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THE ROLE OF EXOCHITINASE TYPE A1 IN THE FUNGISTATIC ACTIVITY OF THE RHIZOSPHERE BACTERIUM *PAENIBACILLUS* SP. M4.

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Abstract: The aim of the study was to detect the activity and characterize potentially fungistatic chitinases synthesized by rhizosphere bacteria identified as *Paenibacillus* sp. M4. Maximum chitinolytic activity was achieved on the fifth day of culturing bacteria in a growth medium with 1% colloidal chitin. Analysis of a zymogram uncovered the presence of four activity bands in the crude bacterial extract. The used three-stage protein purification procedure resulted in a single band of chitinase activity on the zymogram. The purified enzyme exhibited maximum activity at pH 6.5 and temperature 45°C, and thermal stability at 40°C for 4 h. In terms of substrate specificity, it is an exochitinase (chitobiose). The amino acid sequence obtained after mass spectrometry showed similarity to chitinase A1 synthesized by *Bacillus circulans*. The M4 isolate demonstrated the highest growth inhibiting activity against plant pathogens belonging to the genera *Fusarium*, *Rhizoctonia* and *Alternaria*. Fungistatic activity, although to a somewhat lesser degree, was also demonstrated by purified chitinase. The obtained results confirm the participation of the studied exochitinase in antagonism towards pathogenic molds. However, the lower fungistatic effectiveness of the chitinases points to the synergistic action of different metabolites in biocontrol by these bacteria.

Key words: chitinase purification; biocontrol; plant pathogens; *Paenibacillus* sp.; fungistatic activity

INTRODUCTION

Chitinases are enzymes hydrolyzing the β-1,4-glycosydic bonds in chitin, which is one of the most common natural polymers in nature. Chitin can be found in numerous species, from fungi to plants and lower animals. In the exoskeletons of arthropods or in cell walls of fungi it exists in the form of ordered, crystalline structures creating microfibrils, while in crustaceans in the form of fibrous material integrated into a six-thread protein fibril [1,2]. The arthropod exoskeleton is a rich source of chitin, which constitutes 20-50% of its dry weight. From a practical standpoint, shells of crustaceans such as crabs and prawns are readily available as waste from seafood processing and are used for the production of commercial chitin [3].

Chitin decomposition is a gradual process that involves chitinolytic enzymes belonging to the O-glycoside hydrolases subclass [E.C. 3.2.1.14]. So far, 115 families of glycoside hydrolases (GHs) have been classified, three of which encompass chitinolytic enzymes: families 18, 19 and 20 [4]. Due to the position of the hydrolyzed bond, chitinases can be broadly divided into endochitinases (which hydrolyze random bonds located within the chain, and the products of their action are chitooligosaccharides) and exochitinases that detach the disaccharide chitobiose from the reducing or non-reducing end of the chitin chain, or single units of β-N-acetyl-D-glucosamine [5].

Many organisms, including bacteria, fungi, insects, plants and animals, are capable of synthesizing chitinolytic enzymes, but in each of them, the enzymes fulfill a different, specific role. From the practical perspective of chitinase utilization, it is certainly important that they can be applied in biotechnology,

particularly in the production of chitooligosaccharides and protoplasts from yeasts and fungi [6].

The vast majority of bacterial chitinases – both endochitinases and exochitinases – are grouped in 18 GH families. In addition, the bacterial chitinases belonging to the 18 GH have been divided into three main subfamilies, A, B and C, depending on the amino acid sequence of their catalytic domains [7].

Chitin degrading enzymes play a role not only in bacterial nutrition where they break down chitin that can then be used as a source of energy, but they can also be considered pathogenic factors [8]. Furthermore, chitinases are useful in agriculture as biocontrol agents against fungal phytopathogens and can therefore be an alternative to or a component of plant protection agents [9]. The ability to degrade chitin, the main component of the cell walls of molds, is considered one of the more important mechanisms of biocontrol, in which other enzymes and bacterial metabolites, such as glucanases, proteolytic enzymes, antibiotics and siderophores, can be involved. However, chitinases are a particularly effective tool in fighting pathogenic fungi because their target is very precise, resulting in degradation of the hyphae of the mycelium [10].

The ability to secrete chitinases is fairly common among soil microorganisms. Many chitinolytic bacteria have been described, mainly of the genera *Stenotrophomonas*, *Serratia, Bacillus* and *Paenibacillus* [6]. *Paenibacillus* bacteria are exceptionally active in terms of hydrolase synthesis; depending on the species they secrete proteases, glucanases and chitinases [11]. The following can be classified as chitinolytic bacteria of the *Paenibacillus* genus: *Paenibacillus thiaminolyticus, P. macerans, P. alvei*, *P. koreensis*, *P. borealis*, *P. chitinolyticus* and *P. anaericanus* [12,13].

Our research also focuses on biological control. The main goal of our studies was to seek rhizosphere bacteria with high biocontrol potential and to assess the role of the chitinases of these bacteria in controlling the growth of phytopathogenic molds. The characterization of the purified enzyme was also carried out. Biochemical characterization and fungistatic activity testing of one of the chitinases of this bacterial isolate was aimed at evaluating the role of the enzyme in antagonism towards fungi causing diseases of farm crops.

MATERIALS AND METHODS

Isolation, screening and identification of chitinolytic bacteria

For isolation of bacteria from the rhizosphere of spring barley (*Hordeum vulgare L.*), Soldo variety, roots were collected, together with attached soil, from healthy barley plants in the stem elongation phase. Rhizosphere bacteria were isolated following the methodology described by Buyer [14]. The bacterial isolates were cultivated on agar media with 0.5% chitin at 28°C for 72 h. After this, the presence and size of halo zones around the grown bacterial colonies were checked. The selected bacterial isolates were identified on the basis of morphological and biochemical traits according to Bergey's Manual of Determinative Bacteriology [15]. Additionally, identification of the studied strain was confirmed by analysis of the 16 S rRNA gene sequence. Amplification of the 16 S rRNA gene was performed using 27F and 1492R universal primers [16]. The obtained nucleotide sequences were compared with sequences deposited in the available GenBank, European Molecular Biology Laboratory (EMBL) and DNA Data Bank of Japan (DDBJ) databases using the BLAST program.

Determination of chitinase activity

Chitinase activity was determined with 1% colloidal chitin in 50 mM of sodium acetate buffer, pH 5.8 as substrate. The amount of reductive sugars released after enzymatic hydrolysis was measured according to Miller [17]. One unit of chitinase activity (U) was defined as the amount of enzyme yielding 1 μmol N-acetyl-D-glucosamine (GlcNAc) per hour.

Optimization of growth medium composition

The bacteria were cultured for 36 h in a shaking incubator at 28°C. Optimized growth medium contained 3 g/L KH_2PO_4, 3 g/L K_2HPO_4, 0.5 g/L $MgSO_4$, 2 g/L NaCl, 0.005 g/L $FeCl_3$, 5 g/L Bacto Peptone, 2 g/L yeast extract, enriched with 10 g/L of colloidal chitin or crystalline chitin flakes from shrimp shells (Sigma Aldrich), crab shell powder (Roth) chitosan (Sigma, Aldrich; ≥75% deacetylated chitin from prawn shells). Control growth media contained all the components except a source of chitin or chitosan. Colloidal chitin was prepared according to Lee et al. [18].

Enzyme purification

A 5-day old bacterial culture in a growth medium enriched with 1% colloidal chitin was centrifuged, the obtained supernatant was precipitated with ammonium sulfate (up to 85% solution saturation). The preparation was centrifuged for 30 min at 13000*x*g, and the obtained protein pellet was dissolved in 5 mL of 50 mM sodium acetate buffer, pH 5.8. The obtained solution was dialyzed for 12 h against the same buffer. The enzyme solution was supplemented with $(NH_4)_2SO_4$ to a final concentration of 0.8 M and then subjected to hydrophobic chromatography on a Phenyl-Sepharose CL-4B column. Prior to separation, the column was equilibrated with 1 M $(NH_4)_2SO_4$ in 50 mM sodium acetate buffer, pH 5.8. The associated proteins were eluted with a decreasing $(NH_4)_2SO_4$ gradient. The most active fractions collected during the hydrophobic chromatography were pooled, dialyzed and purified using molecular sieve chromatography (Superdex 200). Chromatographic separation was performed in 50 mM sodium acetate buffer pH 5.8. Protein concentrations were determined using the method of Bradford [19] with bovine serum albumin as the protein standard.

Gel electrophoresis and zymography

Isoforms of chitinases synthesized by the tested bacteria were analyzed on zymograms following native electrophoretic separations [20]. The proteins were separated on a 8% polyacrylamide gel with incorporated 0.05% glycol chitin. Glycol chitin was prepared according to Trudel and Asselin [21]. The gel was incubated at 40°C in 0.1 M sodium acetate buffer pH 5.8 for 2 h. Finally, the gel was submerged for 30 min in a 0.01% solution of Congo Red dye and transferred to 1 M NaCl solution.

Proteomic analysis using mass spectrometry

The highly purified enzyme preparation (containing on average 30 μg/mL of protein) was sent to the Laboratory of Mass Spectrometry of the IBB PAS (Poland). A protein sample previously digested with trypsin, was separated on a nanoAcquity UPLC (Ultra Performance LC) system and analyzed with an Orbitrap-based mass spectrometer. The obtained peptide sequences were analyzed using the BLAST program.

Biochemical characterization of the purified chitinase of M4 isolate

Substrate specificity of the purified chitinase was determined using chromogenic, synthetic substrate (Chitinase Assay Kit, Sigma) N-acetylglucosamine derivatives of 4-nitrophenols: 4-Nitrophenyl N,N′-diacetyl-β-D-chitobioside, for determination of exochitinase/chitobiosidase activity, 4-Nitrophenyl N-acetyl-β-D-glucosaminide for exochitinase/β-N-acetylglucosaminidase activity detection, and 4-Nitrophenyl β-D-N,N′,N″-triacetylchitotriose for endochitinase activity detection. One unit of chitinase activity (U) was defined as the amount of enzyme yielding 1 μmol of p-nitrophenol per min. The optimum pH and temperature as well as the thermal stability of the purified enzyme were determined in enzymatic reactions using colloidal chitin as the substrate.

Fungistatic activity of M4 isolate and of purified chitinase A1-type

The following phytopathogenic species of fungi of the genus *Fusarium* were used: *Fusarium solani*, *Fusarium oxysporum* and *Fusarium. culmorum*. Other fungal species used were *Chaetomium globosum*, *Alternaria alternata*, *Rhizoctonia solani* and *Rhizoctonia cerealis*.

The tested fungal cultures were from the Bank of Plant Pathogens in Poznan (Poland).

In studies of the degree (percentage) of inhibition of mycelium growth by the M4 isolate, the method of dual cultures in modified PDA medium was employed. In the modified PDA medium, the concentration of glucose was reduced to 0.2%, and 0.3% chitin was added. The rate of fungal growth inhibition by the M4 isolate was determined following the formula for growth inhibition=(K-F/K)x100, where K is the culture diameter in the control combination and F is the culture diameter in the test combination.

The fungistatic activity of purified A1-type chitinase was studied using the well method in which the enzyme diffuses into the agar medium. The experiment also included a crude chitinase preparation obtained after precipitation and dialysis of proteins in the bacterial culture supernatant. The protein solutions were sterilized by filtration. The specific activities of the crude chitinase and purified enzyme were 6.1 and 91.8 U/mg, respectively. Bioactivity analysis of the studied enzymes preparations was performed in PDA medium by the well diffusion method, as described by Narayana and Vijayalakshmi [22]. Antifungal activity was classified according to Ghasemi et al. [23] as no inhibition, –, weak inhibition, ±; <2 mm, moderate inhibition, +; 2-8 mm, strong inhibition, ++;>8 mm.

All of the presented results are means obtained from three independent replicates. The mean error, reflecting maximal deviation of the results of measurements from the mean, did not exceed 5%.

RESULTS

From 45 isolated strains of bacteria, only 17 exhibited chitinolytic activity. Approximately 2-cm light zones on growth medium with colloidal chitin were observed around colonies of the bacterial isolate selected for further testing.

The studied strain was identified as *Paenibacillus* sp. M4. The nucleotide sequence of the gene coding 16 S RNA was deposited in DDBJ under accession number LC043402. Analysis of the obtained 16 S RNA gene sequence of the studied bacterial strain revealed 99% similarity with other bacterial strains of the genus *Paenibacillus*, including *Paenibacillus* sp. HA18 (gb|KF011602) and *Paenibacillus* sp. PAMC 26811 (gb|KF011685).

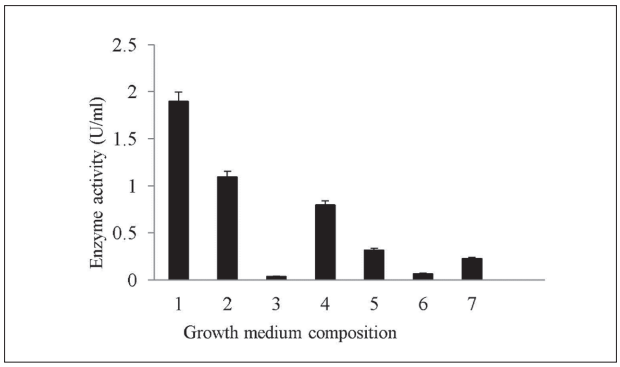

Fig. 1. Optimization of culture medium composition for the production of chitinases by *Paenibacillus* sp. M4 isolate. The following sources of chitin and chitosan were used: 1 – colloidal chitin; 2 – colloidal chitin enriched with 0.2% glucose; 3 – colloidal chitin enriched with 0.8% glucose; 4 – powdered chitin; 5 – chitin flakes; 6 – chitosan. The control medium not supplemented with chitin or chitosan has been designated as 7.

The effect of growth medium composition on chitinolytic activity of the M4 isolate was tested using 5-day-old bacterial cultures. The highest chitinase activity was achieved in a growth medium enriched with 1% of colloidal chitin or powdered crab shells (Fig. 1). It is interesting that the presence of 0.8% glucose in the culture medium resulted in reduced chitinolytic activity of the bacteria. Enzymatic preparations obtained from 5-day-old bacteria cultures in growth medium with 1.0% colloidal chitin were subjected to a three-stage purification procedure, according to Table 1.

The procedure used resulted in about 15-fold purification of the enzyme, with a 32% yield. The most effective step was hydrophobic chromatography in which the enzyme was purified 5-fold. After this stage, chitinolytic activity was separated into two main fractions, eluted in a decreasing ammonium sulfate gradient (Fig. 2). Protein from fraction II was taken for further purification by molecular sieve chromatography. The effectiveness of the separation of the individual

Table 1. Purification of the chitinase type A1 produced by *Paenibacillus* sp. M4

Purification Step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification fold	Recovery (%)
Cell free supernatant	430	70	6.1	1	100
Ammonium sulfate precipitation (0-85%)	390	45	8.7	1.4	90
Hydrophobic chromatography (samples 42-54)	230	5.5	41.8	6.9	53.5
Gel filtration	134	1.5	91.8	15	31.2

chitinases was checked by using zymograms. In the raw bacterial extract, four active chitinase forms were observed, whereas after hydrophobic chromatography, three forms were observed in fraction I and two in fraction II. After molecular sieve chromatography, only a single band of chitinase activity was observed in the zymogram (Fig. 3). The purified enzyme was subjected to proteomic identification using mass spectrometry (MS). Proteomic analysis of the purified enzymatic preparation demonstrated that the amino acid sequence of the studied chitinase is highly similar to the sequence of the chitinase A1 of *Bacillus circulans* Wl-12 (GenBank accession no P20533). The peptides derived from the studied chitinase overlap with 40% of the aa sequence of chitinase A1 by *Bacillus circulans* Wl-12. Chitinase A1 in strain Wl-12 is composed of 699 amino acid residues. Conserved domains typical for the family of 18 GHs were identified (Fig. 1S, Supplementary Material).

The purified enzyme exhibited activity in a broad range of pH values, ranging from 3.6 to 8.0, with maximum activity at pH 6.5 (Fig. 4). The temperature optimum was determined to be 45°C, although a broad range of temperatures in which the enzyme maintains activity could be observed (Fig. 4). The enzyme showed high thermal stability after 4 h of preincubation at 40°C. The studied chitinase demonstrated highest activity against 4-Nitrophenyl N,N′-diacetyl-β-D-chitobioside as the substrate and

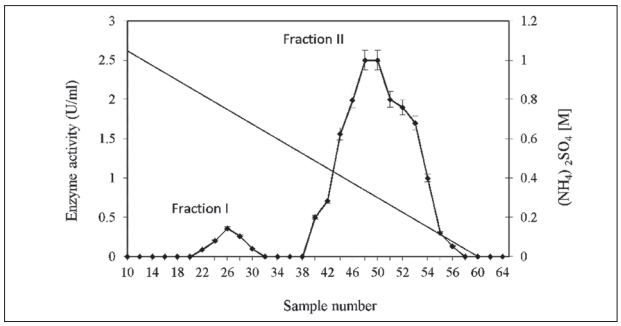

Fig. 2. Elution profile of *Paenibacillus* sp. M4 chitinases after hydrophobic chromatography with a decreasing ammonium sulphate gradient (1-0 M). On the chromatogram two protein fractions with chitinolytic activity, I and II, are indicated. Fraction II was taken for the subsequent step of protein purification, sieve chromatography.

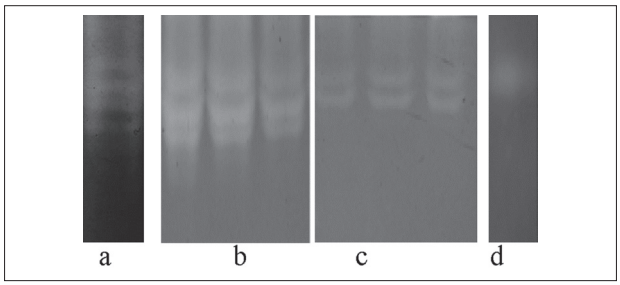

Fig. 3. Zymogram of chitinases activity at various stages of protein purification. Chitinolytic activity was detected after native electrophoretic separation in 8% polyacrylamide gel containing 0.1% of glycol chitin. Bands of active chitinases in crude bacterial extract – a; activity of chitinases after hydrophobic chromatography – b: FI, sample number 26-28 – c: FII, sample number 46-48; single activity band of purified chitinase after molecular filtration – d.

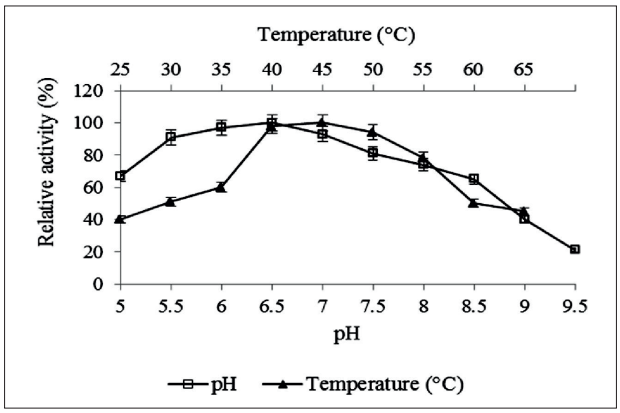

Fig. 4. Effect of pH and temperature on chitinase activity. In the case of optimum pH chitinolytic activity was studied in Britton-Robinson buffer in the 5.0-9.5 range. Optimum temperature was determined at pH 6.5 in the temperature range from 25 to 65°C.

Table 2. Substrate specificity of the chitinase type A1 from *Paenibacillus* sp. M4

Substrate	Activity (U/mL)	Catalytic type
4-Nitrophenyl N-acetyl-β-D-glucosaminide	0	Exochitinase
Nitrophenyl N,N′-diacetyl-β-D-chitobioside	22	Exochitinase (chitobiosidase)
4-Nitrophenyl β-D-N,N′,N″-triacetylchitotriose	2	Endochitinase

Table 3. Antagonism of *Paenibacillus* sp. M4 isolate towards fungal phytopathogens.

Phytopathogens	Antifungal activity (%)
Fusarium solani	55
Fusarium oxysporum	60
Alternaria alternata	57
Rhizoctonia solani	40
Rhizoctonia cerealis	32
Chaetomium globosum	15
Fusarium culmorum	0

Table 4. Fungistatic activity of the chitinases from *Paenibacillus* sp. M4.

Phytopathogens	Crude chitinases after salting out with ammonium sulfate	Chitinase type A1, after molecular sieve chromatography
F. solani	±	-
F. oxysporum	+	+
A. alternata	++	++

no inhibition, –, weak inhibition, ±; <2 mm, moderate inhibition, +; 2–8 mm, strong inhibition, ++; >8 mm (23).

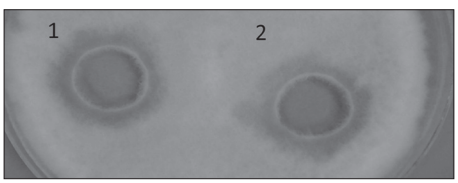

Fig. 5. Fungistatic activity of crude chitinases (well 1) and purified chitinase (well 2) against *F. oxysporum*. 200 µl portions of the preparation were added to the wells and this was repeated every 12 h. Observations were carried out after 48-h culture of the pathogen at 28°C.

minimal activity against 4-Nitrophenyl β-D-N,N′,N″-triacetylchitotriose (Table 2).

The M4 isolate in dual cultures exhibited a varying degree of antagonism towards such cereal pathogens as *F. oxysporum, F. solani, A. alternata, R. solani, R. cerealis* and *Ch. globosum. F. culmorum* was found to be fully indifferent to the presence of the bacteria (Table 3).

The fungistatic activity of A1-type chitinase and crude *Paenibacillus* sp. M4 chitinases was studied with regard to the same species of pathogens but using the agar well diffusion method, which has proven to be more effective in the case of liquid enzymatic preparations. The species of pathogens susceptible to the action of these enzyme preparations are presented in Table 4. The purified chitinase type A1 showed a fungistatic effect on the growth of *F. oxysporum* and *A. alternate.* This enzyme did not have any effect on other pathogens. The fungistatic activity of the chitinase type A1 against *F. oxysporum* is shown in Fig. 5.

DISCUSSION

Interest in chitinolytic microorganisms is motivated by the possibility of their use in biological plant protection. Chitinases synthesized by these organisms are an effective and precise biocontrol tool. From this point of view, *Paenibacillus* sp. M4 is an interesting study entity because it is among the chitinolytic strains of bacteria that are at the same time antagonistic towards phytopathogenic molds. The chitinase we isolated showed fungistatic activity. This enzyme is called chitinase type A1 because of the similarity of its amino acid sequence to that of *B. circulars* chitinase A1. Zymogram analysis indicated that under the experimental conditions, the studied M4 isolate secreted at least four chitinases. As indicated by the literature data, bacteria of the *Paenibacillus* genus usually synthesize from a few to over ten extracellular chitinases [24,25].

Chitinolytic activity was also detected in bacterial cultures on growth media that did not contain chitin. The obtained results indicate that chitinases are se-

creted constitutively. Itoh et al. [24] also described the constitutive nature of chitinases in the FPU-7 strain. Furthermore, the presence of glucose in the growth medium caused a catabolite repression effect, which is consistent with the results described for chitinase synthesized by *Paenibacillus* sp. D1 [26]. However, most of the chitinases described so far, including *B. circulars* chitinase A1, are induced in the presence of a substrate, most of all by colloidal chitin [27].

Strain M4 synthesizes a chitinase similar to chitinase A1 from chitinase group A, which belongs to family 18 GH. Chitinase A1 has been characterized in detail in *Bacillus circulans*, with 6 different chitinases described (A1, A2, B1, B2, C and D). Among them, chitinase A1 is synthesized in the largest amounts and exhibits strong affinity to insoluble forms of chitin. As with most chitinases from 18 GH, it comprises a C-end chitin-binding domain (ChBD, N-end large domain containing the enzyme catalytic site, and a third fibronectin domain type III [28].

The results of substrate specificity tests indicate that the studied chitinase belongs to exochitinases called chitobioses, capable of detaching two N-acetylglucosamine units from the non-reducing end of the molecule. However, no β-N-acetylglucosaminidase activity was observed. This substrate specificity confirms that the studied enzyme has similar properties to chitinase A1 [28]. Similar results were reported by Itoh et al. [24] for chitinases of the FPU-7 strain. *P. chitinolyticus* does not exhibit β-N-acetylglucosaminidase activity either, but it has endochitinase activity [29] similar to *P. illinoisensis* [30].

The optimal temperature of action of this enzyme is 45°C, which is typical for most group A chitinases [31, 32]. On the other hand, chitinases from bacteria of the genus *Paenibacillus* have different temperature optima, e.g. 37°C for the enzyme from *P. pasadenensis* [33] and 60°C for *P. thermoaerophilus* [34]. Similarly to the studied enzyme, chitinases produced by bacteria of the genus *Paenibacillus* are frequently thermally stable [35].

The tested enzyme showed an optimum pH close to neutral, similarly to chitinase A from *Bacillus licheniformis* [36]. The results presented in the literature indicate that chitinases synthesized by *Paenibacillus* bacteria may show optimal activity in either acidic [35] or alkaline environments [33].

In our studies, fungistatic activity towards ubiquitous fungal pathogens was demonstrated by both the M4 isolate and the chitinase type A1. In dual cultures, the M4 isolate strongly inhibited the growth of *R. solani, F. oxysporum* and *A. alternata*. The studied chitinase type A1 showed less fungistatic activity compared to the M4 isolate. Furthermore, there was no negative influence on the growth of *R. solani* and *R. cerealis*. Our results bring us to conclude that chitinases are an important biocontrol tool of these bacteria, but not the only one. Literature data point to the dependence of the antagonism of bacteria against phytopathogenic fungi on different enzymatic activities. The results of Aktuganov et al. [37] indicate a synergism of antifungal mechanisms of fungal cell-wall lytic enzymes, in which β-1,3-glucanases are initiators of cell-wall hydrolysis, which is amplified by chitinase activity. Nevertheless, it appears there is no single rule applicable to the role of bacterial lytic enzymes in the antagonistic interactions of microorganism. It should also be kept in mind that the final effect of bacterial antagonism towards fungal pathogens is also affected by the activity of other metabolites, such as siderophores and antibiotics. The fungistatic properties of group A chitinases were described earlier for bacteria belonging to the genera *Stenotrophomonas* [32]. Similarly, there are numerous scientific studies that describe antifungal activity for many bacteria of the genus *Paenibacillus*. *P. peoriae* inhibits the growth of *Fusarium moniliforme*, *Diplodia macrospora*, *Cephalosporium*, *Penicillium corylophilum* and *Colletotrichum graminicola* [38]. In a similar plate test, *P. ehimensis* exhibited fungistatic activity, among others, towards *Phytophthora capsici*, *Rhizoctonia cerealis* and *Rhizoctonia solani* [39].

To summarize the results of our studies, it can be said that chitinase type A1 plays a significant but not the only role in biocontrol by the bacterial isolate M4. Moreover, chitinase type A1 exhibited activity in a broad range of pH and had high thermal stability, suggesting that the enzyme may find practical

application in plant protection. These results are very useful because the damage to and diseases of farm crops caused by fungal pathogens are not only an economic problem for food producers but also a health problem for consumers. The need to produce healthy food requires the elaboration of more effective plant protection methods, which at the same time are safer for the environment. For this reason, it seems worthwhile continuing these studies with the use of plants in field conditions.

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Authors' contributions: U. Jankiewicz participated in the design of the study, performed the experiments and data analysis, and wrote the manuscript. M. Swiontek Brzezinska participated in the research and helped to draft the manuscript. Both authors read and approved the final manuscript.

Conflict of interest disclosure: The authors declare no conflict of interest

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Supplementary data

Fig.1S. Sequence alignment for precursor protein of chitinase A1 from *Bacillus circulans* (GenBank, accession number P20533) and studied chitinase type A1 from *Paenibacillus* sp. M4. For proteomic analysis of the purified enzyme mass spectrometry was employed; amino acids were identified following digestion of the protein with trypsin. Identical amino acid sequences in both proteins are marked in red. In the presented amino acid sequence the main conserved domains were identified in the regions: 1-41 – Signal sequence, 42-460 – ChiA1, 46-438 – GH18 type II, 465-549 – Fibronectin type 3, 455-697 aa – Chitin-binding domain of Chi A1-like proteins. The lead amino acid of each domain is indicated by an arrow.
Available at: http://www.serbiosoc.org.rs/sup/Fig1S.tif