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Characteristic of extracelluler metabolic of coastal ecosystem bacteria producing histidine decarboxylase crude

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Abstract

This research is aims to characteritisc of coastal ecosystem bacteria with treatment crude metabolic extraction, purification of crude metabolic with precipitation, dialysis and treatment FT-IR. This precipitate is applied on substance L-histidine becomes histamine on *In vitro* system. Research method is explorative with parameter which is bacteria isolation test, biochemical test with microbact identification system and application bacteria on L-Histidine with aeration time that is utilized, namely 6 hours, 12 hours, 18 hours, and 24 hours to test histamine rates. This research result extracelluler metabolics bacteria *Planococcus citreus* on aeration 24 hour point out to histamine rate 26.72 mg/kg, *Acinetobacter baumanii* 22.75 mg/kg, *Enterobacter gergoviae* 7.31 mg/kg, *Bacillus megaterium* 6.20 mg/kg and *Nitrococcus* sp 2.33 mg/kg respectively. The amount of protein was estimated from metabolic, precipitate and dialisat of bacteria for *Planococcus citreus*, *Enterobacter gergoviae*, *Acinetobacter baumanii* and *Bacillus megaterium*, showed that is *Planococcus citreus* beginning 3.27 mg/ml (metabolic crude), 6.65 mg/ml (precipitate 30%), 7.64 mg/ml (precipitate 40%), 8.11 mg/ml (precipitate 50%), 9.25 mg/ml (precipitate 60%), 12.17 mg/ml (precipitate 70%) and 15.57 mg/ml (dialysis purification). The FT-IR spectrum of the protein of *Planococcus citreus* revealed the presence of 5 peaks at the wave numbers of 345.34, 2075.26, 1639.38, 1400.22 and 1105.14 cm^{-1} that indicated the presence of their bending mode amide (N-H), alkena (C=C), imine (C=N), alkana secundere ($-CH_2-$) and carboxyl.

Introduction

Heterotrophic bacteria are key players in the processes of organic matter recycling, decomposition and mineralization in aquatic environments. Although only small and chemically simple compounds can be passively transported through bacterial membranes, substrates for bacterial utilization in aquatic environments are dominated by particulate or highmolecular-weight dissolved organic matter. Complex substrates must first be hydrolyzed outside the cell into smaller size molecules by extracellular enzymes and this process represents a limiting step in nutrient cycling. Bacterial extracellular enzymatic activity is regulated at the ecosystem level, by environmental factors and at the micro-environment level by enzyme-substrate interactions. Over the last century, changes in the atmosphere concentration of CO_2 and other greenhouse gases caused changes in climate patterns that have repercussions in ecosystem function and biodiversity. Microorganisms are generally able to respond very quickly to environmental changes because of their close contact with the surrounding environment and rapid growth. As mediators in important biogeochemical processes, namely decomposition and transformation of organic matter, release of inorganic nutrients for higher trophic levels and detoxification of xenobiotics, bacterial enzyme activities have the potential to be used as descriptors of biological responses to changing environmental conditions. The present paper reviews the currently available information on environmental regulation of bacterial extracellular enzymatic activity in aquatic environments and discusses the potential implications of direct and indirect effects global changes on heterotrophic bacterial communities and on the processes of organic matter recycling (Cunha *et al.*, 2010).

Microbial activity is responsible for major nutrient transformations within a mangrove ecosystem (Holguin *et al*., 1992; Alongi *et al.*, 1993). It has been documentedthat microorganisms play an important role in the productivity, conservation and rehabilitation of mangrove ecosystems (Holguin *et al*., 1992). Knowledge of microbial community diversity and the relationship between environmental factors and members of bacterial communities in mangrove sediments is important for understanding how the

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mangrove ecosystems function; this is necessary to formulate effective management and conservation strategies. To understand the community and diversity of bacteria in mangrove ecosystems, isolation and cultivation methods are often used.

Mangrove swamps provide a unique ecological niche to different microbes which play various roles in nutrients recycling as well as various environmental activities (Sahoo and Dhal, 2009; Kannan and Vincent, 2011; Akpan *et al*., 2012). The mangrove swamp is characterized by intertidal variation at intervals; at high tide the mud flat is submerged while at low tide the water flows away making the mud flat visible. Besides the intertidal variation, salinity level in the sea water could be considerably high, so most organisms inhabiting the swamps are therefore salt tolerant. The swamp soils are rich in nutrients due to the leaf litter fall. The litter supports the initial phase of the food chain through its decomposition by bacterial species and some fungi (Wafar *et al*., 1997; Akpan *et al*., 2012). The microbial action on the litter mineralizes it, decreasing its carbon content and releasing mineral nutrients such as nitrogen, phosphorus and other nutrients. The nutritive/survival value indicator of the leaf litter/microorganisms is the ratio of carbon to nitrogen (C:N) content of the leaf litter. The plant residues with the C:N ratios of 20:1 or narrower have sufficient nitrogen to supply the decomposing microorganisms and also to release nitrogen for plant use (Akpan *et al*., 2012).

Six bacterial genera were identified based on their morphological, biochemical and physiological characteristics for the mangrove swamp soils supporting tall mangrove, short mangrove and Nypa palms in the Cross River estuary, South-East Nigeria. The six genera of bacteria were *Micrococcus* sp., *Bacillus* sp., *Pseudomonas* sp., *Staphylococcus* sp. and *Streptococcus* sp. The percentage distributions of the isolates were as follows: *Streptomyces* sp. (33-50%), *Micrococcus* sp. (17%), *Bacillus* sp. (17-33%), *Pseudomonas* sp. (17%), *Straphylococcus* sp. (17%) and *Streptococcus* sp. (17%). *Streptomyces* sp., *Micrococcus* sp., *Bacillus* sp. and *Pseudomonas* sp. were classified as indigenous (authochthonous) bacteria.

Biogenic amines are physiologically degraded through oxidative deamination catalysed by amines oxidase by the following reaction: $R\text{-}CH_2\text{-}NH\text{-}R' + O_2 + H_2O \rightarrow R\text{-}CHO + H_2N\text{-}R' + H_2O_2$ (Murooka *et al*., 1979; Ishizuka *et al*., 1993; Yamashita *et al*., 1993; Zaman *et al*., 2010). Monoamine and diamine oxidases are ubiquitous and play an important role in the metabolism of amines in human, plant, and animal cells. Furthermore, these enzymes have also been found in some bacterial strains. Monoamine oxidase was found in some strains of *Enterobacteriaceae* family such as *Klebsiella*, *Enterobacter*, *Eschericia*, *Salmonella*, *Serratia* and *Proteus* (Murooka *et al*., 1979; Zaman *et al*., 2010). Ishizuka *et al*. (1993) reported that *Micrococcus rubens* possesses putrescine oxidase. Some strains of the food fermenting microorganisms such as *Micrococcus* sp. and *Brevibacterium linens* exhibit the ability to degrade histamine and tyramine, while the strains of *Lactobacillus plantarum*, *Lactobacillus sake*, *Lactobacillus pentosus* and *Pediococcus acidilactici* only degrade histamine (Leuschner *et al*., 1998; Zaman *et al*., 2010).

Histamine is formed mainly through the decarboxylation of histidine by exogenous decarboxylase released by many bacterial species known to possess histidine decarboxylase. These bacteria have been isolated not only from fish and other seafood products, but also other types of foods such as cheese, fermented sausage, and wine (Taylor, 1986). In these fermented foods, several species of histamine-producing lactic acid bacteria belonging to the *Lactobacillus*, *Leuconostoc* and *Pediococcus* genera have been isolated (Stratton *et al*., 1992; Kung *et al.*, 2005; Tsai *et al*., 2005a). Recently, our research group isolated histamine formers *Staphylococcus* spp., *Enterobacter cloacae* and *Candida* spp. from mustard pickle products in Taiwan (Kung *et al.*, 2005).

Biogenic amines are formed mainly through the decarboxylation of specific free amino acids by exogenous decarboxylases released by the microbial species associated with the seafood (Rawles *et al*., 1996). Many different bacterial species are known to possess histidine decarboxylase and have the ability to produce histamine (An and Ben-Gigirey, 1998). Although only *Morganella morganii*, *Klebsiella pneumoniae* and *Hafnia alvei* have been isolated from the fish incriminated in scombroid poisoning (Taylor and Speckard, 1983). Among them are the enteric bacteria that include *Proteus vulgaris*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Serratia fonticola*, *Serratia liquefaciens*, *Klebsiella planticola*, *Raoultella ornithinolytica* and *Citrobacter freundii* (Ababouch *et al.*, 1991; Lopez-Sabater *et al.*, 1994; Kim *et al.*, 2003; Tsai *et al*., 2005b; Chen *et al.*, 2008). In addition to the enteric bacteria, *Clostridium* spp., *Vibrio alginolyticus*, *Acinetobacter lowffi*, *Plesiomonas shigelloides*, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Aeromonas* spp., and *Photobacterium* spp. have also been reported as histamine producers (Ryser *et al.*, 1984; Middlebrooks *et al*., 1988; Yatsunami and Echigo, 1991; Lopez-

Sabater *et al.*, 1994; Okuzumi *et al*., 1994; Chen *et al.*, 2008). Recently, we demonstrated the presence of histamine-forming *Proteus*, *Enterobacter*, *Klebsiella*, *Rahnella* and *Acinetobacter* in sailfish fillets in Taiwan, but failed to isolate any of the three above mentioned major histamine-formers, the *H. alvei*, *M. morganii* and *K. pneumonia* (Tsai *et al.*, 2004; Chen *et al*., 2008). This research is aims to characteritisc of coastal ecosystem bacteria with treatment crude metabolic extraction, purification of crude metabolic with precipitation and dialysis, and treatment FT-IR. This precipitate is applied on substance L-histidine becomes histamine on *In vitro* system.

Materials And Methods

Materials and equipment

Sample bacteria of coastal ecosystem namely *Bacillus megaterium* (P1), *Enterobacter gergoviae* (P2), *Planococcus citreus* (P3), *Nitrococcus* sp (Q1), and *Acinetobacter baumanii* (Q2). Trypton soy broth Media, NaCl 0.9%, and powder L-Histidine Merk SIGMA, Ammonium sulfat buffer fosfat 0,1 M pH 7, buffer fosfat 0.005 M pH 7, etanol, Na_2CO_3 5%, EDTA 50 mM pH 8, cellophane bag 10 kDa, glasswool, NaOH 1 N, HCl 0.1 N, orto-ptalatdikarbosildehid (OPT) 0.1 %, acid phospat (H_3PO_4) 3.57 N, resin of exchange ion of type dowex 1 - X8 50-10 mesh, reagen biuret 50 ml SDS-PAGE and KBr.

Inoculum preparation: The modified method (Ruiz *et al.*, 2012). Prepare inoculum, strain coastal bacteria was grown 24 h at 30°C in MRS broth. This culture was diluted 1:10 in sterile saline solution (0.85%) to a final cell density of approximately 8 log cfu/ml. This dilution was used as the *Bacillus megaterium* (P1), *Enterobacter gergoviae* (P2), *Planococcus citreus* (P3), *Nitrococcus* sp (Q1), and *Acinetobacter baumanii* (Q2).

Preparation of histamine standard solutions: the modified method (Du *et al*., 2002). Histamine stock solution (100 ppm) was prepared by dissolving 1.69 mg histamine dihydrochloride (Sigma, St. Louis, Mo., U.S.A.) into 10 ml volumetric flask with 75% methanol (75 methanol:25 water, v/v). Standard histamine solutions (0.5 to 100 ppm) for CE analysis were prepared by diluting the stock solution with 75% methanol. Equiment is utilized namely incubator, autoclave, resin's column 20 cm x 0.8 cm, reservoar 2 cm x 5 cm; Erlenmeyer 25 ml, 50 ml, 100 ml, and 1,000 ml; micro pipette, spektrofluorometer, stirer plate, tube reacts 5 ml gets to close, analytical scale, and waterbath

Separation of metabolic of coastal bacteria

The isolate of bacteria is taken one ose and is inserted in a 25 ml Trypton Soy Broth (TSB) that was prepared by autoclaving for 15 min at 121°C that also was permitted cold. Each culture is inoculated 0.6% in TSB oxoid and incubated with shaking at 37°C for 24 h. The samples were kept at 4°C for one day, and then centrifuged (12,000 g, 4°C, 15 min). Supernatants were collected and centrifuged again (12,000 g, 4°C, 15 min). The second supernatants were collected and then were kept at 4°C for one day so that produced crude metabolite bacteria (HDC Crude). The modified method (Niven *et al.*, 2012; Sahnouni *et al*., 2012).

Aplication of metabolic of coastal ecosystem bacteria with solution L-histidine

To prepared 100 ml tube that have contained 100 ml TSB and autoclaving for 15 min at 121°C that also was permitted cold and then the each tube added 1% histamine stock solution (100 ppm). This liquid will be given metabolic of coastal bacteria 0.4% namely *Nitrococcus* sp, *Acinetobacter baumanii*, (*Nitrococcus* sp + *Acinetobacter baumanii*), *Bacillus megaterium*, *Enterobacter gergoviae*, *Planococcus citreus*, (*Bacillus megaterium* + *Enterobacter gergoviae* + *Planococcus citreus*). This culture broth incubated with shaking in waterbath at 37°C for 24 h. The culture broth were taken for quantitation of histamine.

Histamine analysis with spektroflourometric

The system of spektroflourometric : histamine is extracted from fish muscle or waterwaste of fish processing with added methanol. Histamine will be converted to OH. meanwhile histamin's substance another is purificated via ion exchange resin and changed to derivate OPT compound. Flourosence quantity of histamine measured on wave length of excitsion 350 nm and emission 444 nm. Analysis Procedure: A 10 ml culture broth added 50 ml methanol and then shaking homogeneity. This liquid heated in waterbath at 60°C for 15 minutes and screened to produce filtrate and residu. Filtrate filled in tube and storaged in refrigerator at 4°C.

Prepare resin dowex: to weigh 3 g resin filled in beaker glass and added 15 ml NaOH 2 N/g resin for changing resin as OH form and stirred up with stirrer-plate for 30 minute. Liquid at up section is pour and adds again $NaOH_2$ and resin washing with aquadest as much 3 times and screened with filter paper number 588 and then kept on aquadest for one week.

Prepare resin column: glasswool put into resin column as high as 1.5 cm and resin in aquadest put into resin column as high as 1.5 cm and aquadest 1 cm for prevented resin dry. To place tube 50 ml that already contains 5 ml HCl 1 N correct down resin's column to keep all elusi sample that overlooked by resin's column. Purification of sample: a 1 ml filtrate sample put into resin column but drainese is opened so elusi filtrate is kept all on tube 50 ml (that already contains 5 ml HCl 1 N) and then kept in refrigerator.

Formation of derivatisation compound: to prepare 3 erlenmeyer for kept all sample, standard (weigh 169.1 mg histamine and diluted into 100 ml HCl 0.1 N and keeping on refrigerator) and blanco (HCl 0.1 N). To take 5 ml sample, 5 ml standard and 5 ml HCl 0.1 N. Sample is added 10 ml HCl 0.1 N with stirrer-plate and standart is added 3 ml NaOH 1 N with stirer and added again 1 ml OPT 0.1% with stirer and kept in refrigerator. Blanco is added 3 ml H_3PO_4 3.57 N with stirrer respectively. To measure fluorosense sample, standart and blanco with spektrofluorometric on wavelength of excitsion 350 nm and emission 444 nm for 90 minute (LPPMHP, 2010).

Purification of metabolic of coastal bacteria with precipitation of ammonium sulphate $(NH_4)_2SO_4$ and analysis of dialysis

Precipitation of Ammonium Sulphate $(NH_4)_2SO_4$: the supernatant was made 30%, 40%, 50%, 60% and 70% by adding salt $(NH_4)_2SO_4$/0.1 mM-PLP dropwise over 1 h with continual stirring, and stirring was continued for at least 1 h more. The solution was centrifuged at 15,000 g for 30 min, and the pellet containing HDC Crude added 4 ml buffer phosphates 0.05 M and kept at refrigerator and then dialysed overnight against the same buffer. This product is called a precipitate.

Analysis of dialysis: to prepare buffer fosfat 0.1 M pH 8 {(solution A = 0.028392 g $Na_2H_2PO_4$ dissolved in 2 ml aquadest) + (solution B = 0.143256 g $Na_2HPO_4.12H_2O$ dissolved in 4 ml aquadest) = total solution made 10 ml with rasio 2 :3} and buffer fosfat 0.05 M pH 8 {(solution A = 1.41 g Na_2HPO_4 dissolved in 200 ml aquadest) + (solution B = 6.267 g $Na_2HPO_4.12H_2O$ dissolved in 350 ml aquadest) = total solution made 1L with rasio 2 :3} and then solution Na_2CO_3 5% = 5 g Na_2CO_3 dissolved in 100 ml aquadest and EDTA 50 mM pH 8 : 1.861 g EDTA dissolved 100 ml aquadest. To prepare cellophane bag 10 kDa. Heating 100 ml solution Na_2CO_3 5% upon hot plate then inserts cellophane bag for 15 minutes, then washed by aquadest and heating 100 ml solution EDTA 50 mM pH 9 upon hot plate then inserts cellophane bag for 15 minutes, then washed by aquadest. Heating aquadest upon hot plate then inserts cellophane bag for 15 minutes, then wash with aquadest. Procedure : a precipitate filled in cellophane bag 10 kDa and is nipped every its tip and soaked in 1 L buffer phosphates 0.05 M pH 8 for 24 hours. The product dialysis filled in eppendorf as much 0.5 M and added etanol cold with rasio 1:1 with stirrer in waterbath and kept in refrigerator 4°C for one hour then The solution was centrifuged (12,000 g, 4°C, 15 min). The product is called a dialysate. The modified method from Padmakar *et al.* (2005) and Rajeswari *et al.* (2012).

Table 1. BSA concentration and dilution

No.	Material	Dilution						
1.	BSA Stock (µL)	13	26	52	104	208	416	832
2.	Aquadest (µL)	987	974	948	896	792	584	168
3.	Protein concentration (µg/ml)	15.625	31.25	62.5	125	250	500	1000

Table 2. The composition of stacking gel

No.	Composition	1 slab (µL)
1.	Acrylamide 30%	515
2.	Tris HCL 1.5 M, pH 8.8	625
3.	H_2O	1,325
4.	SDS 10%	25
5.	APS 10%	7.5
6.	TEMED	5

Table 3. The composition separating gel

No.	Composition	1 slab (µL)
1.	Acrylamide 30%	4,126
2.	Tris HCL 1.5 M, pH 8.8	2,500
3.	H_2O	3,270
4.	SDS 10%	100
5.	APS 10%	100
6.	TEMED	20

Protein estimation with BSA (Bovine Serum Albumine) methods

Determination of protein concentrates utilize biuret's method with BSA as standard. The principle of biuret methods is compound with 2 or more peptida if reacted with salt cupri in basa condition, will form colour violet's. Biuret's reaction hinges on forming a complex among Cu's ion ++ with 4 atom n peptida on basa's conditions, therefore will form a purple complex that its absorbance can be read on wavelength 540 nm. BSA is stable solution on heating 70°C for 30 minutes, more specific because 95% proteins consisting of albumine. There is excess even biuret this alone is more specific for peptida, polipeptida, and protein, don't react with ammonia, urea, and simple nitrogen compound BSA (Wiseman, 1985; Holme and Peck, 1993).

Preparation: sample (crude metabolic, precipitate and dialisate) 1 ml is dissolved in 1.000 µL Phosphate Buffer Saline (PBS) ; reagent biuret {0.075 g $CuSO_4$; 0.3 g ($KNaC_4H_4O_6$); 15 ml NaOH 2.5 M; 0.05 g KI and 50 ml aquadest} and BSA: protein standard 1.2 mg/ml and then 2 ml diluted as Table 1.

Procedure: To prepare eppendorf as much 14 numbers, content each by akuades and stock solution (BSA) so gotten by desirable concentration (accord

dilution count), then vortek. Taken 1 ml solution of each eppendorf, then moves into tube reacts. Added each tube with 2 ml reagen biuret (1:2) and incubate on temperature 37°C for 20 minutes. Read absorbance with UV Vis's spectrophotometer on wavelength 540 nm.

Analysis of protein molecule weight with SDS=PAGE Method

Prepare: A 1 mg dialysate of *Planococcus citreus*, *Acinetobacter baumanii*, *Nitrococcus* sp and *Enterobacter gergoviae* dissolved in 500 µL PBS. To make stacking is gel and separating is gel (12.5%) and the composition can be seen on Table 2 and 3. To make RSB (Reducing Sample Buffer) : 1 ml tris Cl pH 8.8 + 0.8 ml glyserol + 1.6 ml SDS (Sodium Dodesil Sulfate) 10% + 0.4 mercaptoetanol + 0.2 ml bromopenol blue then is added 0,8 ml aquadest until and homogeneity. Destaining's solution: 20 ml methanols 20% + 10 ml glasial's acetates acid 10%, then added by aquadest until volume 100 ml and doing homogenity. Staining's solution: 0.1 g coomasie blue R 0.1 % + 40 ml methanols 40% + 10 ml glasial's Acetates acid 10% then enhanced aquadest until volume 100 ml and homogeneity. Running buffer: 3.03 g tris base + 14.2 g glycine is dissolved in 700 ml aquadest then crossed a check by pH 8.8. Afterwards is added 1 g SDS, until homogenity and added by aquadest until its volume 1 L.

The procedure : To prepare power supply and buffer system so separating gel with concentration 12.5% are filled in plate and is waited until thickly. Upon separating is gel inserted stacking is gel with concentration 12.5% and comb are inserted within plate so its following will form well as place of enzyme. Sample 15 µL is given 15 µL RSB and is heated up to 5 minutes. Marker (prestained is ladder's protein) 15 µL that utilizing with Molecule Weigth (MW) 17- 250 kDa. Then sample and marker is inserted in gel well. Running buffer decanted into buffer system. After anode and cathode linked by power supply and streamed by electricity current as big as 120 Volts, 20 Ampere for 75 minutes. Elektroforesis's process is done until ribbon reaches bounds a stop to gel. Coloration did by soaks gel in staining solution while at joggle with shaker up to 1-2 hours. Hereafter decolorizing did by soaks gel in destaining solution. Process destaining done until protein ribbon on looked gel and colour gel not very blue, to achromatize background. Protein ribbon will in view in gel.

To measurement molecule weight proteins sample : to each protein sample, observed preceding gets what amount seeming protein ribbon, then each protein ribbon measured by protein move distance (Rf) with ruler (cm). Each Rf's point (distance of moving protein) for each one acquired protein ribbon, accounted by its molecule weight with linear equation of molecule weight curve (marker).

$$Rf = \frac{\text{Distance of protein moving from early place}}{\text{Distance of colour moving from early place}}$$

FT-IR analysis

In the study of molecule vibrations, Infrared spectroscopy has contributed more to this field than Raman due to the rapid developments in infrared instrumentation (Merritt *et al*., 1986). The vibrational spectra can be utilized directly and simply as molecular "finger prints" to characterize and identify the molecule (Roberts *et al*., 1985). The lyophilized metabolic crude of bacteria extract from *Planococcus citreus* was subjected to FT-IR analysis. The IR spectrum of metabolic crude of bacteria extract was recorded with a perkin-Elmer model 297 IR spectrophotometer. One part of extract was mixed with 99 part of dried potassium bromide and it was scanned between 600-4000 wave number (cm^{-1}) at a speed of 1 micron and with a programmed slit opening 2x and air as reference (Indira *et al*., 2011).

Results and Discussions

Aplication of extracelluler metabolic of coastal ecosystem bacteria with solution L-histidine

Extracelluler Metabolic bacteria single was applied by solution l histidine 100 ppm and done by aerations up to 6 hours, 12 hours, 18 hours and 24 hours on temperature ambient and then done by histamine analysis, apparently can describe histidine as histamine on Table 4. Extracelluler metabolicis bacteria *Planococcus citreus* on aeration 24 hour point out to histamine rate 26.72 mg/kg (ppm), *Acinetobacter baumanii* 22.75 mg/kg, *Enterobacter gergoviae* 7.31 mg/kg, *Bacillus megaterium* 6.20 mg/kg and *Nitrococcus* sp 2.33 mg/kg, respectively. Meanwhile metabolit of bacteria affiliate *Bacillus megaterium* + *Enterobacter gergoviae* pointing out histamin's rate 5.47 mg/kg, *Bacillus megaterium* + *Enterobacter gergoviae* + *Planococcus citreus* histamin's rate 5.1 mg/kg, *Bacillus megaterium* + *Planococcus citreus* histamin's rate 4.52 mg/kg, *Enterobacter gergoviae* + *Planococcus citreus* 3.59 mg/kg and *Nitrococcus* sp + *Acinetobacter baumanii* have histamine rate 3.33 mg/kg. Ability metabolic describes histidine to histamine on single bacteria because there are enzyme component on metabolic and predicting to constitute histidin decarboksilase enzymes, meanwhile ability decrease describes

Table 4. Histamine rate of histidine with extracelluler metabolic of coastal ecosystem bacteria

No	Sample code	Treatment			
		6 hours /mg/kg	12 hours /mg/kg	18 hours /mg/kg	24 hours /mg/kg
1.	Control	0.05			
2.	*Bacillus megaterium*	1.04	5.37	5.45	6.20
3.	*Enterobacter gergoviae*	0.02	2.37	6.13	7.31
4.	*Planococcus citreus*	2.59	3.14	3.49	26.72
5.	*Bacillus megaterium* + *Enterobacter gergoviae*	2.85	4.17	4.57	5.47
6.	*Bacillus megaterium* + *Planococcus citreus*	0.06	1.73	3.57	4.52
7.	*Enterobacter gergoviae* + *Planococcus citreus*	1.50	0.78	0.47	3.59
8.	*Bacillus megaterium* + *Enterobacter gergoviae* + *Planococcus citreus*	0.73	1.11	3.93	5.10
9.	*Nitrococcus* sp	1.77	1.28	2.05	2.33
10.	*Acinetobacter baumanii*	0.70	1.67	7.96	22.75
11	*Nitrococcus* sp + *Acinetobacter baumanii*	1.68	0.60	1.72	3.33

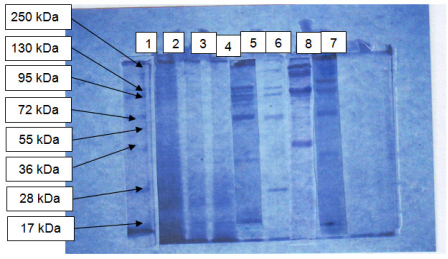

Figure 1. Profile of peptida ribbons, 1. Marker, 2. Metabolit Crude, 3. Precipitate 30%, 4. Precipitate 40%, 5. Precipitate 50%, 6. Precipitate 60%, 7. Precipitate 70%, and 8. Dialisat

histidine to histamine on merging bacteria because there are inhibitor who gets antagonist symbiosis character.

Purification of metabolic of coastal bacteria with precipitation of ammonium sulphate $(NH_4)_2SO_4$ and analysis of dialysis

Purification of bacteria metabolic done by ammonium sulphate concentrates 30%, 40%, 50%, 60% and 70%, and aliquot partly drawned out on dialysis process and then applicated on L-histidine solution and to done aeration at 24 hours. Result purification applicated on L-histidine solution, showed histamine rate on Table 5. For metabolic crude *Planococcus citreus* 2.74 mg/kg. Process precipitation with ammonium sulphate concentrates 30%, 40%, 50%, 60% and 70%, gotten by histamine rate which is 4.15 mg/kg, 4.38 mg/kg, 4.54 mg/kg, 9.42 mg/kg and 10.31 mg/kg and dialisa's process is gotten histamine rate 11.32 mg/kg, Metabolic histamine rate crude *Enterobacter gergoviae* 3.74 mg/k. Process precipitation with ammonium sulphate concentrates 30%, 40%, 50%, 60% and 70%, gotten by histamine rate which is 0.59 mg/kg, 6.31 mg/kg, 3.38 mg/kg, 1.61 mg/kg, 0.56 mg/kg, and dialisa's process is gotten titrates histamine 3.89 mg/kg. Metabolic histamine rate crude *Acinetobacter baumanii* 2.15 mg/kg. Process precipitation with ammonium sulphate concentrates 30%, 40%, 50%, 60% and 70%, gotten by histamin's rate which is 0.07 mg/kg, 0.84 mg/kg, 1.63 mg/kg, 0.39 mg/kg, 0.53 mg/kg, and dialisa's process is gotten histamine rate 3,93 mg/kg. Metabolic histamine rate crude *Bacillus megaterium* 3.57 mg/k. Process precipitation with ammonium sulphate concentrates 30%, 40%, 50%, 60% and 70%, gotten by histamin's rate which is 1.55 mg/kg, 5.30 mg/kg, 3.67 mg/kg, 2.19 mg/kg and 0.56 mg/kg, and dialisa's process is gotten titrates histamine 3.07 mg/kg.

Table 5. Histamine rate of metabolic crude, precipitate and dialisat from coastal ecosystem bacteria with L-histidine solution

No	Sample	*Planococcus citreus*	*Enterbacter gergoviae*	*Acinetobacter baumanii*	*Bacillus megaterium*
1.	Metabolic Crude	2.74	3.74	2.15	3.57
	30 %	4.15	0.59	0.07	1.55
	40 %	4.38	6.31	0.84	5.30
2.	Precipitate 50 %	4.54	3.38	1.63	3.67
	60 %	9.42	1.61	0.39	2.19
	70 %	10.31	0.56	0.53	0.56
3.	Dialysate	11.32	3.89	3.93	3.07

Table 6. The amount of protein of bacteria

No	Sample	*Planococcus citreus* (mg/ml)	*Enterobacter gergoviae* (mg/ml)	*Acinetobacter baumanii* (mg/ml)	*Bacillus megaterium* (mg/ml)
1.	Metabolic crude	3.27	6.12	7.31	2.41
2.	Precipitate 30 %	6.64	1.66	12.5	3.66
3.	Precipitate 40 %	7.64	2.88	13.33	3.83
4.	Precipitate 50 %	8.11	3.5	13.33	4.83
5.	Precipitate 60 %	9.25	4.16	12.5	3.52
6.	Precipitate 70 %	12.17	3.61	14.17	5.49
7.	Dialisat	15.57	6.23	7.25	6.62

Protein estimation with BSA (Bovine Serum Albumine) methods

The amount of protein was estimated from metabolic, precipitate and dialisat of bacteria for *Planococcus citreus*, *Enterobacter gergoviae*, *Acinetobacter baumanii* and *Bacillus megaterium*, showed that is *Planococcus citreus* beginning 3.27 mg/ml (metabolic crude), 6.65 mg/ml (precipitate 30%), 7.64 mg/ml (precipitate 40%), 8.11 mg/ml (precipitate 50%), 9.25 mg/ml (precipitate 60%), 12.17 mg/ml (precipitate 70%) and 15.57 mg/ml (dialysis purification), appealed by protein amount from others sample. It was caused to process purification with dialysis has made towards protein purification as Table 6. Dialysis is utilized to increase enzyme concentration and often been utilized to come to contaminant with size little molecule.

Analysis of protein molecule weight with SDS-PAGE method

The peptida ribbon profile, from extract *Planococcus citreus* can be seen on Figure 1, showed that metabolic crude samples appears thicker because protein rate is still outgrow. Ribbon on precipitate sample was looked marks to separate peptide and

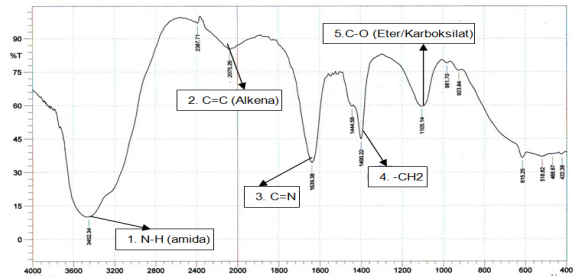

Figure 2. The FT-IR spectrum of *Planococcus citreus* metabolic

dialisate sample appears the less ribbon peptidanya. It was showed that was become hydrolysis process up to increase dialisat metabolit *Planococcus citreus*, so that breaks down protein cords become peptida little molecule weight. There are amino acids that known namely metabolic crude is 9 amino acid amount 9, the precipitate 30% is 8 amino acids, the precipitate 40% is 7 amino acids, the precipitate 50% is 7 amino acids, the precipitate 60% is 8 amino acids and, the precipitate 70% is 5 amino acids and then dialysate is 4 amino acid.

FT-IR analysis

The FT-IR spectrum of the protein of *Planococcus citreus* revealed the presence of 5 peaks at the wave numbers of 345.34, 2075.26, 1639.38, 1400.22 and 1105.14 cm^{-1} that indicated the presence of their bending mode amide (N-H), alkena (C=C), imine (C=N), alkana secundere (-CH_2-) and carboxyl so that the bending mode showed histidine decarboxylase enzyme can be seen on Figure 2.

Discussion

This research have produce extracellulermetabolic bacteria of coastal ecosystem that have potency as enzyme especially histidine decarboxylase where extracelluler metabolic bacteria will can decompose histidine to histamine and histamine is groups from biogenic amine component. Biogenic amines are formed mainly through the decarboxylation of specific free amino acids by exogenous decarboxylases released by the microbial species associated with the seafood. Many different bacterial species are known to possess histidine decarboxylase and have the ability to produce histamine (Hsu *et al*., 2009). The microbial metabolism at intracellular and extracellular level is gaining much importance to provide various useful products of industrial importance viz. enzymes, sugars, antibiotics and organic acids etc. Such diversified microbial systems are also reported from mangrove ecosystem (Christophersen *et al*., 1999; Kathiresan and Selvam, 2006). Mangroves, over millions of years, have evolved both morphologically and physiologically to adapt to swampy and saline environments. Similar adaptive characteristics in the form and function may occur with the associated microflora in such environments. Some reports are available on the occurrence of free living and symbiotic microorganisms in such saline habitats (Gupta *et al.*, 2007).

Intracellular biosynthetic enzymes are usually exposed to low substrate concentrations, in contrast to extracellular enzymes. Optimizing their catalytic efficiency (kcat/Km) in psychrophilic (cold-adapted) organisms may thus be challenging, since improving kcat at low temperatures by decreasing theactivation enthalpy may have a cost in terms of affinity for the substrate(s) of the reaction . The study of cold-active enzymes is thus an important topic in terms of physiology and metabolic evolution. No cold-active ornithine carbamoyltransferase (OTCase; EC 2.1.3.3) had been characterized until now. However, the presence of this enzyme in microorganisms adapted to the full range of environments compatible with life makes it an excellent candidate for investigations of protein evolution and of molecular adaptations to extreme conditions (Xu *et al*., 2003).

Histamine-producing bacteria (HPB) produce a large amount of histamine frequently isolated from fish subjected to appropriate examination for coastal ecosystem bacteria, that contaminat waste boiled fish processing implicated in actual HFP incidents reported to date *Nitrococcus* sp, *Acinetobacter baumanii*, *Bacillus megaterium*, *Enterobacter gergoviae*, *Planococcus citreus* such as by the addition of NaCl to the culture medium and avoidance of exposure of the bacterium to high temperatures. The former is also a prolific HPB but a mesophilic marine bacterium. It has been shown that accumulation of histamine by HPB occurs after the level of bacterial growth exceeds 10^8 CFU/ml in culture medium. These factors have led to speculation that the accumulation of histamine in thawed fish arises from the release of Histidine Decarboxylase (HDC) from the autolyzed HPB, which might occur when fish is frozen just before the level of bacterial growth reaches the concentration of formed histamine. HDC catalyzes the decarboxylation of histidine to histamine and contains two types of enzyme, a pyridoxal-5-phosphate (PLP)-dependent enzyme and a pyruvoyl-dependent enzyme. This bacteria including marine bacteria have the PLP-dependent enzyme. In this study, we constructed HDCs in metabolic extracelluler of *Nitrococcus* sp, *Acinetobacter baumanii*, *Bacillus megaterium*, *Enterobacter gergoviae*, *Planococcus citreus* in an *Escherichia coli* expression system under control of

the T7lac promoter and compared HDCs between mangrove bacteria and estuarine species (Kanki *et al*., 2007).

Identification of histamine-producing histidine decarboxylase-positive (HDC+) bacteria is difficult since they belong to diverse species and only some strains of a given species are histamine producers. HDC+ bacteria differ from non-histamine-producing (HDC−) bacteria by the presence of HDC, the enzyme that converts histidine into histamine and CO_2. Two different kinds of HDCs were found in gram-negative and gram-positive bacteria. HDCs of gram-negative bacteria use pyridoxal phosphate as a cofactor for activity, whereas HDCs of gram-positive bacteria use a different catalytic mechanism based on a pyruvoyl group linked at the active site (Lucas *et al*., 2005). Histamine can be metabolized by two alternative ways: oxidative deamination by DAO (former name: histaminase) or ring methylation by histamine-N-methyltransferase (HNMT) (Schwelberger, 2004). Whether histamine is catabolized by DAO or HNMT, is supposed to depend on the localization of histamine. The DAO protein is stored in plasma membrane-associated vesicular structures in epithelial cells of kidney and intestine and is secreted into the circulation upon stimulation (Schwelberger and Bodner, 1997; Schwelberger *et al.*, 1998). Therefore it has been proposed that DAO might be responsible for scavenging extracellular histamine after mediator release. Conversely, HNMT, the second important enzyme inactivating histamine, is a cytosolic protein, which can convert histamine only in the intracellular space of cells (Kufner *et al*., 2001; Klocker *et al*., 2005).

Extracelluler metabolic is protein and protein purification methodologies are currently applied to three distinctly different classes of proteins: (a) naturally occurring proteins, (b) recombinant proteins produced by introduction of appropriate genes into micro-organisms or cultured eukaryotic cells, and (c) complex mixtures of proteins arising proteins may be found: (a) free in solution (egg albumin, immunoglobulins, milk casein, secreted mucopolysaccharides), (b) sequestered within an extracellular matrix (seed coat proteins, oyster shell proteins, and proteins of the epidermal stratum corneum), (c) imbedded within phospholipid membranes (cell surface receptor proteins, visual pigment proteins, membrane transport proteins), (d) as components of connective tissue (keratin, yellow elastic fiber proteins, myosin, actin), or (e) contained within the fluid cytoplasm of cells (whether naturally occurring or recombinant). Some proteins associated with the extracellular matrix may be readily released by aqueous wash steps. Others require very vigorous extraction methods (Ward *et al*., 2009).

Six forms of electrophoresis dominate the field of analytical protein separations: SDS-PAGE, native PAGE, native acrylamide gradient PAGE, isoelectric focusing, two dimensional electrophoresis, and Capillary Zone Electrophoresis (CZE). The most common analytical electrophoretic method, SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis), replaced analytical ultracentrifugation, almost overnight in the 1970's, as the "method of choice" in protein molecular weight determinations. Analytical ultracentrifugation is an expensive method generally requiring a dedicated technician in a core facility. SDS-PAGE, on the other hand, is a much less expensive technique that "everyone" can do. SDS (Sodium Dodecyl Sulfate) also called laurel sulfate) is a powerful anionic detergent that binds tightly to proteins, denaturing them fully at elevated temperature (100°C) in the presence of a reducing agent like mercaptoethanol. Multi-subunit proteins (whose subunits are noncovalently associated or covalently attached by disulfide bonds) are dissociated into monomeric units by the combined action of SDS and a reducing agent at elevated temperature. So much SDS binds to a protein that the protein's intrinsic charge is overwhelmed by the bound SDS. In a cross linked polyacrylamide gel (PAGE), electrophoretic mobility of a protein, so-coated with SDS, is almost entirely based upon size of the SDS-coated protein (a structure described as a rigid rod or as a detergent micelle). Monomeric molecular weight is then determined relative to standards of known molecular weights. Fixation of the slab gel with an aqueous methanol/acetic acid mixture, followed by staining with Coomassie Brilliant Blue R allows for the sample (GFP IgG and IgY in various stages of purity) and a lane of standards.

Conclusion

Extracelluler Metabolics bacteria *Planococcus citreus* on aeration 24 hour point out to histamine rate 26.72 mg/kg, *Acinetobacter baumanii* 22.75 mg/kg, *Enterobacter gergoviae* 7.31 mg/kg, *Bacillus megaterium* 6.20 mg/kg and *Nitrococcus* sp 2.33 mg/kg, respectively. Meanwhile metabolit of bacteria affiliate *Bacillus megaterium* + *Enterobacter gergoviae* pointing out histamin's rate 5.47 mg/kg, *Bacillus megaterium* + *Enterobacter gergoviae* + *Planococcus citreus* histamin's rate 5.1 mg/kg, *Bacillus megaterium* + *Planococcus citreus* histamin's rate 4.52 mg/kg, *Enterobacter gergoviae* +

Planococcus citreus 3.59 mg/kg and *Nitrococcus* sp + *Acinetobacter baumanii* have histamine rate 3.33 mg/kg.

Metabolic crude with process precipitation with ammonium sulphate concentrates 30%, 40%, 50%, 60% and 70% for ; 1) *Planococcus citreus* 2.74 mg/kg, gotten by histamine rate which is 4.15 mg/kg, 4.38 mg/kg, 4.54 mg/kg, 9.42 mg/kg, 10.31 mg/kg and dialysa process is gotten histamine rate 11.32 mg/kg, 2) *Enterobacter gergoviae* 3.74 mg/k, gotten by histamine rate which is 0.59 mg/kg, 6.31 mg/kg, 3.38 mg/kg, 1.61 mg/kg, 0.56 mg/kg and dialysa process is gotten titrates histamine 3.89 mg/kg, 3) *Acinetobacter baumanii* 2.15 mg/kg, gotten by histamin's rate which is 0.07 mg/kg, 0.84 mg/kg, 1.63 mg/kg, 0.39 mg/kg, 0.53 mg/kg, and dialisa's process is gotten histamine rate 3,93 mg/kg, 4) *Bacillus megaterium* 3.57 mg/k, gotten by histamin's rate which is 1.55 mg/kg, 5.30 mg/kg, 3.67 mg/kg, 2.19 mg/kg, 0.56 mg/kg, and dialisa's process is gotten titrates histamine 3.07 mg/kg.

The amount of protein was estimated from metabolic, precipitate and dialisat of bacteria for *Planococcus citreus*, *Enterobacter gergoviae*, *Acinetobacter baumanii* and *Bacillus megaterium*, showed that is *Planococcus citreus* beginning 3.27 mg/ml (metabolic crude), 6.65 mg/ml (precipitate 30%), 7.64 mg/ml (precipitate 40%), 8.11 mg/ml (precipitate 50%), 9.25 mg/ml (precipitate 60%), 12.17 mg/ml (precipitate 70%) and 15.57 mg/ml (dialysis purification).

The FT-IR spectrum of the protein of *Planococcus citreus* revealed the presence of 5 peaks at the wave numbers of 345.34, 2075.26, 1639.38, 1400.22 and 1105.14 cm^{-1} that indicated the presence of their bending mode amide (N-H), alkena (C=C), imine (C=N), alkana secundere (-CH_2-) and carboxyl.

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References

Ababouch, L., Afila, M. E., Rhafiri, S. and Busta, F. F. 1991. Identification of histamine-producing bacteria isolated from sardine (*Sardina pilchardus*) stored in ice and at ambient temperature (25°C). Food Microbiology 8: 127-136.

Akpan, E. J., Okokon, J. E. and Etuk, I. C. 2012. Antiplasmodial and antipyretic studies on root extracts of Anthocleista djalonensis against *Plasmodium berghei.* Asian Pacific Journal of Tropical Disease 2 (1): 36-42.

Alongi, D. M., Christoffersen, P. and Tirendi, F. 1993. The influence of forest type on microbial-nutrient relationships in tropical mangrove sediments. Journal of Experimental Marine Biology and Ecology 171 (2): 201-223.

An, H. and Ben-Gigirey, B. 1998. Scombrotoxin poisoning. In I. Millar, D. Gray, & N. Strachan (Eds.). Microbiology of seafoods. London: Chapman and Hall Ltd.

Chen, H. C., Kung, H. F., Chen, W. C., Lin, W. F., Hwang, D. F., Lee, Y. C. and Tsai, Y. H. 2008. Determination of histamine and histamine-forming bacteria in tuna dumpling implicated in a food-borne poisoning. Food Chemistry 106 (2): 612-618.

Christophersen, C., Crescente, O., Frisvad, J.C., Gram, L., Nielsen, J., Nielsen, P.H. and Rahbæk, L. 1999. Antibacterial activity of marine-derived fungi. Mycopathologia 143: 135-138.

Cunha, A., Almeida, A., Coelho, F. J. R. C., Gomes, N. C. M., Oliveira, V. and Santos, A. L. 2010. Bacterial extracellular enzymatic activity in globally changing aquatic ecosystems. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology (1): 124-135.

Du, W. X., Lin, C. M., Phu, A. T., Cornell, J. A., Marshall, M. R. and Wei, C. I. 2002. Development of biogenic amines in yellowfin tuna (*Thunnus albacares*): effect of storage and correlation with decarboxylase-positive bacterial flora. Journal of Food Science 67 (1): 292-301.

Gupta, N., Das, S. and Basak, U.C. 2007. Useful extracellular activity of bacteria isolated from Bhitarkanika mangrove ecosystem of Orissa coast. Malaysian Journal of Microbiology 3 (2): 15-18.

Holguin, G., Guzman, M. A. and Bashan, Y. 1992. Two new nitrogen-fixing bacteria from the rhizosphere of mangrove trees: their isolation, identification and in vitro interaction with rhizosphere *Staphylococcus* sp. FEMS Microbiology Letters 101 (3): 207-216.

Holme D. J. and Peck, H. 1993. Analytical biochemistry. Second Edition. John Willey and Sons, Inc. New York.

Hsu, H.H., Chuang, T.C., Lin, H.C., Huang, Y.R., Lin, C.M., Kung, H.F. and Tsai, Y.H. 2009. Histamine content and histamine-forming bacteria in dried milkfish (*Chanos chanos*) products. Food Chemistry 114 (3): 933-938.

Indira, K., Jayalakshmi, S., Gopalakrishnan, A. and Srinivasan, M. 2011. Biopreservative potential of marine *Lactobacillus* spp. African Journal Microbiology Research 5 (16): 2287-2296.

Ishizuka, H., Horinouchi, S. and Beppu, T. 1993. Putrescine

oxidase of *Micrococcus rubens*: primary structure and *Escherichia coli.* Journal of General Microbiology 139: 425-432

Kanki, M., Yoda, T., Tsukamoto, T. and Baba, E. 2007. Histidine decarboxylases and their role in accumulation of histamine in tuna and dried saury. Applied and Environmental Microbiology 73 (5): 1467-1473.

Kannan, R. R. and Vincent, S. P. 2011. Molecular characterization of antagonistic *Streptomyces* isolated from a mangrove swamp. Asian Journal of Biotechnology 3: 237-245.

Kathiresan, K. and Selvam, M. M. 2006. Evaluation of beneficial bacteria from mangrove soil. Botanica Marina 49 (1): 86-88.

Kim, S. H., Barros-Velazquez, J., Ben-Gigirey, B., Eun, J. B., Jun, S. H. and Wei, C. I. 2003. Identification of the main bacteria contributing to histamine formation in seafood to ensure product safety. Food Science and Biotechnology 12: 451-460.

Klocker, J., Mätzler, S. A., Huetz, G. N., Drasche, A., Kolbitsch, C. and Schwelberger, H. G. 2005. Expression of histamine degrading enzymes in porcine tissues. Inflammation research: official journal of the European Histamine Research Society 54: S54-S57.

Kufner, M. A., Ulrich, P., Raithel, M. and Schwelberger, H. G. 2001. Determination of histamine degradation capacity in extremely small human colon samples. Inflammation Research 50: S96-S97.

Kung, H. F., Tsai, Y. H., Hwang, C. C., Lee, Y. H., Hwang, J. H. and Wei, C. I. 2005. Hygienic quality and incidence of histamine-forming *Lactobacillus* species in natural and processed cheese in Taiwan. Journal of Food Drug Analysis 13: 51-56.

Kung, H.F., Lee, Y.C., Huang, Y.R., Lin, W.F., Lin, C.M., Chen, W.C. and Tsai, Y.H. 2010. Biogenic amines content, histamine-forming bacteria, and adulteration of pork and poultry in tuna dumpling products. Food Control 21: 977-982.

Kung, H.F., Lee, Y.H., Chang, S.C., Wei, C.I. and Tsai, Y.H. 2007. Histamine contents and histamine-forming bacteria in sufu products in Taiwan. Food Control 18: 381-386.

Leuschner, R.G., Heidel, M. and Hammes, W.P. 1998. Histamine and tyramine degradation by food fermenting microorganisms. International Journal of Food Microbiology 39: 1-10.

Lopez-Sabater, E. I., Rodriguez-Jerez, J. J., Hernandez-Herrero, M. and Mora-Ventura, M. A. T. 1994. Evaluation of histidine decarboxylase activity of bacteria isolated from sardine (*Sardina pilchardus*) by an enzymatic method. Letter in Applied Microbiology 19: 70-75.

LPPMHP, 2010. Test titrates histamine utilizes spektrofluorometric method corresponds to SNI (01-2354.10-2009). Quality Examination of Laboratory fishery. Surabaya.

Lucas, P. M., Wolken, W. A., Claisse, O., Lolkema, J. S. and Lonvaud-Funel, A. 2005. Histamine-producing pathway encoded on an unstable plasmid in *Lactobacillus hilgardii* 0006. Applied and Environmental Microbiology 71 (3): 1417-1424.

Merritt, T. A., Hallman, M., Bloom, B. T., Berry, C., Benirschke, K., Sahn, D. and Jaaskelainen, J. 1986. Prophylactic treatment of very premature infants with human surfactant. New England Journal of Medicine 315 (13): 785-790.

Middlebrooks, B. L., Toom, P. M., Douglas, W. L., Harrison, R. F. and McDowell, S. 1988. Effects of storage time and temperature on the microflora and amine development in Spanish mackerel (*Scomberomorus maculatus*). Journal of Food Science 53: 1024-1029

Murooka, Y., Doi, N. and Harada, T. 1979. Distribution of membrane bound monoamine oxidase in bacteria. Applied and Environmental Microbiology 38: 565-569.

Niven, C. F., JeVreg, M. B. and Corlett, D. A. 1981. Differential plating medium for quantitative detection of histamine-producing bacteria. Applied and Environmental Microbiology 41 : 321-322.

Okuzumi, M., Hiraishi, A., Kobayashi, T. and Fujii, T. 1994. *Photobacterium histaminum* sp. nov., a histamine-producing marine bacterium. International Journal of Systemic Bacteriology 44: 631-636.

Padmakar, B., Madhavilatha, P. and Ramakrishna, V. 2005. Purification of alkaline protease from the cotyledons of germinating indian bean (*Dolichos lablab* L. var *lignosus*) seeds. Biotechnology 4: 365-369.

Rajeswari, V.D., Jayaraman, G. and Sridharan, T.B. 2012. Purification and characterization of extracellular protease from halotolerant bacterium *Virgibacillus dokdonensis* VITP14. Asian Journal of Biochemistry 7: 123-132.

Rawles, D. D., Flick, G. J. and Martin, R. E. 1996. Biogenic amines in fish and shellfish. Advances in Food and Nutrition Research 39: 329-365.

Roberts, J. M., Hahn, C. J., Fehsenfeld, F. C., Warnock, J. M., Albritton, D. L. and Sievers, R. E. 1985. Monoterpene hydrocarbons in the nighttime troposphere. Environmental science & technology 19 (4): 364-369.

Ruiz, G.D., Omar, N.B., Abriouel, H., Cañamero, M.M. and Gálvez, A.2012. Inhibition of *Listeria monocytogenes* and *Escherichia coli* by bacteriocin-producing *Lactobacillus plantarum* EC52 in a meat sausage model system. African Journal of Microbiology Research 6 (6): 1103-1108

Ryser, E. T., Marth, E. H. and Taylor, S. L. 1984. Histamine production by psychrotrophic pseudomonads isolated from tuna fish. Journal of Food Protection 47: 378-380.

Sahnouni, F., Matallah-Boutiba, A., Chemlal, D. and Boutiba, Z. 2012. Technological characterization of lactic acid bacteria isolated from intestinal microbiota of marine fish in the Oran Algeria coast. African Journal of Microbiology Research 6 (13): 3125-3133.

Sahoo, K. and Dhal, N. K. 2009. Potential microbial diversity in mangrove ecosystems: a review. Indian Journal of Marine Sciences 38 (2): 249.

Schwelberger, H. G. 2004. Histamine N-methyltransferase (HNMT) enzyme and gene. Histamine: biology and

medical aspects. Budapest, Hungary: SpringMed Publishing.

Schwelberger, H. G. and Bodner, E. 1997. Purification and characterization of diamine oxidase from porcine kidney and intestine. Biochimica Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology 1340 (1): 152-164.

Schwelberger, H. G., Hittmair, A. and Kohlwein, S. D. 1998. Analysis of tissue and subcellular localization of mammalian diamine oxidase by confocal laser scanning fluorescence microscopy. Inflammation Research 47 (13): 60-61.

Stratton, J. E., Hutkins, R. W., Summer, S. S. and Taylor, S. L. 1992. Histamine and histamine-producing bacteria in retail Swiss and low-salt cheeses. Journal of Food Protection 55: 435-439.

Taylor, S. L. and Speckard, M. 1983. Isolation of histamine-producing bacteria from frozen tuna. Marine Fisheries Review 45: 35-39.

Taylor, S.L. 1986. Histamine food poisoning: toxicology and clinical aspects. Critical Review in Toxicology 17: 91-128.

Tsai, Y. H., Kung, H. F., Lee, T. M., Lin, G. T. and Hwang, D. F. 2004. Histamine related hygienic qualities and bacteria found in popular commercial scombroid fish fillets in Taiwan. Journal of Food Protection 67: 407-412.

Tsai, Y. H., Kung, H. F., Lin, Q. L., Hwang, J. H., Cheng, S. H. and Wei, C. I. 2005a. Occurrence of histamine and histamine-forming bacteria in kimchi products in Taiwan. Food Chemistry 90: 635-641.

Tsai, Y. H., Lin, C. Y., Chang, S. C., Chen, H. C., Kung, H. F. and Wei, C. I. 2005b. Occurrence of histamine and histamine-forming bacteria in salted mackerel in Taiwan. Food Microbiology 22: 461-467.

Wafar, S., Untawale, A. G. and Wafar, M. 1997. Litter fall and energy flux in a mangrove ecosystem. Estuarine, Coastal and Shelf Science 44 (1): 111-124.

Ward, R. D., Hanner, R. and Hebert, P. D. 2009. The campaign to DNA barcode all fishes, FISH-BOL. Journal of Fish Biology 74 (2): 329-356.

Wiseman, A. 1989. Handbook of enzymes biotechnology. 2nd. Edition. Ellis Howard, New York.

Xu, Y., Feller, G., Gerday, C. and Glansdorff, N. 2003. Metabolic enzymes from psychrophilic bacteria: challenge of adaptation to low temperatures in ornithine carbamoyltransferase from *Moritella abyssi.* Journal of bacteriology 185 (7): 2161-2168.

Yamashita, M., Sakaue, M., Iwata, M., Sugino, H. and Murooka, Y. 1993. Purification and characterization of monoamine oxidase from *Klebsiella aerogenes*. Journal of Fermentation and Bioengineering 76: 289-295.

Yatsunami, K. and Echigo, T.1991. Isolation of salt tolerant histamine forming bacteria from commercial rice-bran pickle sardine. Bulletin of the Japanese Society of Scientific Fisheries 57: 1723-1728.

Zaman, M. Z., Bakar, F. A., Selamat, J. and Bakar, J. A. 2010. Occurrence of biogenic amines and amines degrading bacteria in fish sauce. Czech Journal of Food Science 28 (5): 440-449.