by a high index of relative enzymatic activity.

Previous investigation of cellulolytic activity of *C. cochliodes* (which is a bioagent of the biological fungicide Hetomic) showed that the fungus antagonist synthesized a complex of cellulases, with the highest exoglucanase activity of 0.67 ± 0.03 U mL^{-1}, endoglucanase - 0.52 ± 0.02 U mL^{-1} and β-glucosidase activity - 1.02 ± 0.03 U mL^{-1} in the culture fluid (Kopilov at al. 2020), which is significantly lower than data in this study.

Yurieva et al., (2017) determined the maximum endoglucanase, exoglucanase activity of *P. funiculosum*, which was 0.35 ± 0.07 U/ml and 0.063 ± 0.02 U mL^{-1}, respectively.

The analysis of the results indicates that *T. viride* IMB F-100076 produces pectolytic enzymes. A high amount of galacturonic acid was observed in the pectin solution after the introduction of the fungus culture filtrate, which indicates the ability of *T. viride* IMB F-100076 to synthesize the enzyme polygalacturonase. The data

obtained is similar to the results of other researchers (Nasia et al., 2003; Zhihui et al., 2007; Mohamed et al., 2009; Nayebyazdi, Ghanbary, 2012; Anisa, Girish, 2014).

According to data from this study, the strain *T. viride* IMB F-100076 can be described by its high cellulosolytic activity. Exoglucanase activity was 0.70 ± 0.01 U/ml, endoglucanase - 1.16 ± 0.02 U mL^{-1} and β-glucosidase activity - 1.18 ± 0.04 U/ml, which in combination with high antagonistic interaction on a wide range of phytopathogenic fungi of pathogens shows its viability for use in agricultural production.

Conclusion

The cellulase and polygalacturonase activity of *T. viride* IMB F-100076 were determined. The radial growth rate of *T. viride* IMB F-100076 was the highest on mediums with both Na-CMC, cellobiose and pectin (optimum pH 5.0). *Trichoderma viride* IMB F-100076 synthesizes exoglucanase, endoglucanase, β-glucosidase and polygalacturonase, indicates the ability of the fungus to decompose cellobiose to glucose and pectin to galacturonic acid.

References

Agrios G. N. (2005). Plant Pathology. London.

Alkorta I., Garbisu C., Llama M. J., Serra J. L. (1997). Industrial Applications of Pectin Enzymes: A Review. Process Biochem. 33 (1): 21-28. doi: 10.1016/S0032-9592(97)00046-0

Anisa S. K., Girish K. (2014). Pectinolytic Activity of *Rhizopus* sp., and *Trichoderma viride*. J Pure Appl Microbiol. 4 (2): 28-31.

Antoniak H. L., Kalynets-Mamchur Z. I., Dudka I. O., Babych N. O., Panas N. Ye. (2013). Ecology of Fungi. Ivan Franko Lviv National University, Lviv (Ua).

Avdeeva L. V., Osadchaya A. I., Safronova L. A., Ilyash V. M., Kharkhota M. A. (2010). Pectolytic Activity of Bacteria genus *Bacillus.* Mikrobiolohiia i biotekhnolohiia, 3: 71-78 (in Russian).

Badrian P., Valaskova V. (2008). Degradation of Cellulose by Basidiomycetous Fungi. FEMS Microbiol Rev. 32 (3): 501-521. doi: 10.1111/j.1574-6976.2008.00106.x

Baer D., Gudmestad N. C. (1995). In vitro Cellulolytic Activity of the Plant Pathogen *Clavibacter michiganensis* subsp. *sepedonicus*. Can J Microbiol. 41 (10): 877-888. doi: 10.1139/m95-121

Bai, Z.H., Zhang, H.X., Qi, H.Y., Peng, X.W., Li, B.J. (2004). Pectinase Production by *Aspergillus niger* Using Wastewater in Solid State Fermentation for Eliciting Plant Disease Resistance. Bioresour Technol. 95 (1): 49-52. doi: 10.1016/j.biortech.2003.06.006

Bhat M. K., Bhat S. (1997). Cellulose Degrading Enzymes and Their Potential Industrial Applications. Biotechnol Adv. 15 (3-4): 583-620. doi: 10.1016/s0734-9750(97)00006-2

Califano V., Constantini A. (2020). Immobilization of Cellulolytic Enzymes in Mesostructured Silica Materials. Catalysts 10 (6): 706. doi: 10.3390/catal10060706

Cherkupally R., Amballa H., Reddy B. N. (2017). In vitro Screening for Enzymatic Activity of *Trichoderma* Species for Biocontrol Potential. Ann Plant Sci. 6 (11): 1784-1789.

Costa E., Texido N., Usall J., Atares E., Vinas I. (2001). Production of the Biocontrol Agent *Pantoea agglomerans* Strain CPA-2 Using Commercial Products and By-Products. Appl Microbiol Biotechnol. 56 (3-4): 367-371. doi: 10.1007/s002530100666

Dashtban M., Schraft H., Qin W. (2009). Fungal Bioconversion of Lignocellulosic Residues; Opportunities & Perspectives. Int J Biol Sci. 5 (6): 578-595. doi: 10.7150/ijbs.5.578

De-Gregorio A., Mandalari G., Arena N., Nucita F., Tripodo M. M., Lo Curto R. B. (2002). SCP and Crude Pectinase Production by Slurry-State Fermentation of Lemon Pupls. Bioresour Technol. 83 (2): 89-94. doi: 10.1016/s0960-8524(01)00209-7

de Vries R. P., Jansen J., Aguilar G., Parenicová L., Joosten V., Wülfert F., Benen J. A., Visser J. (2002). Expression profiling of pectinolytic genes from *Aspergillus niger*. FEBS Lett. 530 (1-3): 41-47. doi: 10.1016/s0014-5793(02)03391-4

Gauchan D. P., Bhattarai A. K., Pandey S., Bhandari S. (2020). Exploration of Indigenous *Trichoderma* Species for the Use as Biofertilizers, Optimization of Growth Conditions and Cultivation on Cheap Substrates. Novel Research in Microbiology Journal 4 (4): 921-938. doi: 10.21608/nrmj.2020.107543

Gautam S. P., Bundela P. S., Pandey A. K., Jamaluddin Awasthi M. K., Sarsaiya S. (2012). Diversity of Cellulolytic Microbes and the Biodegradation of Municipal Solid Waste by a Potential Strain. Int J Microbiol. 2012: 325907. doi: 10.1155/2012/325907

Gomes I., Gomes J., Steiner W., Esterbauer H. (1992). Production of Cellulase and Xylanase by a Wild Strain of *Trichoderma viride*. Appl Microbiol Biotechnol. 36: 701-707. doi: 10.1007/BF00183253

Jacques A. E., Benen Gert-Jan W. M. van Alebeek, Alphons G. J. Voragen, and Jaap Visser. (2002). Pectic Esterases. Handbook of Food Enzymology,1, 8 pp.

Jayani R.S., Saxena S., Gupta R. (2005). Microbial Pectinolytic Enzymes: A Review. Process Biochem. 40 (9): 2931-2944. doi: 10.1016/j.procbio.2005.03.026

Juhász T., Szengyel Z., Réczey K., Siika-Aho M., Viikari L. (2005). Characterization of Cellulases and Hemicellulases Produced by *Trichoderma reesei* on Various Carbon Sources. Process Biochem. 40(11): 3519-3525. doi: 10.1016/j.procbio.2005.03.057

Kaur P., Taggar M.S., Kaur J. (2020). Cellulolytic Microorganisms: Diversity and Role in Conversion of Rice Straw to Bioethanol. Cellul Chem Technol. 54 (7-8): 613-634.

Khokhar I., Haider M. S., Mushtaq S., Mukhar I. (2012). Isolation and Screening of Highly Cellulolytic Filamentous Fungi. J Appl Sci Environ Manag. 3: 223-226.

Kolombet L. V., Jigletsova S. K., Derbyshev V. V., Erzhov D. V., Kosareva, N. I., Bystrova, E. V. (2001). Studies of Mycofungicid, a Preparation Based on *Trichoderma viride*, for Plant Infection Control. Applied Biochemistry and Microbiology 37 (1): 98-102. doi: 10.1023/A:1002813013156

Kopilov E., Kyslynska A., Nadkernychna O., Tsekhmister H. (2020). Formation and Functioning of *Chaetomium cohliodes/Fagopyrum esculentum* Endophytic Association. J. Microbiol., Biotechnol. Food Sci. 10 (2): 190-196. doi: 10.15414/jmbfs.2020.10.2.190-196

Kshirsagar S., Waghmare P., Saratale G., Saratale R., Kurade M., Jeon B.-H., Govindwar S. (2020). Composition of Synthesized Cellulolytic Enzymes Varied with the Usage of Agricultural Substrates and Microorganisms. Appl Biochem Biotechnol. 191 (4):1695-1710. doi: 10.1007/s12010-020-03297-8

Kurchenko I. N. (2013). Polygalacturonase Activity of Microscopic Fungi of Different Trophic Groups. Mikrobiologicheskiy zhurnal 2: 57-66 (in Russian).

Limón M.C., Chacón M. R., Mejías R., Delgado-Jarana J., Rincón A. M., Codón A. C., Benítez T. (2004). Increased Antifungal and Chitinase Specific Activities of *Trichoderma harzianum* CECT 2413 by Addition of Cellulose Bolding Domain. Appl Microbiol Biotechnol. 64 (5): 675-685. doi: 10.1007/s00253-003-1538-6

Markovich N. A., Kononava G. L. (2003). Lytic Enzymes of *Trichoderma* and Their Role in Plant Defence from Fungal Diseases: A Review. Prikl Biokhim Mikrobiol. 39 (4): 389-400.

Mohamed S. A., Al-Malki A. L., Kumosani T. A. (2009). Characterization of a Polygalacturonase from *Trichoderma harzianum* Grown on Citrus Peel with Application for Apple Juice. Australian Journal of Basic and Applied Science 3 (3): 2770-2777.

Monhen, D. (2008). Pectin Structure and Biosynthesis. Curr Opin Plant Biol. 11 (3): 266-277. doi: 10.1016/j.pbi.2008.03.006

Moroz I. V., Mikhaylova R. V., Shakhnovich Ye. V., Lobanyuk A. G. (2013). Search for Fungal Producers of Cellulolytic Enzymes. Trudy BGU 8: 221-223 (in Russian).

Moyo S., Gashe B. A., Collison E. K., Mpuchane S. (2003). Optimising Growth Conditions for the Pectinolytic Activity of *Kluyveromyces wickerhamii* by Using Response Surface Methodology. Int J Food Microbiol. 85 (1-2): 87-100. doi: 10.1016/s0168-1605(02)00503-2

Nava-Cruz N. Y., Contreras-Esquivel J.C., Aguilar-González, M. A., Nuncio A., Rodriguez-Herrera R., Aguilar C. N. (2016). *Agave atrovirens* Fibers as Substrate and Support for Solid-State Fermentation for Cellulose Production by *Trichoderma asperellum*. 3 Biotech 6 (1): 115. doi: 10.1007/s13205-016-0426-6

Nayebyazdi N., Tajick Gnanbary M. A. (2013). Pectinase Activity of Some Micromycetes Isolated from Agricultural Soils. Journal of Sciences 23 (4): 305-311.

Nazia G. N., Asgher M., Shah A. H., Sheikh M. A., Asad M. J. (2003). Production of Pectinase by *Trichoderma harzianum* in Solid State Fermentation of Citrus Peel. Pak J Agri Sci. 40 (3-4): 193- 201.

Neethu K., Rubeena M., Sajith S., Sreedevi S., Priji P., Unni K. N., Sarath Josh M. K., Jisha V. N., Pradeep S., Benjamin S. (2012). A Novel Strain of *Trichoderma viride* Shows Complete Lignocellulolytic Activities. Adv Biosci Biotechnol. 3 (8): 1160-1166. doi: 10.4236/abb.2012.38142

Nelson N. (1944). A Photometric Adaptation on the Somogyi Method for the Determination of Glucose. Journal of Biological Chemistry 153 (2): 375-380.

Osadchaya A. I., Safronova L. A., Avdeeva L. V., Ilyash V. M. (2009). Screening of Bacterial Strains with High Cellulase Activity. Mikrobiologicheskiy zhurnal 5: 41-48 (in Russian).

Ramanathan G., Banupriya S., Abirami D. (2010). Production and Optimization of Cellulose from *Fusarium oxysporum* by Submerged Fermentation. Sci Ind Res. (India) 69: 454-459.

Saito K., Takakuwa N., Oda Y. (2004). Purification of the Extracellular Pectinolytic Enzyme from the Fungus *Rhizopus oryzae* NBRC 4707. Microbiol Res. 159 (1): 83-86. doi: 10.1016/j.micres.2004.01.001

Sinha A., Harshita Singh Dr. R., Rao S. G., Verma A. (2018). Comprehensive Evaluation of *Trichoderma harzianum* and *Trichoderma viride* on Different Culture Media & at Different Temperature and pH. The Pharma Innovation 7 (2): 193-195.

Somogyi M. (1952). Notes on Sugar Determination. Journal of Biological Chemistry 195 (1): 19-23.

Sysoeva M. A., Ivanova G. A., Gamaiurova V. S., Ziiatdinova G. K., Budnikov G. K., Toscano L., Stoytcheva M., Montero G., Cervantes L., Gerardo M., Felix R. M. (2017). Characterization of Cellulolytic Multi-Enzymatic Complexes from Filamentous Fungi by Solid-State Fermentation Using Wheat Straw as Carbon Source. Int J Adv Res Publ. 1 (5): 40-47.

Volkogon V. V. Vorobey Yu. O., Nadkernichna O. V. (2015). Catalog of Microorganisms Cultures. Chernihiv: publisher Braginets O. V., 48 p.

Zakharova L. Ia., Voronin M. A. (2010). Plant Chemistry, no. 2, pp. 105–108. (in Russian).

Zhihui B., Bo J., Yuejie L., Jian C., Zuming L. (2007). Utilization of Winery Wastes for *Trichoderma viride* Biocontrol Agent Production by Solid State Fermentation. Journal of Environ Sci (China). 20 (3): 353-358. doi: 10.1016/s1001-0742(08)60055-8

aCS87_5