

AKTIVITAS BAKTERI KITINOLITIK YANG DIISOLASI DARI TAMBAK UDANG DI TUBAN

ACTIVITY of CHITINOLYTIC BACTERIA ISOLATED from SHRIMP PONDS in TUBAN

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Abstrak. Penelitian ini bertujuan untuk mengetahui aktivitas bakteri kitinolitik yang diisolasi dari tambak udang di Tuban. Bakteri kitinolitik berhasil di isolasi dengan menumbuhkan pada media yang mengandung kitin koloidal 1%. Diperoleh 24 isolat yang diberi nama dengan TBN 1 sampai dengan TBN 24. Uji aktivitas kitinase menggunakan metode Monreal dan Reese yang didasarkan pada pelepasan N-asetil-glukosamin yang dikomplekskan dengan asam 3,5-dinitrosalisilat. Aktivitas kitinase tertinggi ditunjukkan oleh isolat TBN 11 sebesar 0.2206 U/mL.

Kata kunci: aktivitas kitinase, bakteri kitinolitik, kitin koloidal,

Abstract. The aim from this research was to determine the activity of chitinolytic bacteria isolated from shrimp ponds in Tuban. Chitinolytic bacteria were isolated by growing in media containing 1% colloidal chitin. Obtained 24 isolates which was named with TBN 1 up to TBN 24. Chitinase activity assay by using Monreal and Reese method based on release of N-acetyl-glucosamine were complexed with 3,5-dinitrosalicylic acid. The highest chitinase activity was shown by isolates TBN 11 that was 0.2206 U/mL.

Keyword: chitinase activity, chitinolytic bacteria, colloidal chitin.

INTRODUCTION

Chitinolytic bacteria are bacteria that can produce chitinase enzyme and it can be degrade chitin into the monomer N-acetyl glucosamine. Chitinolytic bacteria are obtainable from rhizosphere, phyllosphere, ground, or from water environment like sea, lake, shrimp ponds etc. Not only from mesophile area, chitinolytic bacteria can be isolated from extreme area, like from hot spring, mud volcano, geothermal area etc. Until now, the number and diversity of chitinolytic bacteria are not yet knowledgeable [1][2].

Exploration habitats of chitinolytic bacteria are needful to know the diversity of chitinolytic bacteria that can produce chitinase enzyme in high activity. In shrimp pond, there are molting processes from shrimp periodically that produce chitin [3]. Chitin can

be degradation rapidly in to the monomer N-acetyl-glucosamine, it's presumable because of the activity of chitinolytic bacteria.

Chitinolytic bacteria are interesting to isolated because it can be degrade chitin with biocompatible and non toxic. Application of chitinolytic bacteria are widely use in many sectors like biocontrol of insect and fungi, mosquito control and processing chitin waste from industries [4]. Not only chitinolytic bacteria are widely use in many sectors, but also derivate from chitin are use in many sectors too. In pharmacies the monomer of chitin N-acetyl-glucosamine is use as supplement and medicine to control blood sugar level, in cosmetics it's used to reduce the activity of tirosinase enzyme that produce melanin [2].

Chitinolytic bacteria are obtainable with growing it in media containing chitin. The presence of chitinolytic bacteria can be detected with clear zone around the colonies in solid media containing chitin [5]. Some bacteria utilize chitin as source of carbon and nitrogen [2].

EXPERIMENTAL SECTION

Tools

Micropipette, centrifuge (eppendorf 5810 R), shaker, laminar air flow, UV-Vis (Shimadzu 1800), autoclave (Hirayama), and glass equipments.

Material

Yeast extract (difco), bacto tripton (difco), agar, NaCl, Shrimps Shell, HCl p.a (Sigma), NaOH, Sodium potassium tartrate, 3,5-Dinitrosalicylic Acid (sigma), deionized water, NaH_2PO_4 , Na_2HPO_4 , N-acetyl-glucosamine (sigma).

Procedure

Isolation Chitin from Shrimps Shell

There were two stage for isolation chitin from shrimps shell first deproteination using sodium hydroxide and then demineralization using chloric acid [6].

Chitin Colloidal Substrate

Colloidal chitin was made from chitin that diluted in concentrated HCl based on method of Hsu and Lockwood [7].

Isolation Bacteria from Shrimp Ponds

Sample water from shrimp ponds Tuban was taken randomly and then mix until homogen. As many as 100 μL from sample water were spread in Luria bertani broth agar media and then it incubated in room temperature for 24 hours for get mix culture.

Selection of Bacteria Produce Chitinase from Shrim Ponds

Every single colony was inoculated in media containing colloidal chitin 1% then it incubated in shaker 150 rpm for 20 hours at

room temperature. The cultures were harvested by centrifuge at 4000 rpm for 15 minute at 4°C . The supernatant was collected and determine the activity with collorimetric method . The forming of clear zone could be detected with inoculated the single colony to solid media containing colloidal chitin 0,3%.

Assay of Chitinase Activity

Activity of chitinase was determine based on release of N-acetyl-glucosamine complexed with 3,5-dinitrosalicylic acid [8]. As many as 2 mL of chitin solution 1% (m/v) diluted in the 0,2 M phosphate buffer and added 0,5 mL of enzyme solution. Then it incubated for 2 hours. After that, it was take place on the boiling water for 5 minutes and then cooling in room temperature. The suspension were centrifugated for 10 minutes in 4000 rpm. As many as 1 mL of supernatan was added with 2 mL of deionized water and 1,5 mL colouring reagen that consist of 5,3 M sodium photasium tartrate and 3,5- dinitrosalicilic acid 96 mM. Then it incubated for 5 minutes in boiling water after that cooling it in room temperature. Finally it was take place on the cuvetee and read in wave leght 540 nm.

RESULT AND DISCUSSION

Isolation Bacteria from Shrimp Ponds

In this research was used sample water taken from shrimp ponds Tuban whereas the sample water had a temperature 30°C with pH 7. Spread plate sample water in the luria bertani broth agar media look like in Figure 1. Obtained 24 isolates which was named with TBN 1 up to TBN 24.

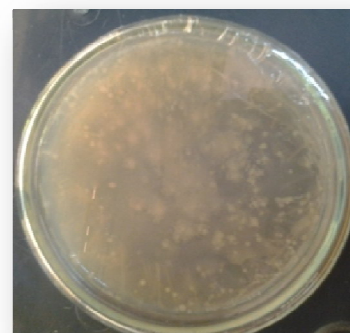


Figure 1. Mixed culture isolated from shrimp Ponds

Assay Activity of Chitinase

All of the isolates were determined the chitinolytic activity by growing in media containing colloidal chitin. Colloidal chitin act as inducer to chitinase gene in bacteria with the result that these gene was expressed [9]. The conventional method using colloidal chitin as substrate was found very effective for determining the activity of chitinase. *Aphamocladium album* could produce 9 types of chitinase when grown on colloidal chitin, but when in the chitin powder only produces 3 types of chitinase, it suspected due to the conformation and cross-linking different at polysaccharides with colloidal chitin in the growth media [1][2].

Table 1. Chitinolytic activity of TBN 1 up to TBN 24

Isolate	Chitinase Activity (U/mL)
TBN 1	0.2988
TBN 2	0.2018
TBN 3	0.1901
TBN 4	0.1956
TBN 5	0.2597
TBN 6	0.2597
TBN 7	0.2417
TBN 8	0.1791
TBN 9	0.7974
TBN 10	1.1436
TBN 11	1.1600
TBN 12	1.0756
TBN 13	1.1241
TBN 14	1.1029
TBN 15	1.0584
TBN 16	1.1256
TBN 17	0.0954
TBN 18	0.1119
TBN 19	0.1392
TBN 20	0.1455
TBN 21	0.1588
TBN 22	0.0767
TBN 23	0.1244
TBN 24	0.1635

Based on Table 1, chitinolytic activity of 24 isolates have range from 0.0767 U/mL until 1.1600 U/mL. Isolate TBN 11 yields the highest chitinolytic activity, that is 1.1600 U/mL. Three isolate that yield the highest chitinolytic activity was tested the activity again, to determine isolate that had the highest activity.

Table 2. Chitinolytic activity of 3 isolate representative

Isolate	Chitinase Activity (U/mL)
TBN 10	0.2198
TBN 11	0.2206
TBN 16	0.2159

Based on table 2, tested again the chitinolytic activity, isolate TBN 11 keep yields the highest activity that is 0.2206 U/mL. Chitinolytic activity from TBN 11 was lower than chitinolytic activity from LA 21 isolated from shrimp pond in Lamongan that was 0.7321 U/mL [10] and it was higher than *Stenotrophomonas* sp. B2-4 isolated from water soil in Pasuruan that was 0.0048 U/mL [11] and *Enterobacter* sp. G-1 isolated from water in Matsue city, Japan the activity was 0.039 U/ml [12].

Most of the microorganisms from soil and water is superior to degrade chitin and some microorganisms utilize chitin as a carbon and nitrogen source [2]. Chitin degradation that hydrolize β -1,4-glycoside bonds involves three types of chitinase, first endochitinase will hydrolize β -1,4-glycoside bonds and produce oligomer chitin then exochitinase will cut the oligomer from the nonreducing end of oligomers and produce the dimmer N-acetyl-glucosamine finally chitobiose hydrolized dimmer N-acetyl-glucosamine into the monomer N-acetyl-glucosamine [13].

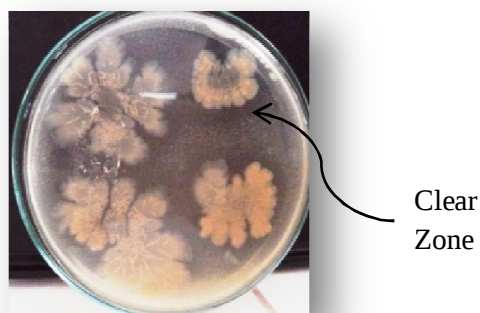


Figure 2. Clear zone isolate TBN 11

Based on figure 2, bacteria that yield the activity of chitinolytic gave clear zone. It caused that chitinases were produced by chitinolytic bacteria had been degraded macromolecule of chitin into the oligomer chitin or that monomer N-acetyl-glucosamine. Because of that the area from the bacteria was looked clear.

CONCLUSION

Chitinolytic bacteria had been isolated from shrimp ponds in Tuban. Obtained 24 isolates which was named with TBN 1 up to TBN 24. Isolate TBN 11 yields the highest chitinolytic activity with the activity 0.2206 U/mL.

REFERENCES

1. Herdyastuti N, Raharjo TJ, Mudasir, Matsjeh S. 2009. Chitinase and Chitinolytic Microorganism: Isolation, Characterization and Potential. *Indo. J. Chem.* Vol 9. No 1. 37-47
2. Haliza W dan Suhartono MT. 2012. Karakteristik Kitinase dari Mikrobial. *Buletin Teknologi Pascapanen Pertanian* Vol 8. No 1. 1-14
3. Muzzarelli RAA. New Aspects of Chitin Chemistry and Enzymology. In *Binomium Chitin-Chitinase: Recent Issues*. Musumeci S and Paoletti MG (Ed). New York: Nova Science Publishers, Inc. page 2.
4. Patil R.S., Ghormade V dan Deshpande, M.V. 2000. Chitinolytic enzymes: an exploration. *Enzyme and Microbial Technology*. 26. 473-483.
5. O'Brien, M. and Colwell, M. M. 1987. A Rapid Test for Chitinase Activity That Uses 4-Methylumbelliferyl-N-Acetyl-D-Glucosaminide. *Applied and Environmental Microbiology*. Vol 53. No. 7. 1718-1720.
6. Acosta N, Jimenez C, Borau V and Heras A. 1993. Extraction and Characterization of Chitin from Crustaceans. *Biomass and Bioenergy*. Vol 5. No 2. 145-153
7. Hsu, S.C. dan Lockwood, J.L. 1975. Powdered Chitin Agar as a Selective Medium for Enumeration of *Actinomycetes* in Water and Soil. *American Society for Microbiology*. Vol. 29, No. 3. 422-426.
8. Monreal, J., and Reese, E.T. 1969. The Chitinase of *Serratia marcescens*. *Canadian Journal of Microbiology*. 15:689-696.
9. Tsujibo H, Kondo N, Tanaka K, Miyamoto K, Bao N, and Imamori Y, 1999, Molecular Analysis of The Gene Encoding a Novel Transglycosylative Enzyme from *Alteromonas* sp. Strain 0-7 & Its Physiological Role in The Chitinolytic System, *J. Bacteriol*, Vol 81. 5461-5466.
10. Fauziah and Herdyastuti N. Uji Aktivitas Bakteri Kitinolitik dari Tambak Udang di Lamongan dan Sidoarjo. *Unesa Journal Of Chemistry*. Vol.2, No. 1. 36-39.
11. Soeka, YS, and Sulistiani. 2011. Seleksi, Karakterisasi, dan Identifikasi Bakteri Penghasil Kitinase yang Diisolasi dari Gunung Bromo Jawa Timur. *Jurnal Natur Indonesia*. 13(2). 155-161
12. Mahata, M., Dharma A., Ryanto, I, and Rizal Y. 2008. Characterization of Extracellular Chitinase from Bacterial Isolate 99 and *Enterobacter* sp. G-1 from Matsue City, Japan. *Microbiology Indonesia*. Vol. 2, No. 1. 34-38
13. Nielsen, M.N. dan Sorensen, J. 1999. Chitinolytic activity of *Pseudomonas Fluorescens* isolates from barley and sugar beet rhizosphere. *FEMS Microbiology Ecology*. 30. 217-227.