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Characterization of Extracellular Proteins Produced By *Aeromonas hydrophila* Cultured At Different Conditions

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Abstract

Esti Handayani Hardi, Gina Saptiani and Angela Mariana Lusiastuti. 2014. Characterization of Extracellular Proteins Produced By Aeromonas hydrophila Cultured At Different Conditions. International Conference of Aquaculture Indonesia 2014. Bacterial disease that is often found in tilapia fish cultivation among other groups of bacteria *Aeromonas* and *Pseudomonas*. In early 2011, found a tilapia in cages in Loa Kulu, Kutai Kartanegara in East Kalimantan death exceeds 60%, the observation known bacterial cause of death was *Aeromonas* sp. and *Pseudomonas* sp. both bacteria have different specific characteristics which influence to they pathogenicity. The aim of this research was to characterize extra cellular proteins produced by *Aeromonas hydrophila* cultured in different conditions. The bacterium was grown on TSA and in TSB media with temperature at 30°C and 40°C during 24, 48 and 72 hours. The ECP proteins were analyzed by SDS PAGE using the method described by Laemmli with silver nitrate stained and protein sequencing using mass spectrometry. A Protein of approximately 31 kDa was detected on the gel where the ECPs from the TSA cultured bacteria that has been incubated at 40°C ware analyzed. It is possible that this could be a toxic protease caused sickness and death to nila tilapia.

Keywords *: Aeromonas hydrophila;* Extracellular product; Protease; SDS PAGE

Introduction

Aeromonas hydrophila occurs widely in many kind of hosts (Pridgeon and Klesius, 2012; Kaper *et al*., 1981; Len, 1987; Pridgeon and Klesius, 2011[a,b]). *Aeromonas* sp. are ubiquitous, facultative anaerobic, Gram-negative rods, currently found in aquatic environments. Some species of this genus have been found to associate with haemorrhagic diseases and motile Aeromonas septicemia (MAS) (Harikrishnan *et al*., 2003). *Aeromonas hydrophila* has been shown to cause septicemia. Fish affected by *A. hydrophila* have been shown to have pale bodies, bleeding, and nekrosa (Karunasagar *et al*., 1989).

Aeromonas hydrophila produces a wide range of proteases, which play important roles in the invasiveness and establishment of infection, by overcoming the initial host defenses, and by providing nutrients for cell proliferation. There are many reports describing the numbers and different natures of proteases found in the culture supernatants of *A. hydrophila*. Moreover, extracellular proteases contribute to the metabolic versatility that allows Aeromonas to persist in different habitats and that facilitate ecological interactions with other organism (Sakai 1985[a]; Nieto and Elli 1986).

The aim of this study was to analyze the extracellular protease complex produced by *A. hydrophila* grown under different conditions.

Material and Methods

Bacterial Isolation, Identification and Culture Conditions

The *A. hydrophila* E1 was isolated from internal organs of moribund and death fish from cages on Mahakam river in Loa Kulu East Kalimantan. Bacterium isolation is done by taking samples of nila tilapia (changes in eksternal anatomy pathology, purulens eyes, a wound). The bacterium identification using the Aeromonas identification with biochemical test, refers to Standar

Nasional Indonesia (2009). Characteristic bacteria test include a gram test, catalase, motile, and oxidative fermentative test.

The bacterium was grown in Tryptone Soya Broth (TSB) and Tryptone Soya Agar (TSA) medium for 24, 48 and 72 h at 30°C and 40°C. Then, the bacterium cells were removed from the culture broth and agar by centrifugation at 10000 g and 4°C for 30 min.

Separation of Extracellular Products (ECP)

The cell-free supernatant (10 µL) from each culture was mixed with 10µl, one gram SDS-PAGE sample loading buffer (192 m Mglycine+0.1% +24.8 m MSDS Trishidroksi aminometan) and then boiled for 1 min. The samples and low-range protein molecular weight standards were then loaded into each well of an SDS PAGE gel (10%) using the method described by Laemmli (1970). The gel was run at 100 V for 120 min. Separated proteins in the gel were then stained with silver nitrate following the method described by Bradford (1976).

Results and Discussion

SDS-PAGE analysis of extracellular proteins (ECPs) produced by *A. hydropila* cultured in different media revealed more protein bands in ECPs produced cells cultured in TSB than TSA. Among the cells that were cultured TSB,more protein bands were observed in supernatant of cells that were incubated longer (48 and 72 h) than shorter time (24 h) and more bands were also observed in those that were incubated at 40°C than at 30°C. In contrast,the number of ECPs produced were not affected by length of incubation but the incubation temperature as similar number of proteins bands were observed in the samples that were incubated at the same temperature but more bands observed in those that were incubated at 40°C than at 30°C. The estimated sizes of ECPs protein obtained *A.hydrophila* bacteria that were incubated at 30°C and 40°C are summarized in Tables 1 and 2.

Table 1 and Figure 1 shows that *A hydropila* was cultured in TSB medium at temperatures 30°C or 40°C for more than 24 hours did not produce different proteins. However, when incubated for 24 hours appeared a different type and amount of protein. Three kinds of proteins (25.43; 24.14 and 21.74 kDa) were not produced by the bacteria that was incubated at 30°C .

Table 1. Molecular weights of extracellular proteins produced by *A. hydrophila* grown in TSB incubated at different temperature for 24.48, and 72 h

Incubation temperature 30°C			Incubation temperature 40°C		
24 Hour (a)	48 Hour (b)	72 Hour (c)	24 Hour (d)	48 Hour (e)	72 Hour (f)
38.61	109.65	109.65	109.65	109.65	109.65
29.74	80.17	80.17	80.17	80.17	80.17
25.43	50.12	50.12	50.12	50.12	50.12
24.14	38.61	38.61	38.61	38.61	38.61
21.74	29.74	29.74	29.74	29.74	29.74
	25.43	25.43	25.43	25.43	25.43
	24.14	24.14	24.14	24.14	24.14
	21.74	21.74	21.74	21.74	21.74
	17.65	17.65		17.65	17.65
	15.09	15.09		15.09	15.09
5 band	10 band	10 band	8 band	10 band	10 band

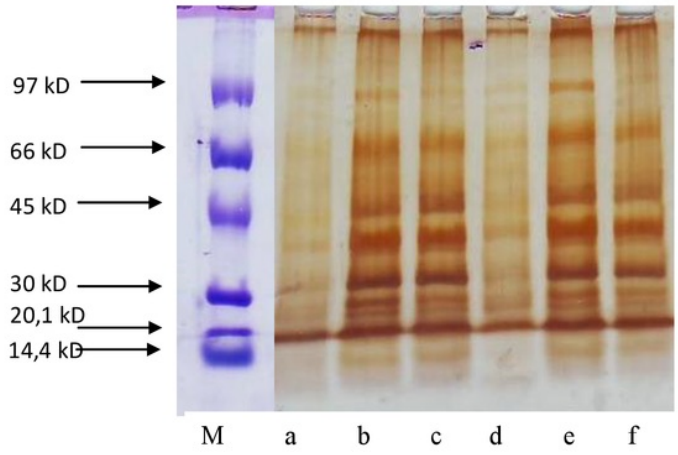

Figure 1. Silver stained SDS-PAGE gel of ECP of *A. Hydrophila* grown in TSB medium. M is low range standard proteins (Phamacia) stained with Coomassie brilliant blue. Description coding refers to Table

Type and amount of protein that produced in agar it was not the same when incubated on 30°C and 40°C. The 48 hours of incubation produce more proteins as compared to 24 or 72 hours. Some studies suggest that *A. hydrophila* produce hemolysin and proteases at certain time (Wretlind *et al*., 1973; Riddle *et al.*, 1981). Research conducted by Riddle *et al.* (1981) showed that bacteria *A. hydrophila* produce proteases after 48 hours of incubation in medium TSYE compared with BHI media. This was consistent with the results of this research that the bacterium were incubated at 48 and 72 hours have more kind of type of protein than the bacterium who was incubated at 24 hours. However, this study contrasts with research conducted by Wretlind *et al.* (1973) and Riddle *et al*. (1981), the optimal hemolytic activity was detected in the exponential phase, and reached a maximum before reaching maximum growth and decreased during the incubation period was extended.

Results ECP of *A. hydrophila* grown on medium and long incubation time showed a different proteins with different bands. The extracellular product of *A. hydrophila* grown in TSB medium at long incubation has amount of protein types more than those incubated on TSA medium. The 48 hours was a long incubation time of bacteria to produce ECP optimally, it seen that the bacteria will produced more ECP in incubation time of 48 hours compared to 24 and 72 hours. Bacterial incubation temperature 30°C and 40°C also affect the production of ECP. Higher temperatures caused in result of higher ECP production by the bacteria .

Table 2 and Figure 2 shows that the bacteria *A. hydrophila* grown on TSA medium and incubated at 40°C resulted in proteins with known molecular weight of 31.75 kDa alleged protease (Esteve and Birkbeck, 2004). In this study also found the molecular weight of 38,61-38.63 kDa protein which was the mature protein (Esteve and Birkbeck, 2004) and found no intermediate protein (43 or 44 kDa). Casco'n *et al*. 2000 showed that elastolytic activity carried out by proteins AhpB (AG2 AhpB mutant), but showed no proteolytic activity. However AhpB mature protein (38 kDa) were identified by Rivero *et al.* (1990) have no activity hydrolyses casein and elastin (Rivero *et al*., 1990). More proteins produced by the bacterium *A. hydrophila* when bacteria was incubated at 40°C. Protein produced at incubation temperature 40°C and it can not produced at it temperature 30°C were 23.66; 31.75; 40.57; 49.36 and 66.25 kDa. According Zacaria *et al.* (2010) protein with a molecular weight of 56 kDa was serine, 22 and 84 kDa metaloprotein and 93 kDa gelatinase.

Table 2. Molecular weights of extracellular proteins produced by *A. hydrophila* grown in TSA incubated at different temperature for 24.48, and 72 h

Incubation Temperature 30°C			Incubation Temperature 40°C		
24 Hour (aa)	48 Hour (bb)	72 Hour (cc)	24 Hour (dd)	48 Hour (ee)	72 Hour (ff)
113.60	113.60	113.60	113.60	113.60	113.60
76.74	98.06	98.06	98.06	98.06	98.06
	76.74	76.74	76.74	76.74	76.74
	66.25		66.25	66.25	66.25
			49.36	49.36	49.36
			40.57	38.63	38.63
			31.75	31.75	31.75
			23.66	23.66	23.66
2 band	4 band	3 band	8 band	8 band	8 band

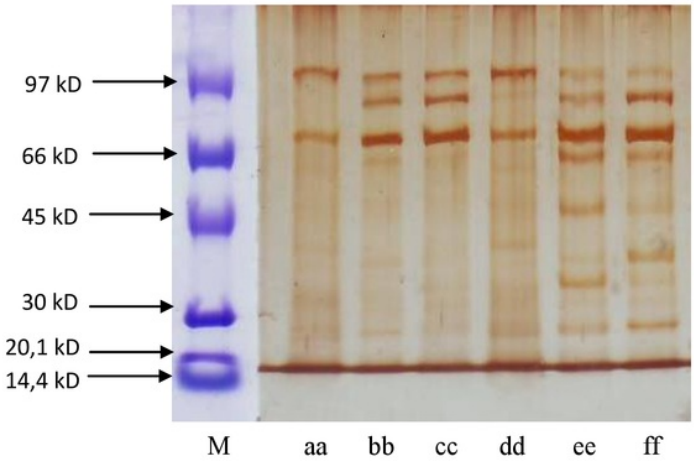

Figure 2. Silver stained SDS-PAGE gel of ECP of *A. Hydrophila* grown in TSA medium. M is low range standard proteins (Phamacia) stained with Coomassie brilliant blue. Description coding refers to Table

Conclusion

Putative protein of 31,75 kDa associated with *A. hydrophila* pathogenicity was detected in the TSA medium culture at incubation temperature 40°C. Different culture condition of incubation caused different bacterium protein produced.

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