ISOLATION AND IDENTIFICATION OF *Vibrio* sp. FROM TRADITIONAL SEAFOOD PRODUCTS OF EASTERN SURABAYA CITY AREA

ISOLASI DAN IDENTIFIKASI *Vibrio* sp. PADA PRODUK SEAFOOD TRADISIONAL AREA TIMUR KOTA SURABAYA

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Abstrak

Vibrio sp. adalah patogen oportunis yang terdapat pada ekosistem muara dan lingkungan laut. *Vibrio* sp. Dapat menyebabkan gastroenteritis dan septikemia sedangkan *V. alginolyticus* memiliki peran penting dalam pembusukan produk *seafood*. Keberadaan *Vibrio* sp. dalam produk olahan seafood perlu diperhatikan karena salah satu agen kausatif penyebab *food-borne illness*. Tujuan penelitian ini adalah untuk mengisolasi dan mengidentifikasi *Vibrio* spp. Pada produk olahan seafood tradisional daerah Surabaya Timur. Total 24 sampel telah dibeli dari pasar ikan tradisional di seluruh wilayah Surabaya Timur, yang diolah dengan menggunakan sepuluh teknik yang berbeda. Dua belas isolat dengan ukuran koloni yang berbeda dan warna diisolasi dan didasarkan pada identifikasi sebelumnya, terdapat sembilan *Vibrio* sp. Hasil uji biokimia menunjukkan bahwa terdapat delapan isolat *Vibrio alginolyticus* dan satu *V. parahaemolyticus*. Keberadaan *Vibrio* sp. dalam produk olahan *seafood* tradisional harus menjadi perhatian sebelum karena produk tersebut siap untuk makan dan dapat sumber wabah penyakit yang disebabkan oleh makanan.

Kata kunci : *Vibrio* sp., produk olahan seafood tradisional, food-borne disease, Surabaya

Introduction

Vibrio sp. is an opportunist pathogen in estuarine and marine environment. *Vibrio* sp. can be transferred into food matrics and caused food-borne illness if consumed by human. *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* are major food-borne pathogen in undercookked seafood. Pathogenic Vibrio causes acute gastroenteritis characterized by diarrhea, headache, vomitting, nausea and fever (Yang, *et al*., 2008).

Infection of *Vibrio* sp. were reported in Japan (Alam *et al*., 2002), Taiwan (Wong *et al*., 2000), China (Chen *et al*., 1991; Liu *et al*., 2004; Yang *et al*., 2008) and Indonesia (Lesmana *et al*., 2001). Pathogenic *Vibrio*, such as *V. parahaemolyticus* can produce toxin, i.e thermostable direct hemolysin (TDH), TDH-related hemolysin (TRH) or both (Miyamoto *et al*., 1969, Yang *et al*., 2008).

Traditional seafood product of Indonesia is widely consumed in daily consumtion. Mostly traditional seafood product can be separated into boiled seafood, boiling salt or *pindang*, fish or shrimp cracker, clam satay, dried fish (*bulu ayam*) or shrimp (*rebon kering*), and salt dried fish. All of these products can contain Vibrio since they produced in seashore area and usually not consern of sanitation and hygiene. Research related to isolating and identification of pathogenic Vibrio in seafood was conducted in many coutry (Yang *et al*., 2008) but isolation and identification of pathogenic *Vibrio* sp. of Indonesian traditional seafood product still low even though this research is really important. Based on the reason before, the objectives of this research were isolated and identified of Vibrio sp. in Indonesian traditional seafood product, especially in western Surabaya area.

Material and Method

Sampling

Total twenty four samples (*pindang*, *satay*, boiled, dried, salted, cracker, *otak-otak*) were collected from traditional market arround western Surabaya area, Indonesia. The samples were purchased from traditional market and taken to laboratory employing polyethylene bag and analyzed three hours after arrived in laboratory. The category and specification of samples was showed in Table 1.

Enrichment

Five gram of samples were homogenized with sterile alkaline peptone water (APW) (1%

Table 1. Samples of traditional seafood products

No	Samples		Code	Count of *Vibrio* sp. Isolated
	Seafood Products	Raw Material		
1	Smoked	*Banyar*	AB	0
2	Smoked	Ray	AP	1
3	Smoked	Mackerel	AT	0
4	*Pepes*	*Tongkol*	ET	0
5	Dried	*Bulu Ayam*	KB	1
6	Dried	*Rebon*	KR	0
7	Dried	*Duk-Duk*	KD	0
8	Dried	*Layur*	KL	0
9	Dried	*Teri*	KT	0
10	Sauce	Sarden	MS	0
11	Salted	*Klothok*	NK	0
12	Salted	*Glomo*	NG	1
13	Salted	*Wader*	NW	0
14	*Otak-Otak*	Fish	OI	0
15	Boiled Salt	*Layar*	PL	4
16	Boiled Salt	*Tongkol*	PT	2
17	Boiled	*Kupang*	RK	0
18	Satay	Shell	SK	3
19	*Terasi*	Shrimp	TU	0
20	Chips	*Lorjuk*	UL	0
21	Chips	*Kupang*	UK	0
22	Chips	Squid	UC	0
23	Chips	*Tengiri*	UT	0
24	Chips	Shrimp	UU	0

peptone, 2% NaCl, pH 8) and incubated at 37^{o}C for 5 h withoud shaking (modified Yang et al., 2008). All sampes were stored in polyethylene bag or 15 mL reaction tube before isolation process.

Isolation of *Vibrio* sp. from traditional seafood products

One full loop of enriched samples was streaked onto thiosulfate-citrate-bile salts-sucrose (TCBS) agar (Merck) and incubated at 37^{o}C for 20 h. Colonies with diameter 2-4 mm, form yellow or green colour, produce or not produce yellow hallow in TCBS medium were picked and cultured in tryptic soya broth (TSB) (Merck) and incubated at 37^{o}C for 18 h before stored. Isolates were stored in TSB supplemented with 50% glycerol and stored at -20^{o}C before identification.

Identification

All isolates were cultured from frozen state in TSB medium supplemented with 3% NaCl for 5 hour before Gram staining procedures. Gram staining was done employing standard procedure. Only isolates that showed Gram negative and rod shape were further identification, including catalase test, oxydase test, hydrolysis gelatin, fermentation pattern to deduce the species.

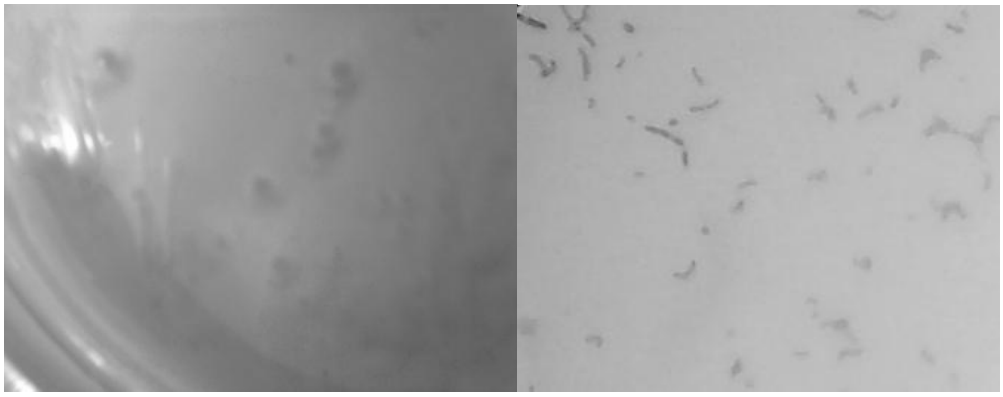
Result and Discussion

Isolation of *Vibrio* sp. from traditional seafood products

Total twelve isolates that grow in TCBS medium with different color and colony were isolated from traditional seafood products (Figure 1). All isolates that form colony in TCBS agar were then purified and store in 50% glycerol at -20^{o}C for further analysis.

Vibrio sp. is commonly found in marine environment and sometimes founded in seafood product. Occurrence of pathogenic *Vibrio* sp. in seafood products is reported from 1% (Raghunath et al., 2008) to 34.7% (Robert-Pillot et al., 2014). This amount indicated that undercooked or raw seafood product is important source of gastroenteritis and septicemia derived by seafood-borne pathogen. Low amount of isolates may due to traditional processing can inactivated of *Vibrio* in fish matrices.

Based on this result, seafood containing *Vibrio* sp. are mackerel tuna (*Tongkol*), cockle, smoked sing ray, and fish. This result is similar with the research that



a b

Fig 1 Colony of isolates in TCBS medium (a) and rod form of isolates

Table 2. Twelve isolates in thiosulfate citrate bile salts sucrose (TCBS) agar

No	Code of Isolate	Source	Description		
			Shape of colony	Colour	Diameter
1	PL1	*Pindang layar*	Round	Yellowish green	3 mm
2	PL2	*Pindang layar*	Round	Yellowish green	2 mm
3	PL3	*Pindang layar*	Round	Yellowish green	0,5 mm
4	PL4	*Pindang layar*	Round	Yellowish green	3 mm
5	PT1	*Pindang mackerel*	Round	Yellow	2 mm
6	PT2	*Pindang mackerel*	Round	Yellowish green	1 mm
7	SK1	Cockle satay	Round	Dark green	2 mm
8	SK2	Cockle satay	Round	Dark green	2 mm
9	SK3	Cockle satay	Round	Dark green	2 mm
10	AP	Smoked stingray	Round	Yellowish white	1 mm
11	KB	Dried small fish	Round	Yellowish white	1 mm
12	NG	Salted fish	Round	Yellowish white	1 mm

conducted by Raghunath *et al* (2008) that found pathogenic *Vibrio parahaemolyticus* from hen-clamp, short-nect clam, oyster, scallop, mackerel. Seafoods that harbor *Vibrio* sp. are mollusk, shellfish (Yang *et al*., 2008), hen-clamp, short-nect clam, oyster, scallop, mackerel (Raghunath, *et al*., 2008; Hara-Kudo *et al*., 2012), shrimps, oysters (Chen *et al*., 2012). This research indicated that traditional processing of satay still not sufficient to inactivating vibrio sp. in cockle flesh. This condition occurs due to satay processing is undercooked process. Raw undercooked seafood is major source of gastroenteritis caused by *Vibrio* sp. ((Tuyet *et al*., 2002; Alam *et al*., 2003; DePaola *et al*., 2003; Yang *et al*., 2008).

Primary identification of isolates

Based on Gram staining and cell form, there were three isolates that not belong to *Vibrio* sp. Isolate NG1, AP1, and KB1 were coccus, non motile, and conform linear form. Nine isolates were showed Gram negative, motile, short or long rod, curved or linear. All of those nine isolates were proceed to identification. This phenomenon is due to other bacteria, i.e. *Pseudomonas*, *Escherichia coli*, and *Proteus* can growth in TCBS agar forming transparent or small colonies (Massad and Oliver, 1987). *Vibrio* sp. has characteristic of curved rods (0.5 x1.0 um), motile, and mesophile.

Identification of *Vibrio* sp. in traditional seafood products

Based on morphology and physiology test, nine isolates of *Vibrio* sp. that isolated from traditional seafood product consist of two different species. Isolates PL1, PL2, PL3, PL4, PT1, PT2 were belongs to *V. alginolyticus* when SK1, SK2, and SK3 belongs to *V. parahaemolyticus* based on morphology and biochemistry test (Table 3).

Both species, *V. parahaemolyticus* and *V. alginolyticus*, found in invertebrates and

Table 3. Identification of isolates

Test or property	PL1, PL2, PL3, PL4, PT1, PT2	SK1, SK2, SK3	*V. alginolyticus*	*V. parahaemolyticus*
Collour in TCBS	Yellow	Green	Yellow	Green
Form	Rod	Rod	Rod	Rod
Gram	-	-	-	-
TSIA	A/A	A/A	A/A	A/A
Gas	-	-	-	-
H_2S	+	-	-	-
Catalase	+	+	+	+
Oxidase	+	+	+	+
O/F	F	F		
Sucrose	+	+	+	-
Maltose	+	+	+	+
Manitol	+	+	+	+
Nitrate reduction	+	+	+	+
Motility	+	+	+	+
Simmon citrate	-	-	-	-
MR	-	-	-	-
VP	+	-	+	-

estuarine environment and can zoonosis to human (Farmer et al., 2005). Pathogenic *Vibrio* sp. that involved in food-borne disease is *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* whereas others can cause food spoilage (*V. alginolyticus*). In humans, *V. alginolyticus* has been implicated with ear, soft tissue and wound infections (Horii et al., 2005; Austin, 2010). This found imply that there are two major species of *Vibrio* that found in traditional seafood product, i.e *V. parahaemolyticus* and *V. alginolyticus*. Vibrio has potential to infect human (zoonosis) since *V. parahaemolyticus* can produce TDH and TRH. *V. alginolyticus* play double role in seafood products, i.e. as human's wound infection and seafood spoilage.

Conclusion

Isolation and identification of *Vibrio* sp. were done in traditional seafood products of eastern-Surabaya. Based on morphology and physiology test resulted eight isolates belongs to *Vibrio alginolyticus* and one *V. parahaemolyticus*. Occurrence of *Vibrio* sp. in traditional seafood product must be prior concern since those product is ready to eat and can be source of food-borne disease outbreak and seafood spoilage.

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