Effectiveness Of Bacteria Decarboxylase That Is Isolated From Mangrove Waters For Decompose Histidine To Histamine

Yahya, Soemarno, Yenny Risjani, Happy Nursyam

Faculty of fisheries and Marine Brawijaya University, Malang
Program of Post Graduate Sarjana Faculty of Agriculture Brawijaya University, Malang

ABSTRACT

This research intent to isolate Mangrove's plant bacteria and applying on substance L-histidine becomes histamine on In Vitro's system. Material of research which is Media TSB, aquabidest, aquadest, NaCl, powder L-Histidine Merk SIGMA and sample bacteria of Mangrove's plant Rhizophora Apiculata and Avicennia alba, incubator, autoklaf, resin's column, spectrofluorometer, stirer plate, waterbath. 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. Acquired result 5 isolat bacterias which is Mangrove (Rhizophora Apiculata) there is 3 isolat bacterias which is B (Bacillus megaterium), E( Enterobacter gergoviae) and P (Planococcus citreus) and Mangrove (Avicennia alba) there is 2 isolat bacterias which is N (Nitrococcus sp) and A (Acinetobacter baumanii). Histamine rate N (Nitrococcus sp) 0,12 mg / kg; 1,19 mg / kg; 3,55 mg / kg; 0,23 mg / kg, A (Acinetobacter baumanii) 6,18 mg / kg; 8,68 mg / kg; 5,31 mg / kg; 4,21 mg / kg and NA(Nitrococcus sp + Acinetobacter baumanii) as big as 2,89 mg / kg; 6,90 mg / kg; 3,26 mg / kg; 2,43 mg / kg. Histamine rate B (Bacillus megaterium) 3,54 mg / kg, 1,30 mg / kg, 3,45 mg / kg, 1,95 mg / kg, E(Enterobacter gergoviae) 6,73 mg / kg, 2,84 mg / kg; 2,78 mg / kg, 0,26 mg / kg, P(Planococcus citreus) 6,04 mg / kg, 7,28 mg / kg, 12,55 mg / kg and B + E + P (Bacillus megaterium + Enterobacter Gergoviae + Planococcus Citreus) 1,61 mg / kg, 1,09 mg / kg, 3,33 mg / kg, 3,81 mg / kg. Concluded Nitrococcus sp and Acinetobacter baumanii can not decompose histidine becomes histamine (bacteria Non Decarboxylase) and Planococcus citreus, Bacillus megaterium and Enterobacter gergoviae can decompose histidin as histamin (Decarboxylase bacteria).

Key words: Mangroves of Rhizophora Apiculata and Avicennia alba, bacterias of Nitrococcus sp, Acinetobacter baumanii, Planococcus citreus, Bacillus megaterium and Enterobacter gergoviae.

Introduction

Mangroves forest constitute unique and dual-purpose ecosystem deep environment because marks sense ocean and continent influence. On mangrove's ecosystem happens complex interaction among chemical factor, physical and biological. Therefore, mangrove's forest at conceive of interface ecosystem, since links continent with littoral [2]. Mangrove's forest constitute its amends place bacteria community. Bacteria fills a number niche and constitute environmental logistic basic component [22]. As a mangrove's ecosystem have biotics component and abiotic. mangroves leaves produce personation, meanwhile animal group as consumer and bacteria as decomposer [6].

Bacteria plays essential role in mangroves ecosystem. In the presence and bacteria variety in mangroves ecosystem regarded by salinity factor, pH, physical, climate, vegetation, nutrisi and location [11]. Known severally photosynthesis bacteria acts out in mangrove's ecosystem passes through to process photosynthesis, nitrogen fixation, methanogenesis, enzyme production and antibiotic producer [16]. Bacteria constitutes conditioner in nitrogen cycle on environmentally mangrove. Cyanobacteria goes out to sea is microbiota component is of important that gets role in nitrogenic source collation on mangrove's ecosystem [13].

Histamine is a biogenic amine (sometimes referred to as a vasoactive amine) that, in mammals, is produced primarily by the action of the enzyme histidine decarboxylase on the amino acid histidine.
Histidine is one of the 20 or so amino acids that combine together to make a protein. Histidine decarboxylase is present in large quantities in leukocytes known as granulocytes (granule-containing cells), especially tissue mast cells and blood basophils. In these cells it converts histidine to histamine. The newly formed histamine is then stored in structures within the cell (called intracellular granules) in readiness for release in response to signals from a variety of body systems. Inflammation, whether produced in defending the body from injury or infection, or as a result of an allergic reaction, these signals come from lymphocytes, cytokines and antibodies [12].

There is a two histidine kinds flesh-made fishes, which is histidine is free that will be changed as histamin and histidine is tied-up in protein [21]. Histidine frees that available of fish flesh hand in glove once its relationship by formed it flesh-made histamin. All flesh which tall dark chromatic obstetric histidine its free [14]. Histamine is compound biogenik amen usufructs histidine amino acid change frees that lies flesh-made fish which at biologis’s ala production passes through to process dekarboxyilase of free amino acid and available on foods material sort as fish, red flesh, cheese, and ferment food [14]. Histamine constitutes little component, having molecule weight contemns that comprise of ring imidazol and etilamin's chain flank. Histamine also constitute component that don't water leach. Histamine constitutes one of amine biogenic that have influence to human physiological effect [2].

A variety bacteria genus which can result histidine decarboxylases enzyme (HDC) including kin Enterobacteriaceae and Bacillaceae [3]. Generally species Bacillus, Citrobacter, Clostridium, Escherichia, Klebsiella, Lactobacillus, Pediococcus, Photobacterium, Salmonella, Shigella, and Streptococcus pointing out decarboxylases activity amino acid [3]. Factor that regard histidine change becomes histamine is time factor, temperature, type and a lot of it microflora bacteria that exists in body fishes out [21]. A variety bacteria genus which can result histidin dekarboksilasi's enzyme (HDC) and a variety bacteria genus which can result histidine decarboxylase enzyme (HDC) including kin Enterobacteriaceae and Bacillaceae [3].

This research is aimed for insulating Mangrove’s plant bacteria and then those bacteria is applying substance L-histidine that can describe L-histidine becomes Histamin In Vitro’s system.

**Materials And Methods**

Research material that is utilized is TSB Media, aquabidest, NaCl, fresh water, egg paper, tissue, molten soap and powder L-Histidine Merk SIGMA to solution L histidine, aquabides, methanol, aquades, glasswool, NaOH 1 n, HCl 0,1 N, Orto pitalatdicarbosildehid (OPT) 0,1 %, acid phospat (H 3 PO 4 ) 3,57 N, resin penukar is type ion Dowex 1 - X8 50 10 mesh and bacteria sample of Mangrove’s plant Rhizophora Apiculata and Avicennia alba at mangrove’s forest Mlaten’s beach Nguling’s district Pasuruan’s Regency .Equipment that utilized by incubator, autoclave, resin's column 20 cm x 0,8 cm, reservoar 2 cm x 5 cm; gourd metes out 25 ml, 50 ml, 100 ml, and 1000 ml; pipette volumetric, stirer plate, tube reacts 5 ml gets to close, analytical scale, waterbath.

This research is executed on November 2011 Februaries 2012 that research locations for isolating and identification at Microbiological Laboratory medicine of Brawijaya's University and Bioaugmentasi's process aeriation system at Microbiological Laboratory of fishery and marine science of Brawijaya's University and Testing Histamine at Quality Examination of Laboratory fishery (LPPMHP) Surabaya.

This research method is explorative method [4] with parameter which is Bacteria namely testing isolation with Bacteria breeder and Bacteria Dilution [9,20], biochemical test with Microbact identification Kits [19] with positive gram bacteria test can utilize GNB 12B only, meanwhile GNB 12A is ignored. Negative gram bacteria quiz utilizes 1set which is GNB 12A / b / e, 24E (see figure 1). Test titrates histamine utilizes spektrofluorometrys method corresponds to SNI’S default, 01 - 2354. 10 - 2009 [1].

**Fig. 1: Microbact identification Kits**

Research procedure which is L. Histadine 500 ppm 150 ml is filled in liquid enhanced bottle bacteria in TSB media (Trypton Soy Broth) as much 0,4%, with density 10 ^6 CFU / ml and at aeration up to 6 hours (code 1), 12 hours (code 2), 18 hours (code 3) and 24 hours (code 4). Bacterias Added
process single and also consortium in solution L-Histidine there is single and also consortium / affiliate which is Code N (1,2,3,4); A (1,2,3,4), NA (1,2,3,4), B (1,2,3,4), E (1,2,3,4), P (1,2,3,4), B + E + P (1,2,3,4).

Results:

Isolating result and identification was gotten by bacteria 5 isolat that dominant of mangrove's plant Mangrove (Rhizophora Apiculata) 3 isolat bacterias which is code B (Bacillus megaterium), code E (Enterobacter gergoviae) and code P (Planococcus citreus) and Mangrove (Avicennia alba) 2 isolat is bacteria which is code N (Nitrococcus sp) and code A (Acinetobacter baumannii).

According to Mortimer (1981) colony morphology watch covers to form, elevation edge, and colony color identifying by Microbact System Kits. Base biochemical test result with Microbact Identification Kits GNB 12A / b / e and 24E gotten by species that acquired with oktal's arithmetic counting on programs and that result geared with species character bases bergey’s Of Determinative Bacteriology's Manuals. Microbact Identification Kits GNB's result. See figure 2.

Observing result one of isolat is bacteria code A having marking: colony color wans, diameter 2 µm, globulous cell, react negative gram, negative nitrate, lisin is negative, ornithin is negative, H₂S negative, positive glucose, mannitol is negative, xylose is positive, ONPG is negative, indole is negative, Urease is negative, Citrate is positive, TDA is VP's negative and quiz negative. Base one series of that test and after as compared to characteristic which is worded on guidance book identifies bergey’s Of Determinative Bacteriology's Manuals therefore that bacteria gets diidentifikasikan as Acinetobacter baumannii (See figure 3.)

**Fig. 2:** For example Microbact Identification Kits GNB 12A/B/E and 24E on Acinetobacter baumanii

**Fig. 3:** Morphology Photograph bacteria mangrove code B (Bacillus megaterium), code E( Enterobacter gergoviae) and code P (Planococcus citreus) code N (Nitrococcus sp) and code A (Acinetobacter baumannii).

Acinetobacter's gender bar shaped with diameter 0,9 - 1,6 µm and long 1,5 - 2,5 µm becomes phase deep balls stationer's growths. Cell doesn't form spore. This gender growing properly on all media which complexeses generically. Mostly colony grows in media that contains to decarbonize singles and nitrogenic energy source. This bacteria by nature available in earth, water, and waste [10].

**Isolat of bacteria E** is having marking: pink's colony color, diameter 0,9 µm, globulous cell, react negative gram, positif's nitrate, lisin is positive, ornithin is positive, H₂S negative, positive glucose, mannitol is positive, xylose is positive, ONPG is
positive, indole is negative, Urease is positive, Citrate is positive, TDA is VP's negative and positive test. Base one series of that test and after as compared to characteristic which is worded on guidance book identifies *Bergey’s of Determinative Bacteriology’s Manuals* therefore that bacteria gets geared as *Enterobacter gergoviae*.

According to Nursanti and Madjid (2009), bacteria *Enterobacter gergoviae* also having other benefit which is as dissolving as substance P deep meremediai begrimed earth. say that, dissolving bacteria of susbtrace P (*Pseudomonas putida* and *Enterobacter gergoviae*) can increase solubility p on ultisol's earth.

**Isolat is bacteria code B** having marking as follows: form rounded colony, having scraggly edge, convex elevation, krem's color, cell form erects, diameter 3.0 µm, result tests gram coloration points out positive(+), meanwhile biochemical test result by use of *Microbact Identification Kit* gotten by characteristic as follows: isolat is bacteria has spore, test negative oxidation, motil, test negative nitrate, indole's test negative, test *Voges Proskauer* (VP) negative, test positive glucose, manitol's test positive, sucrose's test positive, and catalase's test positive. Base one series of that test and after as compared to characteristic which is worded on guidance book identifies *Bergey ’s of Determinative Bacteriology’s Manuals* therefore that bacteria gets to geared as *Bacillus megaterium*.

According to Hold, et al [10], *Bacillus megaterium* constituting aerob's bacteria, positive gram, bar shaped with diameter measure 1.2 1.5 micrometers and long 2.0 2.4 micrometers, cylindrical cell cell form until oval or pear's form, and motil endospora mostly being formed in the period of 48 hours with optimum Temperature for pertumbuhannya among 28 °C – 35 °C and its maximum temperature among 40 °C – 45 °C. In that glucose media, batangya's form sometimes longer and diameter big until 3 µm / more in many strain.

**Isolat is Bacteria code N** having marking as follows: form rounded colony, colony color yellows, memilik'i is diameter 0.6 µm, positive gram bacteria, negative oxidation, catalase is negative, indole is negative, VP's test negative, and motilita's quiz positive. Base one series of morphology watch, biochemical test and after as compared to marking which is worded on guidance book identifies *Bergey ’s Of Determinative Bacteriology’s Manuals* therefore that bacteria gets to geared as *Planococcus citreus*.

According to Holt et al., 1994, cell form *Planococcus citreus* are rounded, with diameter measure 1.0 - 1.2 µm, having single cell, motil, each cell usually has a or two flagella, but there is also that memiliki three or four flagella, no spore forming, and comprises positive gram. Get chemoorganotrophs's character (system metabolism is engaged exhalation never get ferment, can't memproduksi acid or gas of glucose, maltose, laktosa, sukrosa. Life on 20ºC's temperature 37ºC, can be found at oceanic water, but usually frequent found at estuary region.

**Discussion:**

Result Tests to titrate histamine by use of Spektrofluorometric with longing exitasi's wave 350 nm and issue 444 nm according to Standarisasi's body SNI'S National 2354. 10 years 2009. See table 1

<table>
<thead>
<tr>
<th>No</th>
<th>Sample code</th>
<th>Perlakuan</th>
<th>6 hours (mg/ kg)</th>
<th>12 hours (mg/ kg)</th>
<th>18 hours (mg/ kg)</th>
<th>24 hours (mg/ kg)</th>
</tr>
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<tbody>
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<td>Control</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>2.</td>
<td>N</td>
<td></td>
<td>0.12</td>
<td>0.19</td>
<td>0.32</td>
<td>0.23</td>
</tr>
<tr>
<td>3.</td>
<td>A</td>
<td>6.18</td>
<td>8.68</td>
<td>5.31</td>
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<td></td>
</tr>
<tr>
<td>4.</td>
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<td>2.89</td>
<td>6.90</td>
<td>3.26</td>
<td>2.43</td>
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*Table 1: Histamine Rate from Nitrococcus sp dan Acinetobacter baumanii*
According to figure 4 and 5 that four aeration time conducts those are utilized, namely 6 hours, 12 hours, 18 hours, and 24 hours on Added bacteria on histidin's solution which is bacteria code N (Nitrococcus sp) experiencing ascension of histamin's rate 0,05 mg / kg becomes 0,12 mg / kg; 1,19; 3,55 mg / kg; 0,23 mg / kg. For bacteria code A (Acinetobacter baumanii) of 0,05 mg / kg becomes 6,18 mg / kg; 8,68 mg / kg; 5,31 mg / kg; 4,21 mg / kg, for bacteria code NA affiliate as big as 2,89 mg / kg; 6,90 mg / kg; 3,26 mg / kg; 2,43 mg / kg so in common bacteria Nitrococcus sp and Acinetobacter baumannii can't down meaning histamine rate that bacteria is not decarboxylase bacteria (see table 1).

**Table 2:** Histamine rate from *Planococcus citreus*, *Bacillus megaterium* and *Enterobacter gergoviae*

<table>
<thead>
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<th>No</th>
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<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>silence</td>
<td>0,05</td>
<td>12 hours (mg/kg)</td>
</tr>
<tr>
<td>1.</td>
<td>Control</td>
<td>0,05</td>
</tr>
<tr>
<td>2.</td>
<td>B</td>
<td>6,73</td>
</tr>
<tr>
<td>3.</td>
<td>E</td>
<td>6,04</td>
</tr>
<tr>
<td>5.</td>
<td>B + E + P</td>
<td>1,61</td>
</tr>
</tbody>
</table>

**Fig. 4:** Histamine Rate from *Nitrococcus* sp dan *Acinetobacter baumanii*

*Planococcus citreus* resulting greater histamine namely as big as 12,55 mg / kg, as compared to the other bacteria (*Bacillus megaterium* and *Enterobacter gergoviae*). It is caused because decarboxylase's enzyme activity that contained in bacteria *Planococcus citreus* are even greater as compared to *Bacillus megaterium* and *Enterobacter gergoviae*. Bacteria *Bacillus megaterium*, *Enterobacter gergoviae* and *Planococcus citreus* can describe histidin as histamin. Besides, that bacteria constitute producer bacteria groups histidine decarboxylase's enzymes (HDC), one that functioning to change histidine amino acid becomes histamine so histamine rate gets to increase. According to Allen [3], a variety bacteria which can result histidin decarboxylase enzyme (HDC) including kin *Enterobactericeae* and *Bacillaceae*. According to Mangunwardoyo, dkk (2007) known a lot of bacteria genus who can result histidin decarboxylase, enzyme that changes histidine as histamine, as *Proteus morganii* (are next so-called *Morganella morganii*), *Hafnia alvei*, *Klebsiella*...

Density and so long ferment regard Histamine decrease. Histamine decrease because of resistivity of Acinetobacter Baumanii bacteria in constrain job of decarboxylase enzyme. According to Kučerová, et al [15], biogenic amine at production on network fishes out by bacterias of Bacillus, Pseudomonas, Lactobacillus, Pediococcus, Proteus, Streptococcus, Salmonella, Escherichia coli, Photobacterium, Klebsiella, and Hafnia one that result histidin decarboxylase's enzyme. Even at decarboxylase's enzyme production, therefore will pierce through and through histamine resultant even bacteria growth was constrained by cold temperature until 4 °C. Production histamine will progressively increase despite was kept on coolant half. Bacteria Acinetobacter baumanii can constrain dekarboksilase's enzyme job because bacteria acinetobacter baumanii can result inhibitor protease, according to Desniar dkk [8] A. baumanii to result inhibitor protease with supreme activity as big as 1.64 u / ml and protein concentration 0.152 mg / ml with production time up to 20 hours. Inhibitor protease is resulted on logaritmik phase.

Inhibitor protease constitutes compound that at production with every consideration on ferment media in condition extreme, which is upon all nutrisi what do consist in that ferment media was dwindling. On that condition inhibitor protease at production on logaritmik's phase until stationer's phase. Acinetobacter baumanii to result inhibitor protease with supreme activity as big as 1.64 u / ml and protein concentration 0.152 mg / ml with production time up to 20 hours. Inhibitor protease is resulted on logaritmik's phase [8].

Conclusion:

Application bacteria on L-histidine with aeration time that is utilized, namely 6 hours, 12 hours, 18 hours, and 24 hours to Test histamin's rates. Acquired result 5 isolat bacterias which is Mangrove (Rhizophora Apiculata) there is 3 isolat bacterias which is code B (Bacillus megaterium), code E (Enterobacter gergoviae) and code P (Planococcus citreus) meanwhile Mangrove (Avicennia alba) there is 2 isolat bacterias which is code N (Nitrococcus sp) and code A (Acinetobacter baumanii). Histamine rate code N (Nitrococcus sp) 0.12 mg / kg; 1.19 mg / kg; 3.55 mg / kg; 0.23 mg / kg, code A (Acinetobacter baumanii) 6.18 mg / kg; 8.68 mg / kg; 5.31 mg / kg; 4.21 mg / kg and code NA (Nitrococcus sp + Acinetobacter baumanii) as big as 2.89 mg / kg; 6.90 mg / kg; 3.26 mg / kg; 2.43 mg / kg. Histamine rate code B (Bacillus megaterium) 3.54 mg / kg; 1.30 mg / kg; 3.45 mg / kg; 1.95 mg / kg, code E (Enterobacter gergoviae) 6.73 mg / kg; 2.84 mg / kg; 2.78 mg / kg; 0.26 mg / kg, code P (Planococcus citreus) 6.04 mg / kg, 7.28 mg / kg, 8.53 mg / kg; 12.55 mg / kg and code B + E + P (Bacillus megaterium + Enterobacter Gergoviae + Planococcus Citreus) 1.61 mg / kg, 1.09 mg / kg, 3.33 mg / kg, 3.81 mg / kg. Concluded Nitrococcus sp and Acinetobacter baumanii can't decompose L-histidine becomes histamine so called by Non-Decarboxylase Bacteria and Planococcus citreus, Bacillus megaterium and Enterobacter gergoviae can decompose L-histidine becomes histamine so called by Decarboxylases Bacteria.

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