Production of the chitinase by Beauveria bassiana in infecting Tribolium castaneum

by Ahdin Gassa

Submission date: 23-Jan-2022 07:09AM (UTC+0700)
Submission ID: 1746115582
File name: C19.pdf (444.53K)
Word count: 2935
Character count: 14916

IOP Conference Series: Earth and Environmental Science

Production of the chitinase by *Beauveria bassiana* in infecting *Tribolium castaneum*

Production of the chitinase by *Beauveria bassiana* in infecting *Tribolium castaneum*

M Sepe[1,2], I D Daud[3], A Gassa[3] and Firdaus[4]

[1]Program of Agriculture, Graduate School, Hasanuddin University, Makassar 90245, Indonesia
[2]Department of Agrotechnology, Faculty of Agriculture, Gorontalo Ichsan University, Gorontalo 96115, Indonesia
[3]Plant Pests and Diseases Department, Faculty of Agriculture, Hasanuddin University, Makassar 90245, Indonesia
[4]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar 90245, Indonesia

E-mail: musliminsepe@gmail.com

Abstract. *Beauveria bassiana* is one of the microorganisms that produce the enzyme chitinase. Chitinase has a high economic value which is widely used as a biocontrol agent because it can degrade chitin into an environmentally friendly product. The aim of the study was to investigate the production of chitinase by *B. bassiana* isolate in the presence of the cuticle of *T. castaneum*. In this study, the isolates of *B. bassiana* were cultured into potato dextrose agar. Further isolation, purification, and determination of the activity of chitinase. The results show that chitinase can be obtained from *B. bassiana* isolate derived from *T. castaneum* by using chitin colloidal substrate. The highest average specific activity of chitinase originating from isolated *B. bassiana* was 1 Unit/mg. Protein test using standard BSA solution and Lowry method obtained reading results with a spectrophotometer that was r = 0.9925.

1. Introduction

Beauveria bassiana (Balsamo) Vuill (Deuteromycota: Hyphomycetes) is an entomopathogenic fungus that can control pests on a field scale [1]. For a long time, this type of fungus can potentially have potential as a biological control agent against insect pests. Their high ability caused this fungus to be widely developed as a biological agent in the field of agriculture [2]. Fungus *B. bassiana* produces the enzyme chitinase when it penetrates the insect's body. The enzyme chitinase can hydrolyze β-1.4-acetamido-2-deoxy-D-glycoside bonds to chitin and insect chitin oligomers [3]. The ability to hydrolyze chitin polymer compounds on insect cell walls causing fractures and broken of cell walls. The destruction of the cell wall is continued with the formation of mycelium, which will wrap around the host body [4]. The use of enzymes in hydrolyzing chitin polymer is more developed due to its more specific inability to produce products and having no side effects that can cause environmental pollution [3]. Chitinase obtained from fungal biological control agents such as *Trichoderma* sp. was previously conducted [5], b the enzyme produced by *B. bassiana* is still limited. This encourages researchers to conduct further research on the production of the chitinase enzyme by *B. bassiana* while degrading

insect cuticles of *T. castaneum*. The purpose of this study was to evaluate the activity of the chitinase enzyme produced by *B. bassiana* during the degradation of insect cuticles.

2. Methods

2.1. Fungus B. bassiana isolates

Fungus *B. bassiana* isolates used as research samples come from *T. castaneum* warehouse insect pests. In the next stage, the isolated *B. bassiana* fungus isolates from *T. castaneum* were purified in a solid medium, potato dextrose agar (PDA), to further test enzyme activity.

2.2. The manufacture of chitin colloidal medium and agar chitin medium

The production of a colloidal chitin medium, which was as much as 10 grams of chitin from shrimp skin powder, dissolved in 200 mL of thick HCL, then sealed and incubated 24 hours at 4ºC (all stages of treatment was performed at cold temperatures)—further filtered using glass wool. The resulting filtrate was added to 100 mL of Aquadest and neutralized with NaOH 12 N. Then centrifuged at 8,000 rpm at 4ºC temperature for 20 minutes. The resulting pellet was added to 100 mL of Aquadest and then centrifuged at 8,000 rpm for 20 minutes at 4ºC temperature. Sediment in the form of pellets (colloidal chitin) was stored at cold temperatures [6].

Chitin agar medium was made from a colloidal mixture of chitin 0.3%, K_2HPO_4 0.1 g, $MgSO_4.7H_2O$ 0.01 g, yeast extract 0.05 g, peptone 0.1 g, NaCl 0.5 g, $(NH_4)_2SO_4$ 0.1 g, and 1 g agar into an Erlenmeyer containing Aquadest 100 mL. The solution was then homogenized with a *magnetic stirrer* and heated until dissolved, then sterilized in an autoclave at 121ºC for 15 minutes at a pressure of 2 atm [7].

2.3. Chitinolytic index method

The rejuvenated *B. bassiana* isolate was taken in two ose and put in a sterile Eppendorf tube containing 100 µl of sterile distilled water. The result of dilution as much as five µl was put on a petri dish in agar medium containing chitin colloid. Petri dishes were incubated for seven days, and then the colony diameter was measured. The clear zone formed was visualized by adding 0.1% congo red, then the plate was washed with distilled water and NaCl, then measured the diameter of the clear zone formed and documented. The chitinolytic index was obtained by comparing the apparent zone diameter and the colony diameter [7].

2.4. Chitinolytic fungal rejuvenation

The Chitinolytic fungus was derived from *B. bassiana* isolate. The fungus was rejuvenated by taking two ose isolates and grown on chitin media in a petri dish, then incubated for 24 hours [8].

2.5. Preparation of fungus suspension of the chitinase enzyme

Two ose colonies of chitinolytic fungus were suspended into a reaction tube containing 5 mL of liquid chitin medium, then incubated at 37ºC for 18-24 hours. The result of such treatment was called inoculum [8].

2.6. Production of the enzyme chitinase

Chitinase production was performed using a 10% active inoculum, by mixing 5 mL of inoculum with 45 mL of liquid chitin media. Then shaker at 37ºC at 180 rpm for 5 days [6].

2.7. Measurement of chitinase enzyme activity

Chitinase activity was tested according to methods of Veda and aria [9] namely with the colloidal substrate of chitin. Colloidal chitin 0.3% as much as 1 mL, Buffer acetate 0.2 M pH 5 2.0 mL, and Enzyme filtrate 1mL was put into the reaction tube and incubated at room temperature for a certain amount of time. Then the mixture was heated in boiling water for 20 minutes to stop the reaction enzyme

in the mixture and then cooled. The Enzyme activity was determined by spectrophotometry at λ maks = 660 nm. Chitinase activation was determined based on equation 1.

$$Activity\ unit = \frac{x - y}{0.001}\ X\ \frac{1}{incubation\ time\ (minutes)} \quad (1)$$

Illuminance : x = Control Absorption
y = Sample Absorption

One unit of enzyme activity is measured as the number of enzymes resulting in a reduction in the absorbance of the reaction mixture by 0.001 per minute.

2.8. Determination of protein levels

As much as 2 mL protein sample was injected into the reaction tube, then added 2.75 mL of lowry B reaction, and hushed for 15 minutes. Then added 0.25 Lowry A reaction, then mixed and hushed for 30 minutes. Furthermore, measured its absorbers at a maximum wave of 660 nm.

Lowry A reagent was prepared by mixing the Reagen Folin-Ciocâlteu (FCR) with Aquadest (1:1). Lowry B reagent was prepared with Na_2CO_3 2% in NaOH 0.1 N, $CuSO_4$ 1%, and Na-k-tartrate 1% a ratio 100:1:1. [10].

3. Results and discussion

. Production of the chitinase enzyme

Rejuvenation of *B. bassiana* fungus isolates on agar media containing colloidal chitin 0.3% after incubation for seven days indicating the formation of clear zones around the colony. The clear zone indicate that the isolate was capable of degrading the chitin substrate contained in the chitin medium so that chitin in the media will stimulate *B. bassiana* isolates to produce chitinase to utilize chitin as a source of carbon. The clear zone formed as shown in figure 1.

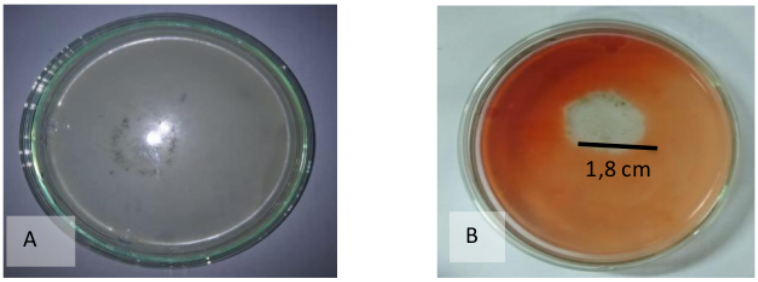

Figure 1. Chitinolithic index of *B. bassiana* (A) before *congo red addition and* (B) after *congo red addition*.

The formation of clear zones around the colony of microorganisms indicates the production of extracellular enzymes [11]. Chitin substrate in the media will hydrolyzed by chitinase, resulting in clear zones around the *B. bassiana* isolate colony. The size of the clear zone formed around the colo depends on the large number of monomers produced. The research results of [2] reveal that the size of the clear zone produced depends on the number of *N*-acetylglucosamine monomers produced from the process of chitin hydrolysis by severing *β*-1.4 homopolymer N-acetylglucosamine. The clear zone will become more apparent after adding congo red ($C_{32}H_{22}N_6O_6S_2Na_2$) solution associated with the polymer chitin substrate bond *β*-1.4 in the medium so that it turns red. Rinsing with distilled water and NaCl will dissolve congo red, especially in the area around the colony that contains reducing sugars so that a clear zone will appear [12, 13].

3.2. Protein content measurement

The protein content in each fraction was measured using the method of [9]. Quantitative analysis in this method is carried out in 3 stages. First, determine the maximum wavelength of the BSA, which in this study obtained 660 nm as the maximum wavelength. Second, making BSA standard curves to determine the concentration and absorbance of standard proteins, the absorbance results obtained are calculated by substituting the standard curve equation Y = ax + b (figure 2). The third is the stage of measuring chitinase protein levels (table 1).

The protein in chitinase will react with Cu in an alkaline solution forming a copper ion complex with amide bonds through this method. The blue darker after reacting with Folin Ciocalteau Reagent reduces the yellow phosphotungstate and phosphorolytic by tyrosine and tryptophan present in the protein into blue molybdenum and blue tungsten [10].

Based on the results of research shows that the greater concentration was, the more excellent absorbency. We obtained a standard curve between the absorption of the protein solution and its concentration. Curves formed in a linear straight line because the protein solution used was a dilute solution with a minor concentration. Bee Law deviations apply if the protein solution used is of high concentration, meaning the protein concentration is large, then the linear line will turn.

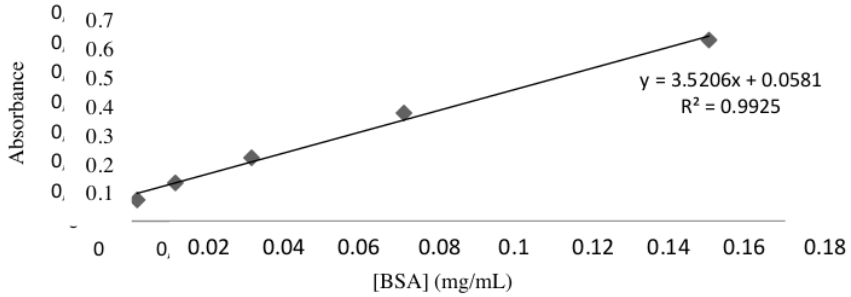

Figure 2. Standard curve.

The equation obtained in this study was Y = 3.5206 x + 0.0581 with a value of R2 = 0.9925 (figure 2). The value of the R2 price in the study is in line with the results of [14] research, namely the results obtained by the equation y = mx + c with a price of R2 = 0.9927. In the lowry protein test, one thing to pay attention to is lowry reagents in new conditions because they are easily damaged by oxidation [14].

Table 1. Enzyme protein content (mg/mL).

Sample code	Absorbance	FP	Measured protein (mg/mL)
Simplo	0.090	10	0.09
Duplo	0.099	10	0.12
Triplo	0.095	10	0.10
Average protein:			0.10

The results in table 1 show that the measured protein from the chitinase enzyme in *B. bassiana* was the highest at an absorbance of 0.099, namely 0.12 mg/mL. The mean protein measured was 0.10 mg/mL. Protein content data is needed to determine the specific activity of an enzyme.

3.3. Measurement of chitinase specific activity

The specific activity of chitinase can be calculated from units of enzyme activity per mg protein. Fractions with the highest specified value of activity were fractions with the highest number of enzymes

than those of others. Thus, a specific enzyme activity describes the level of purity of an enzyme (table 2).

Table 2. Chitinase enzyme activity of each fraction of purification results.

Sample code	Absorbance	Enzyme activity (U/mL)	Protein content (mg/mL)	Specific activities (U/mg protein)
Simplo	0.061	1.017	0.100	10.17
Duplo	0.060	1.000	0.100	10.00
Triplo	0.059	0.983	0.100	9.83
average	0.060	1.000	0.100	10.00

Based on the results of testing on the enzyme chitinase activity, the average enzyme activity was 1 U/mL with a protein content of 1 mg/mL. In contrast, the specific activity of the chitinase enzyme was 10 U/mg Protein indicates that *B. bassiana* to degrade chitin in *T. castaneum* was high compared to research conducted by [15], which was the enzyme activity by *B.bassiana* from Enrekang isolate the highest of 7.15 U/mL due to several factors, including pH, temperature, substrate concentration, and medium components corresponding to the type of enzyme chitinase produced by *B. bassiana* in this study.

4. Conclusions

We conclude that chitinase enzyme can be obtained from *B. bassiana* fungus isolate from *T. castaneum* cadaver using chitin colloidal substrate. The average activity of specific chitinase origin from *B. bassiana* isolate was 1 Unit/mg. Protein test with BSA standard solution and Lowry method obtained as a result of reading with Spectrophotometer was R2=0.9925.

References

[1] Daud I D, Junaid M and Tuwo M 2020 Endophytic seed with *Beauveria bassiana* and liquid compost: Control of pest stem borer of corn, *Ostrinia furnacalis* and increase yield resilient in marginal land? *IOP Conf.Ser.: Earth and Environmental Sci*. **486**(1). 012142

[2] Suryadi Y, Priyatno T P, Samudra I M, Dwi N. Susilowati, Lawati N and Kustaman E 2013 Partial Purification and Characterization of Chitinase from Entomopathogenic Fungus *Beauveria bassiana* Isolate BB200109 *J. Agro Biogen* **9** 77-84

[3] Bielka H, Dixon H B, Karlson P, Liebeeg C, Sharon N, Van Lenten F J, Velix S F, Vligenhart J F G and Webb E C 1984 *Enzyme Nomenclature* (New York: Academic Press)

[4] Prayogo Y and Suharsono 2005 Optimization of the control of soybean pods (*Riptortus linearis*) with the entomopathogenic fungus *Verticillium lecanii J Litbang Pertanian* **24**

[5] Junaid M, Rosmana A and Firman 2020 Testing Chitinase and b-glucanase produced by native *Trichoderma* isolates obtained from South SulawesI *IOP Conf. Ser.: Earth Environ. Sci*. **486** 012159

[6] Haedar N, Natsir H, Fahruddin and Aryanti W 2017 Production and Characterization of Chitinase Enzyme from Chitinolytic Bacteria from Anadara Granosa Shells *J. Ilmu Alam Lingkungan* **8** 14 – 21

[7] Mubarik N R, Irni M, Amaryllis A and Sugeng S 2010 Chitinolytic Bacteria Isolated from Chili Rhizosphere: Chitinase Characterization and Its Application as Biocontrol for Whitefly (*Bemisia tabaci* Genn) *Am. J. Agric. Biol. Sci*. **5** 430-435

[8] Karso, Wuryanti and Sriatun 2014 Isolation and Characterization of Chitinase Isolate of KC3 Chitinolytic Fungus from Cockroaches (Orthoptera) *J. Kimia Sains Aplikasi* **17** 51 – 57

[9] Veda M and Arai M 1992 Purification and some properties of chitinases from *Aeromonas* sp. No. 10S-24 *Biosci. Biotechnol. Biochem*. **56** 460-464

[10] Lowry O H, Rosebrough N J, Farr A L and Randall R J 1951 Protein measurement with the Folin phenol reagent *J. Biol. Chem*. **193** 265-275
[11] Nuniek H, Joko T R, Mudasir and Sabirin 2009 Kitinase and mikroorganisme kitinolitik: Isolasi, karakterisasi, and manfaatnya *Indo. J. Chem* **9** 37-47
[12] Downie B, Hilhorst H W M and Bewley J D 1994 A new assay for quantifying endo-β-D-mananase activity using congo red dye *Phytochem*. **36** 829-835
[13] Sumardi, Suwanto A, Suhartono T M and Tresnawati P 2005 Isolation and characterization of mannanolityc bacteria from palm oil shel and their mannanase enzyme production properties *Biotropica* **25** 1-10
[14] Harjanto S 2017 Comparison of Absorbance Readings Using Spectronic 20 D + and Spectrophotometer UV-Vis T 60U in Determination of Protein Levels with BSA Standard Solution *J. Kimia Sains dan Aplikasi* **20** 114-116
[15] Rachmawaty R 2009 Comparison of Chitinase Enzymes from Beauveria bassiana, a local strain of South Sulawesi, on Mortality of Armyworms (*Spodoptera litura*) *Bionature Vol*. **10** 60-64

Production of the chitinase by Beauveria bassiana in infecting Tribolium castaneum

ORIGINALITY REPORT

21%	15%	17%	8%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	scalenet.info Internet Source	3%
2	Submitted to Consorcio CIXUG Student Paper	2%
3	smujo.id Internet Source	2%
4	I D Daud, A Gassa, A Rizwaldy. "Effectiveness of Beauveria bassiana Vuill. isolate on various culture media and its pathogenicity against Tribolium castaneum", IOP Conference Series: Earth and Environmental Science, 2020 Publication	1%
5	es.scribd.com Internet Source	1%
6	T Abdullah, T Kuswinanti, S N Aminah, Asman Asman. "The potential of Beauveria bassiana vuill to control rice leaf roller cnaphalocrocis medinalis guenee", IOP Conference Series: Earth and Environmental Science, 2021 Publication	1%

7	scholar.google.co.id Internet Source	1 %
8	Submitted to Sriwijaya University Student Paper	1 %
9	eprints.undip.ac.id Internet Source	1 %
10	Muzuni, NA Yanti, WM Prasetya. "Characterization of the gene encoding chitinase enzyme from bacillus isolates insulated from some locations in Southeast Sulawesi", Journal of Physics: Conference Series, 2021 Publication	1 %
11	media.neliti.com Internet Source	1 %
12	N E Elawati, S Pujiyanto, E Kusdiyantini. " Production of extracellular chitinase under submerged fermentation conditions ", Journal of Physics: Conference Series, 2018 Publication	1 %
13	Submitted to Universitas Sumatera Utara Student Paper	1 %
14	A Meyuliana, I Suliansyah, J Jamsari. "Expression ChiPut-II gene from Serratia plymuthica UBCR_12", IOP Conference Series: Earth and Environmental Science, 2020	1 %

Publication

15	Monika Gupta, Nafe Aziz, Devendra Choudhary, Neeraj Shrivastava, Ajit Varma, Bishwajeet Paul. "Identification of Chitin Degrading Bacterial Strains Isolated from Bulk and Rhizospheric Soil", Journal of Pure and Applied Microbiology, 2018 Publication	1 %
16	A K F Bahar, B Patandjengi, A Nasruddin, V Membalik. "Isolation and antagonism of chitinolytic bacteria from Ipomea pes caprae against Lasiodiplodia pseudotheobromae", IOP Conference Series: Earth and Environmental Science, 2021 Publication	<1 %
17	Kihachiro Ogawa, Naoto Yoshida, Kunichi Kariya, Chikako Ohnishi, Ryuichiro Ikeda. "Purification and characterization of a novel chitinase from Burkholderia cepacia strain KH2 isolated from the bed log of Lentinus edodes, Shiitake mushroom.", The Journal of General and Applied Microbiology, 2002 Publication	<1 %
18	N Djaenuddin, Suwarti, S Pakki, A Muis. "Characterization of physiological properties of bacteria isolates TM4 and BNt8 in biopesticide formulas", IOP Conference Series: Earth and Environmental Science, 2021 Publication	<1 %

19	nanopdf.com Internet Source	<1 %
20	darshanpublishers.com Internet Source	<1 %
21	repository.unusa.ac.id Internet Source	<1 %
22	www.jmb.or.kr Internet Source	<1 %
23	www.tarj.in Internet Source	<1 %
24	N Jayanthi, M G M Purwanto, R Chrisnasari, T Pantjajani, A Wahjudi, M Sugiarto. "Characterization of thermostable chitinase from Bacillus licheniformis B2", IOP Conference Series: Earth and Environmental Science, 2019 Publication	<1 %

Exclude quotes	On	Exclude matches	< 5 words
Exclude bibliography	On		