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Original Research Article

Screening and molecular identification of gelatinase-producing bacteria isolated from Indonesian mangrove ecosystem

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Abstract

The objective of this research was to isolate and identify gelatinase-producing bacteria from several mangrove ecosystems in Indonesia. Soil samples were collected from seven coastal locations of mangrove ecosystem in 2017. The gelatinase-producing bacteria were screened by using screening and confirmation methods. The gelatinase-producing bacteria were further analyzed by using the 16s rDNA molecular method. Ten isolates were diagnosed to be gelatinase-producing bacteria, which belonged to three genera: Lysinibacilus sp, Enterobacter sp. and Proteus sp. The isolated bacteria can further be investigated for the possibility of gelatinase production.

Keywords: Coastal, Gelatinase, Hydrolisate gelatin, Marine, Phylogenetic analysis

Introduction

Proteases are commercially important enzymes which are produced by microorganisms, animals and plants. Proteases from microbial sources are preferred over enzymes from plant and animal sources because they have almost all the desired characteristics for their biotechnology applications (Rao et al., 1998; Gupta et al., 2002). Matrix Metalloproteinase (MMPs) and/or gelatinase is a group of proteases that are capable of hydrolyzing the triple-helical collagen types I, II and III by cleaving glycine amino residue. Based on the specificity of the substrate and its homologous, MMPs can be divided into 6 groups, namely collagenase, gelatinase, stromelysin, matrylisin, membrane-type MMPs (MT-MMPs), and other MMPs (Visse and Nagase, 2003). Gelatinase and collagenase are important enzymes of metalloproteases, not only in the chemical and medicine industries but also in the food and biological sciences (Hisano et al., 1989).

Gelatin hydrolisate can be produced by using enzymes. Gelatinase can randomly hydrolyze gelatin to shorter peptides. The research by Sae-Leaw et al., (2016) showed that the product of enzymatic cleavage of gelatin can be used to produce antioxidant peptides. The gelatin is repeated Gly-Pro-X or -Gly-X-Hyp peptides and is usually difficult to be hydrolysed. Hence, by finding gelatinase enzymes, the shorter peptide from gelatin can easily be produced.

Coastal and marine habitats are vast reservoirs for industrially important microorganisms. These habitats are favorite sites for mining the secondary metabolite-producing bacteria (Kelecom, 2002; Konig et al., 2006; Prihanto and Wakayama, 2016). The marine ecosystem is a reservoir of several important microorganisms (Thiyagarajanet al., 2016; Devaghiet al., 2017) which is indicated by the abundance of organic matter in their surrounding area. The bacteria performs various activities in the mangrove ecosystem such as photosynthesis, nitrogen fixation and methanogenesis. Several bacteria were reportedly capable of producing

valuable enzymes such as arysulphatase, L-glutamine, chitinase, L-asparaginase, cellulase, protease, phosphatase) etc. (Sahoo et al., 2008). However, only limited reports are available for enzyme-producing bacteria in Indonesia. Hence, this study aims to explore gelatinase-producing bacteria from the Indonesian mangrove ecosystem.

Material and Methods

Sampling

Ten sediment samples were collected from several locations of the Indonesian mangrove ecosystem from September to October 2017. Two sediment samples were each collected from Aengsareh Beach, Madura; Jenu Beach, Tuban; Sendangbiru Beach, Malang; Tegalpongo Beach, Pasuruan; Bilelando Beach, Lombok; and Sarang Beach, Rembang. The detailed locations are depicted in Figure 1. The samples were collected using sterile spatulas with the depth of 5-15 cm and were then transported to the laboratory in an ice box maintained at 4 °C.

Isolation of gelatinase-producing bacteria

Each sediment sample was serially diluted and plated on gelatin agar containing 15.0 g peptone, 4.0 g yeast extract 1.0 g, 15.0 g agar, and 1000ml sea water . The plates were incubated for 48 hours at 37^0C. The zone of hydrolysis was indicated by a clear zone and was measured and recorded by using a digital caliper (Balan et al., 2012).

Confirmation of gelatinolytic bacteria

A test of the gelatin melting technique was performed. Melt gelatin (liquid form) analysis was tested by a stab tube culture of bacteria into gelatin semisolid agar with 7.5 g / L agar. After an incubation period of 48 h, the culture was refrigerated until the bottom was hardened. If the gelatin was hydrolyzed, the medium will remain liquid during cooling; on the contrary, if the gelatin is not hydrolyzed, the medium will harden as long as it is stored in the refrigerator (Balan et al., 2012).

Identification of bacteria by the 16S-rDNA method

Molecular identification of bacteria was carried out following the method of Dereeper et al. (2008) and Prihanto et al. (2016). The total genomics were extracted using the standard phenol-chloroform method. Universal primers, 27F (5′-AGAGTTTGATCMTGGCTCAG-3′ and 1492R 5′-TACGGYTACCTTGTTACGACTT-3′. were used for the PCR amplification of 16S rDNA gene. The amplified gene was sequenced and Basic Local Alignment Search Tool (BLAST) was done using GenBank (http: //www.ncbi.nlm. nih.gov) data. The phylogenetic tree was reconstructed using online phylogenetic analysis (http: //www.phylogeny.fr).

Results and Discussion

In the present study, gelatinase-producing bacteria were reported from Aengsareh Beach, Madura; Jenu Beach, Tuban; Sendangbiru Beach, Malang; Tegalpongo Beach, Pasuruan; Bilelando Beach, Lombok; and Sarang Beach, Rembang (Table 1).

The *Lysinibacilus fusiformis* UB strain from Aengsareh Beach, Madura was identified as a good source of gelatinase. Sendangbiru Beach, Malang and Jenu Beach, Tuban was dominant for the genus of Enterobacter. Proteus penneri was found on Serang Beach, Rembang, Panggungrejo Beach, Pasuruan and Tegalpongo Beach, Pasuruan. From Bilelando Beach, Lombok, two isolates, namely Proteus penneri strain UB3 and Proteus vulgaris strain UB were isolated.

Phylogenetic analysis was carried out on the bacterial phylogenetic diagram (Figure 2). From these results, it can be seen that Proteus sp. was the highest gelatinase producer. Among the genus Proteus, the dominant species was *Proteus penneri*. Gelatinase-producing bacteria isolates contained 50% of this species alone. Prior to the year of 1983, *Proteus penneri* was classified as P. vulgaris. There were indole positive and negative for *Proteus penneri* (Hickman et al., 1982; Kishore, 2012).

P. penneri can be isolated from urine, blood, and feces. This bacteria is usually considered as an indicator of fecal pollution. The natural habitat of *P. penneri* was not clear. Furthermore, their presence has not been known for a long time. Since 2016, researchers have suggested that *P. penneri* may contribute to the bioremediation of the ecosystem as a result of the ability of this bacteria to tolerate a wide range of heavy metals and/or toxic substances (Drzewiecka, 2016). Reports on the pathogenicity of this bacteria are rare. Hence, this bacteria has the possibility to be further explored as a producer of gelatinase.

The mangrove ecosystem is a reservoir for enzyme-producing microorganisms as well as providing a vast amount of enzyme-producing bacteria. In the mangrove ecosystem, protease enzymes account for one of the dominant enzymes along with cellulose, glucanase, and lipase (Castro et al., 2014). This abundance of the

enzyme producing bacteria is related to the fact that nutrients from the river are accumulated in the estuary where it is the most common habitat of the mangrove. The report from Sathya and Ushadevi (2014) showed that protease and gelatinase are the two most dominant enzymes after amylase from Streptomyces sp. which was isolated from the mangrove ecosystem.

The diversity of the gelatinase-bacteria was relatively low. They were only from three different genus (Lysinibacillus, Enterobacter and Proteus). Most plausible explanation is due to the salinity of the water. The salinity strongly affect the soil sediment microbial diversity. This is corroborated by the Study of Yan et al., (2015). They found that the salinity and water content greatly affect the bacterial community.

It is concluded that gelatinase-producing bacteria can be isolated from the mangrove ecosystem in Indonesia. The dominant bacteria found that can produce gelatinase were Lysinibacilus sp., Enterobacter sp., and Proteus sp.

Table 1: Identification of gelatinolytic bacterial species from different locations of Indonesia

NO	SPESIES NAME	LOCATION
1	*Lysinibacilus fusiformis* stain UB	Aengsareh, Madura
2	*Enterobacter* sp.strain UB	Sendang Biru, Malang
3	*Enterobacter hormaechei* strain UB	Jenu, Tuban
4	*Proteus penneri* strain UB1	Jenu, Tuban
5	*Proteus* sp. UB1	Tegalpongo, Pasuruan
6	*Proteus* sp.UB2	Panggungrejo, Pasuruan
7	*Proteus penneri* strain UB2	Panggungrejo, Pasuruan
8	*Proteus penneri* strain UB3	Sarang, Rembang
9	*Proteus penneri* strain UB4	Bilelando, Lombok
10	*Proteus vulgaris* strain UB	Bilelando, Lombok Island

Figure 1: Sampling location in Indonesia. 01. Aengsareh Beach, Madura; 02. Tegalpongo Beach, Pasuruan; 03. Sendangbiru Beach, Malang; 04. Jenu Beach, Tuban; 05. Bilelando Beach, Lombok Island

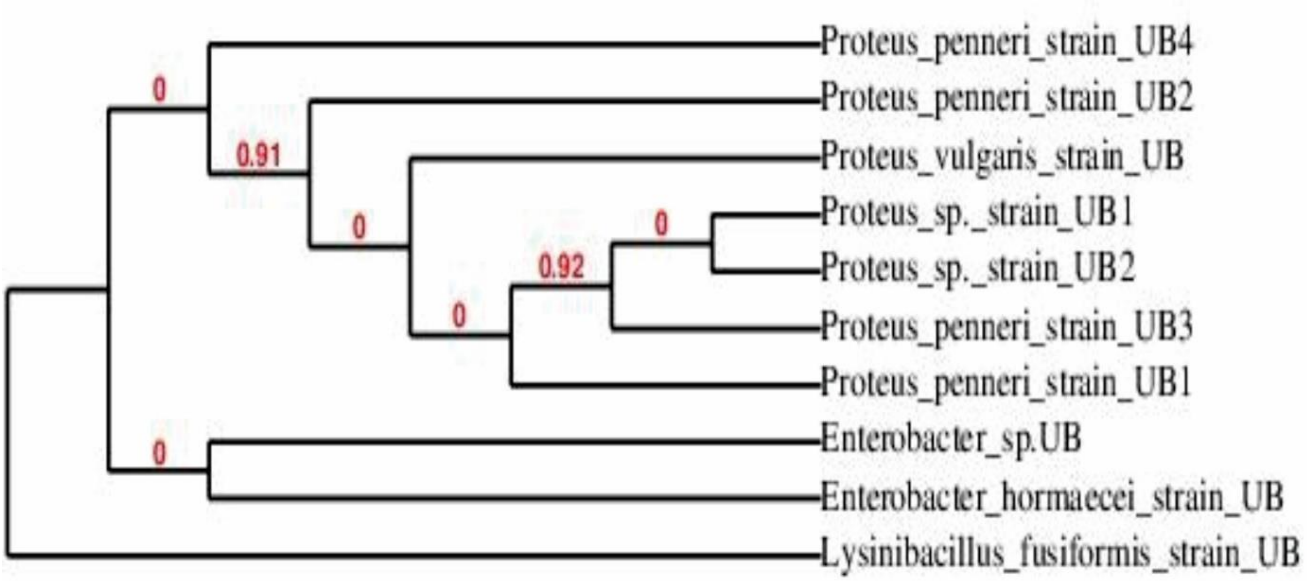

Figure 2: **Phylogenetic tree of gelatinase-producing bacteria identified from mangrove**

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References

Balan SS, Nethaji R, Sankar S and Jayalakshmi S, 2012. Production of gelatinase enzyme from Bacillus sp. isolated from the sediment sample of Porto Novo Coastal sites. Asian Pac. J. Trop. Biomed. 2(3): 1811-1816.

Castro RA, Quecine MC, Lacava PT, Batista BD, Luvizotto DM, Marcon J, Ferreira A, Melo IS and Azevedo JL, 2014. Isolation and enzyme bioprospection of endophytic bacteria associated with plants of Brazilian mangrove ecosystem. SpringerPlus. 28(3): 382-391.

Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon S, Lefort V, Lescot M, Claverie JM and Gascuel O, 2008. Phylogeny. fr: robust phylogenetic analysis for the non-specialist. Nucleic. Acids Res. 36(1): W465–W469.

Dhevagi P, Brundha A, Geetha K, Gobu R. Manju KA and Poorani AE, 2017. A preliminary study on the antimicrobial activity of marine actinomycetes. J. Environ. Biol. 38(3): 483-488.

Drzewiecka D, 2016. Significance and Roles of *Proteus* spp. Bacteria in Natural Environments. Microb. Ecol. 72(4): 741–758.

Gupta R, Beg OK and Lorenz P, 2002. Bacterial Alcaline proteases: Molecular approaches and industrial applications. Appl. Microbiol. Biotechnol. 59(1):15-32.

Hickman FW, Steigerwalt AG, Farmer JJI and Brenner DJ, 1982. Identification of *Proteus penneri sp*. nov., formerly known as *Proteus vulgaris* indole negative or as *Proteus vulgaris* biogroup 1. J. Clin. Microbiol. 15(6): 1097-1102.

Hisano T, Abe S, Wakashiro M, Kimura A and Murata K, 1989. Isolations and properties of a collagenase with caseinolytic activity from a *Pseudomonas sp.* J. Biosci. Bioeng. 68(6): 399-403.

Kelecom A, 2002. Secondary metabolites from marine microorganisms. An. Acad. Bras. Ciênc. 74(1): 51-70.

Kishore J, 2012. Isolation, identification and characterization of *Proteus penneri* - a missed rare pathogen. Indian J. Med. Res. 135(3): 341–345.

König GM, Kehraus S, Seibert SF, Abdel-Lateff A and Müller D, 2006. Natural products from marine organisms and their associated microbes. ChemBiochem. 7(2): 29-238.

Prihanto AA, Jaziri AA and Perwira IY, 2016. Purification and characterization of neutral protease from *Bacillus substilis* UBT 7 isolated from Terasi, Indonesian fermented fish. Biosci. Biotechnol. Res. Asia. 13(3): 1409-1413.

Prihanto AA and Wakayama M, 2016. Marine Microorganism: An Underexplored Source of L-Asparaginase. Adv. Food Nutr. Res. 79(1):1-25.

Rao MB, Tanksale AM, Ghatge MS and Deshpande VV, 1998. Molecular and biotechnological aspects of microbial proteases. Microbiol. Mol. Biol. Rev. 62(3): 597-635.

Sae-leaw T, Benjakul S, O'Brien NM and Kishimura K, 2016. Characteristics and functional properties of gelatin from seabass skin as influenced by defatting. Int. J. Food. Sci. Technol. 51(1): 1204–1211

Sahoo K and Dhal NK, 2009. Potential microbial diversity in mangrove ecosystems. A review. Indian J. Mar. Sci. 38(2): 249–256.

Sathya R and Ushadevi T, 2014. Industrially important enzymes producing Streptomyces species from mangrove sediments. Int. J. Pharm. Pharm. Sci. 6(10): 233-237.

Thiyagarajan S, Bavya M and Jamal A, 2016. Isolation of marine fungi sp. and its antifouling activity against marine bacteria. J. Environ. Biol. 37(5): 895-903.

Visse R and Nagase H, 2003. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ. Res. 92(8): 827-839.

Yan N, Marschner P, Cao W, Zuo C and Qin W, 2015. Influence of salinity and water content on soil microorganisms. Int. Soil. Water Cons. Res. 3(4): 316–323.