



Research Article

The work is licensed under



Novel HMG-CoA Reductase Inhibitor Peptide from *Lactobacillus acidophilus* Isolated from Indonesian fermented food *Bekasam*

Rinto¹, Ratih Dewanti², Sedarnawati Yasni², Maggy Thenawidjaja Suhartono^{2*}

¹Department of Fisheries Product Technology, Faculty of Agricultural, Sriwijaya University, Indralaya South Sumatera 30862, Indonesia.

²Department of Food Science and Technology, Faculty of Agricultural Technology, Bogor Agricultural University, Dramaga Bogor 16002, Indonesia

*Corresponding Author: Maggy Thenawidjaja Suhartono, Department of Food Science and Technology, Faculty of Agricultural Technology, Bogor Agricultural University, Dramaga Bogor 16002 Indonesia

Received: 31 May 2017 Revised: 04 September 2017 Accepted: 11 September 2017

ABSTRACT

Extract of *bekasam*, an Indonesian fermented fish, inhibited HMG-CoA reductase, a key enzyme for cholesterol biosynthesis. Screening of statin producing microorganisms revealed *Lactobacillus acidophilus*. The bacteria produced statin optimally at temperature 25 °C, pH 7 when incubated for 5 days in MRS media. These conditions were also found best for HMGCoA reductase inhibition. To confirm the presence of statin and peptides as inhibitors we fractionated the cell free extracellular supernatant. Five fractions were resulted: F1 cell free supernatant; F2 MW > 10 kD, F3 MW 3-10 kD, F4 MW < 3 kD and F5 MW < 1 kD. SDS PAGE showed F1, F2, and F3 contained protein bands. No peptide band was observed in F4 and F5 which showed the highest HMG-CoA reductase inhibition (93.94 %), while F3 showed 87.88% inhibition, F1 77.27% and F2 45.45%. F3 with high inhibition of HMG-CoA reductase was found to contain 6 kD peptide with predicted amino acid sequence of KGENYNTGVTPLNRPKAAEVVAFLNKEAIEAIADTMKK. We concluded that *Lactobacillus acidophilus* present in the fermented fish *bekasam* is responsible for producing statin and peptide which act as HMGCo A inhibitors. This is the first report of a peptide as inhibitor of HMG-CoA reductase produced from *Lactobacillus acidophilus*

Keyword: *Bekasam*, *Lactobacillus acidophilus*; HMG-CoA reductase inhibitor

INTRODUCTION

Bekasam is one of Indonesian traditional fermented fish product from South Sumatera, South Kalimantan, and North Sulawesi Indonesia known to have health benefits. Commonly, *bekasam* is made from fish, salt, and rice which are naturally fermented for 5 to 7 days. Many products similar to *bekasam* can be found in other Asian countries i.e. *burong-isda* and *burong-bangus* in Philippine, *pla-ra* and *placom* in Thailand, *heshiko* and *narezushi* in

Japan [1-4]. Microorganism in the *bekasam* is dominated by *Lactobacillus*, *Pediococcus* and *Leuconostoc* [5].

Lactobacillus produce bioactive compounds such as bacteriocine i.e. lactacin, lactocin, acidocin, plantaricin, helveticin and acidophilin, which are a group of peptides with antibacterial activities [6, 7]. *Lactobacillus* also produce low molecular weight compounds which act as antifungi [8, 9]. Other functional peptides and

metabolites produced by *Lactobacillus* have not been well studied and reported.

Bekasam extract has been reported capable of reducing hypertension through inhibition of *Angiotensin I Converting Enzyme* (ACE). This activity might be due to the presence of peptide produced by lactic acid bacteria i.e. *Lactobacillus* and *Pediococcus* during fermentation [10]. Many products similar to *bekasam*, i.e. the Japanese *heshiko* and *narezushi* were reported capable of reducing cholesterol through inhibition of HMG-CoA reductase, an enzyme responsible in the first step of cholesterol biosynthesis [11, 12]. Fractionation of *heshiko* and *narezushi* extract produced peptides and non peptide fractions found as inhibitor of HMG-CoA reductase [1,2]. Researches on compounds capable of inhibiting HMG-CoA reductase enzyme revealed small molecule statins (> 80%) and peptides (> 40%) [13, 14],

Statin is a bioactive compound of 300-500 dalton known as pharmacological medicine to treat high cholesterol patient through inhibition of HMG-Co A reductase enzyme. Some statin produced by microbes are mevastatin/compactin, lovastatin, pravastatin, and simvastatin [12, 13, 15-17]. Statin-producing microorganisms can be isolated from various sources such as *Streptomyces regencies*, *Pseudonocardia autotrophica*, *Streptomices gricoulus*, *Xanthomonas compestris*, *Amycalatopsis sulphurea*, *Bacillus megaterium*, and *Salmonella enteric*, isolated from soil and waste vegetable oil [15], *Pseudonocardia carboxydivorans* isolated from oil-contaminated soil [18] and *Actinomadura* sp. isolated from soil [19].

Other compounds known to inhibit HMG-CoA reductase enzyme, and thus, suggested for cholesterol lowering agents includes some natural and synthetic peptides, extract of tea (green tea and black tea), and fermented fish extract [14, 20]. Peptide known as a cholesterol reducing agent could be extracted from herb *Senna obtusifolia* [21], potato and soybean [22]. Synthetic peptide with Ile-Ile-Ala-Glu-Lys squence [23] and Leu-Arg-Lys-Leu-Arg-Lys-Arg-Leu-Leu-Arg were reported to decrease serum cholesterol [24, 25]. Peptide act as HMG-CoA reductase inhibitor from fermented fish product (*bekasam*) has not been reported.

We analysed the HMG-CoA reductase inhibition activity of *bekasam* extract and found that *bekasam* extract contain statin at 90 to 100 ppm

and the extract decreased HMG-CoA reductase activity by more than 75% compare to pravastatin. Screening for statin producing bacteria from *bekasam* extract revealed isolate *Lactobacillus acidophilus*. In addition to statin, we identified small peptide as other metabolite compound present in the extracellular extract of *L. acidophilus* which inhibited HMG-CoA reductase significantly. This is the first report of peptide from *Lactobacillus acidophilus* known to act as HMG-CoA reductase inhibitor.

MATERIAL AND METHODS

Materials

Bekasam was obtained from Palembang region, South Sumatera, Indonesia. De Man Rogosa Sharpe (MRS) broth medium were purchased from Oxoid (England). Lovastatin, HMG CoA reductase kit assay, were purchased from Sigma Aldrich (USA). A standard molecular weight protein marker (Low Range Protein Ladder) was purchased from Thermo Scientific (Lithuania). *Lactobacillus acidophilus* was screened and isolated from *bekasam*. Bacteria identification has been done previously Rinto et al., 2015 [26]. All other chemicals were analytical grade and purchased from the local representative of Sigma and Merck.

Preparation of *bekasam* extract

Briefly, 10 g *bekasam* was homogenized with 40 mL distilled water. The homogenate was centrifuged at 2 000 g, 4 °C for 15 minutes. After separating the first supernatant, 50 mL of distilled water was added to the precipitate to obtain a second supernatant in the same manner. These two supernatants were mixed and filtered through membrane 0.45 µm (biotechlab, Bulgaria). The filtrate was used in enzyme inhibition assay and analysis of lovastatin content.

Lactobacillus acidophilus fermentation

Lactobacillus acidophilus isolated previously from *bekasam* was grown in the MRS (de Man Rogosa Sharpe) medium containing peptone (10 g/L), 'lab-lamco' powder (8 g/L), yeast extract (4 g/L), glucose (20 g/L), sorbitol mono-oleate (1 mL/L), dipotassium hydrogen phosphat (2 g/L), sodium acetate 3H₂O (5 g/L), triammonium citrate (2 g/L), magnesium sulfate 7H₂O (0.2 g/L), and manganese sulfate 4H₂O (0.05 g/L). Incubation was conducted at varying

temperatures (25, 30, 37) °C, pHs (5.5, 7.0, and 8.5) for 5 days [27, 28]. The increase in cell numbers was determined by spectrophotometer (UV-Mini-1240, Shimadzu) measuring the optical density (O.D) at $\lambda = 620$ nm.

Lovastatin assay

Lovastatin content was determined by spectrophotometer (UV-Mini-1240, Shimadzu) and High Performance Liquid Chromatography (HPLC, Agilent 1200 series). Five millilitre sample was placed into 20 mL methanol and shaken for 2 hours, filtered through membrane 0.45 μm . The filtrate was centrifuged at 120 g, 4 °C for 15 minutes, and the supernatant was separated. Supernatant (0.5 mL) was mixed with 0.5 mL trifluoroacetic acid 1% and further incubated for 10 minutes. Homogenate (0.5 mL) was placed into 5 mL volumetric flask and the volume was filled up with methanol. The absorbance was measured by spectrophotometer at $\lambda = 238$ nm. The concentration of lovastatin was calculated in ppm corresponding to the calibrated curve. Lovastatin standard at concentrations of 6, 8, 10, 20, 30, 40, 50, and 60 ppm were used Osman *et al.*, 2011 [27].

Lovastatin Assay using HPLC followed several stages. At the end of the fermentation period, 5 mL of bacterial culture was diluted six fold with 0.2 N NaOH. After shaking for 2 hours, the alkaline broth was diluted tenfold with 50% methanol, followed by centrifugation at 2,000 g, 4 °C for 15 minutes. After centrifugation, supernatant was separated and filtered by membrane 0.02 μm (Whatman, Germany), followed by sonication for 15 minutes in the Branson Ultrasonic (Branson 8510E-MTH). Supernatant was separated using C18 Column (HPLC, Agilent 1200 series). The mobile phase contained methanol and aqua bides (9:1), with flow rate of 1 mL/min. The eluted compounds were detected at $\lambda = 237$ nm. Lovastatin at concentrations of 10, 20, 30, 50, and 100 ppm were used to make standard curve [29].

HMG-CoA reductase inhibition assay

Sample (5 mL) was centrifuged at 2 000 g, 4°C for 15 minutes. Supernatant was separated and filtered by membrane 0.45 μm and the filtrate was used in the HMG-CoA reductase inhibition assay using HMG-CoA reductase assay kit containing pravastatin as positive control, HMG-CoA substrate, HMG-CoA reductase enzyme,

NADPH and assay buffer. The procedure followed manufacturer's instructions. The assay was based on the spectrophotometric measurement of decrease in absorbance at $\lambda = 340$ nm, which represented oxidation of NADPH by the catalytic subunit of HMG-CoA reductase in the presence of the substrate HMG-CoA. One unit was defined as 1.0 μmole of NADPH converted to NADP⁺ per minute. Specific activity was defined as $\mu\text{mol}/\text{min}/\text{mg}$ -protein (Units/mg) [16].

Fractionation of *L. acidophilus* metabolites

Fractionation was based on the molecule size using filtration membrane (3 K and 10 K MWCO, Thermo-Scientific, UK) and membrane filter 0.02 μm (Whatman, Germany). The five fractions: Non fractionated cell free supernatant (F1); fraction with molecule weight (MW) of > 10 kD (F2), fraction with MW of 3 – 10 kD (F3), fractions with MW of < 3 kD (F4) and fraction with MW < 1 kD (F5) were used for assay of their HMG-CoA reductase inhibitory activity. The peptide profile was analyzed by SDS PAGE.

SDS PAGE analysis

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) with 18% resolving gel in the Tris-HCl (1 M, pH 6.8) was applied. The protein fractions were loaded into the gel and electrophoresed using 1x running buffer Tris (24.8 mM), glycine (192 mM), SDS (0.1%), at pH 8.3. A standard molecular weight marker Low Range Protein Ladder was loaded onto the gel to estimate the molecular weights of the proteins/peptides in different samples. After electrophoresis, the gel was stained with silver staining. Gel was placed in the fixation solution methanol (25%) and acetic acid (12%) for 1 hour, continued into ethanol 50% for 20 minutes and into ethanol solution 30% at 2 x 20 minutes, and placed in the enhancer solution ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) followed by rinsing with aquades. Then the gel was placed in the silver nitrate solution AgNO_3 (0.01 M) for 30 minutes and rinsed again by aquades (2 x 20 seconds), in the developer solution Na_2CO_3 (0.2 M), formaldehyde (37%) and in the fixation solution [30].

Sequencing and Structural Modelling of Peptide

One of the peptide bands from SDS PAGE which high HMG-CoA reductase inhibition was cut,

extracted, purified and sequenced (Proteomics International Pty Ltd, Australia). This method includes digestion of the protein with trypsin. Peptide was analysed by electrospray ionisation mass spectrometry using the Agilent 1260 Infinity HPLC system (Agilent) coupled to an Agilent 6540 mass spectrometer (Agilent). Tryptic peptide was loaded onto a C18 column 300 SB, 5 μm (Agilent) and separated with a linear gradient of water/acetonitrile/0.1% formic acid (v/v). Spectra were analysed to identify peptide of interest using Mascot sequence matching software (Matrix Science) with Ludwig NR [31]. Modelling of molecular structure was conducted using SWISS-MODEL [32].

Statistical Analysis

Data were presented as the mean of three measurements for optical density, lovastatin, and HMG-CoA reductase inhibition. The significance of differences among samples were determined by Analysis of variance (ANOVA) used Minitab 16. Differences were considered significant at $P < 0.05$.

RESULTS

HMG-CoA reductase inhibition activity of bekasam extract

Five types of *bekasam* were taken from different sources in Palembang region, which were located on the Musi River banks of South Sumatra, Indonesia *Bekasam* were made from snakehead fish (*Channa striata*) and *seluang* fish (*Rasbora sp*), which were fermented with salt (15%) and white rice (10%). Analysis showed that the five types of *bekasam* inhibited HMG-CoA reductase (36-100%). *Bekasam* extract (B-3, B-4, and B-5) from *seluang* fish (*Rasbora sp*) showed better

inhibition capacity (46-100 %) than *bekasam* extract (B-1 and B-2) from snakehead fish (*Channa striata*) (Figure 1). The data also implied that compound act as inhibitor in *bekasam* extract from *seluang* fish is almost comparable to 1 $\mu\text{g/ml}$ pravastatin (a medicine used for patient with high cholesterol) with 80% inhibiting activity (Fig. 1A). Pravastatin is one of natural statin produced by microorganism. The other natural statins produced by microorganisms are lovastatin, compactin, and simvastatin. Lovastatin (94.25–113.75 ppm) can be detected in the *bekasam* extract. Figure 1 C also implied the presence of small peptides in all *bekasam* extract

Growth of *Lactobacillus acidophilus*

Screening of statin producing compound from the Indonesian *bekasam* extract revealed and identified *Lactobacillus acidophilus* [26], and this could be responsible for the observed inhibition of HMG-CoA reductase. Maximum lovastatin content during *L. acidophilus* growth was found at 25 °C and pH 7 (Table 1). Lovastatin content in the extracellular fraction of *L. acidophilus* grown at this condition appeared to correlate well with the level of HMG-CoA reductase inhibition. The highest HMG-CoA reductase inhibition was also found at 25 °C and pH 7. Statistical analysis confirmed that HMG-CoA reductase inhibition at 25 °C was significantly higher than at 30 and 37 °C. Lovastatin content at the pH 7 was higher than at pH 5.5 and pH 8.5 (Table 1) and lovastatin concentration at pH 5.5 was significantly the lowest. This might be due to decreasing stability of lovastatin at acid condition (pH 5.5)

Table 1: Lovastatin content and HMG-CoA reductase inhibition by cell free extracellular Supernatant of *L.acidophilus* fermentation at different temperatures and pHs.

		Lovastatin Content * (ppm)	HMG-CoA inhibition * (%)
Temperature	25 °C	34.07±0.04 ^a	77.08±3.60 ^a
	30 °C	24.13±0.03 ^b	56.25±6.25 ^b
	37 °C	22.69±0.02 ^b	54.17±9.54 ^b
pH	5.5	26.13±1.78 ^b	66.67±8.24 ^a
	7.0	34.10±1.05 ^a	76.19±8.24 ^a
	8.5	30.59±1.27 ^a	61.90±8.24 ^a

* Result are means of three experiments, different superscript letters shows significant differences

Fractionation and SDS analysis

In light of our result presented at Figure 1 C which indicated the presence of small peptide in the *bekasam* extract, and in attempt to find molecules other than lovastatin which also act as HMGCoA inhibitor in the *bekasam* extract, we followed our study with fractionation to separate lovastatin and other larger compounds in the extracellular supernatant of *L. acidophilus* fermentation. The fractionation was based on the molecular size. The products were 5 fractions with different molecular weight i.e. fraction 1 (whole cell free extract), fraction 2

(>10 kD), fraction 3 (3-10 kD), fraction 4 (<3 kD), and fraction 5 (<1 kD). SDS PAGE analysis revealed that the fractions containing peptides band were fraction 1 (whole cell free extract), 2 (>10 kD), and 3 (3-10 kD). Fraction 1 (whole cell free extract) contain 4 major peptides with apparent molecular weight of 6 to 36 kD. Fraction 2 (> 10 kD) also contain peptides of the expected sizes, and fraction 3 (3-10 kD) contain major peptide of about 6 kD. No peptide were detected in fraction 4 (< 3 kD) and fraction 5 (< 1 kD) (Table 2 and Fig. 2A).

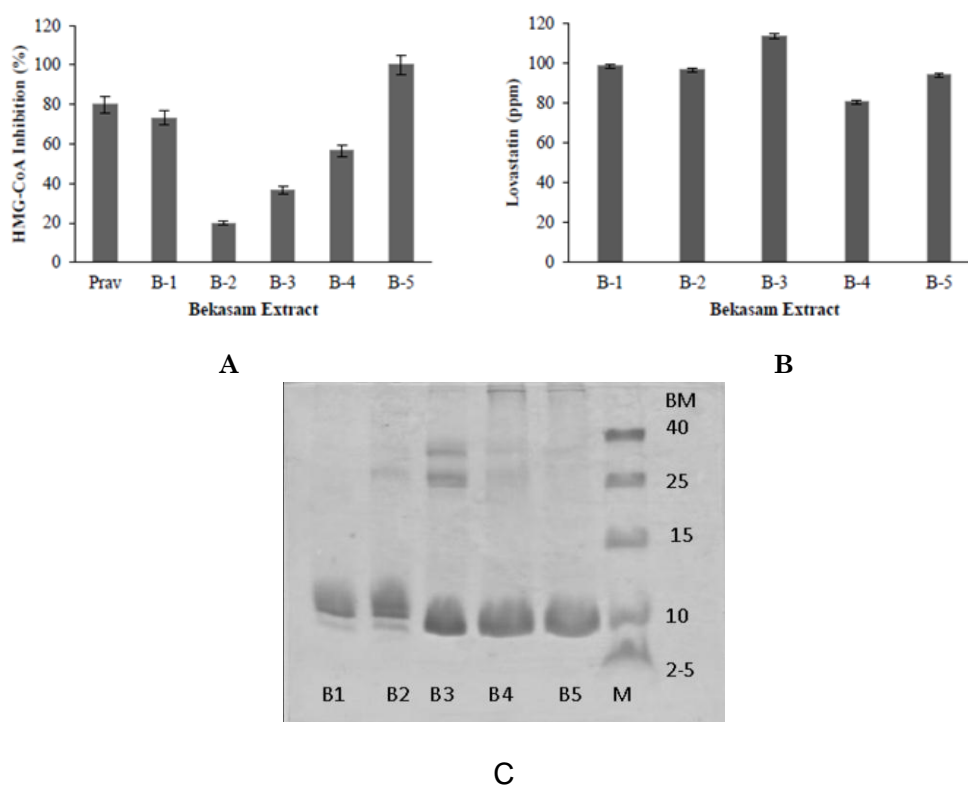


Fig. 1: Inhibition of HMG-CoA reductase by *bekasam* extract (A); Lovastatin content in *bekasam* extract (B) and Peptides profile of *bekasam* extract (C).

B1-B2 : *bekasam* produced from snakehead fish (*Rasbora sp*)
 B3-B5 : *bekasam* produced from *seluang* fish (*Chana striata*)
 M : Marker

Table 2: Analysis of enzyme inhibition in cell free extracellular fractions of *L. Acidophilus* fermentation

	Fractions				
	F1 Whole extract	F2 >10 kD	F3 3-10 kD	F4 <3 kD	F5 < 1 kD
HMG-CoA Inhibition (%) [*]	77.27±4.55 ^a	45.45±4.55 ^b	87.88±2.62 ^a	93.94±10.50 ^a	93.94±10.50 ^a

^{*} Result are means of three experiment, different letters have significant differences

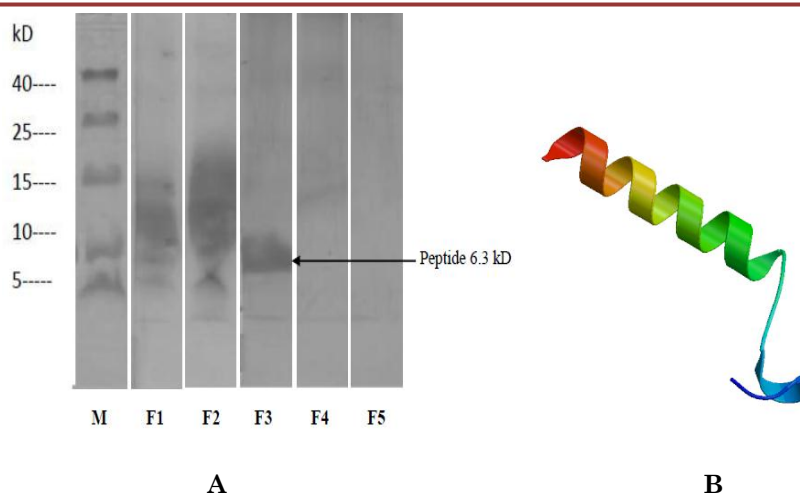


Fig.2. SDS PAGE profile of fractionated *Lactobacillus acidophilus* extracellular proteins (A) and Structure model of the 6 kD peptide (B).

The peptide profile was analyzed through SDS-PAGE using a 18% resolving gel in the 1 M Tris-HCl pH 6.8 as mentioned in the methods. After electrophoresis, the gels were stained with silver staining. The fractions analysed: F1 (whole); F2 (> 10 kD); F3 (3 – 10 kD); F4 (< 3 kD); and F5 (< 1 kD). Sequence (F4) identified as (KGENYNTGVTPNLRPKAAEVVAFLNKEAIEA IADTMKK). Modelling of molecular structure were conducted using SWISS-MODEL (www.expasy.org).

Fraction 4 (MW< 3 kD) and fraction 5 (MW< 1 kD) showed the highest HMG-CoA reductase inhibition (93.94%), followed by fraction 3 (MW 3 to 10 kD (87.88%) and fraction 1 (whole cell free extract (77.27%)). Fraction 2 of MW> 10 kD showed the lowest (45.45%) (Table 2). Strong HMG-CoA reductase inhibition of fraction 4 and 5 are related with the presence of low molecular weight lovastatin compound (Table 2 and Fig. 2A). Figure 2 A show that a major peptide of 6

KD is present in the fraction 3. This implied that compound responsible for HMG-CoA reductase inhibition was not only statin but also peptide. The 6 kd peptide was extracted, isolated and analysed further, which reveal a sequence of: KGENYNTGVTPNLRPKAAEVVAFLNKEAIEA IADTMKK. Modelling of the peptide was conducted using SWISS-MODEL [32] and RasMol Program, and the result is presented in Figure 2 B and 3

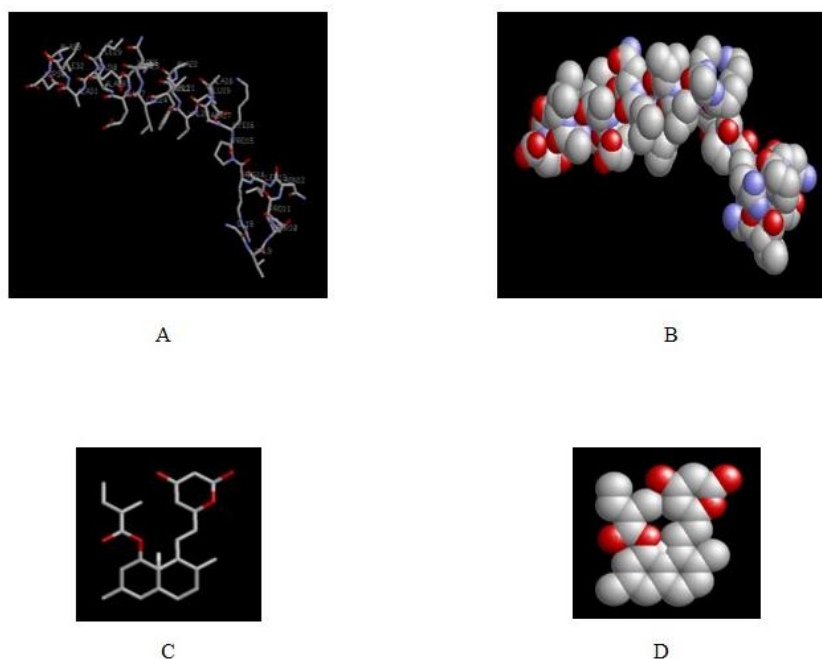


Fig.3. Modelling of peptide 6 kD (A-B) and lovastatin (C-D) were conducted using RasMol

DISCUSSION

Statin compounds and many peptides have been reported as HMG-CoA inhibitors. Lovastatin is one of statin produced by microorganism. The biosynthesis is influenced by several environment factors such as nutrient in the media, pH, and temperature. Snakehead fish (*Chana striata*) and *seluang* fish (*Rasbora sp.*) as a raw material for *bekasam* have different protein content: in snakehead fish 16.2% [35] and in *seluang* fish 17.75% [36]. Methionine content in *seluang* fish is 1.64% [37], which is greater than in snakehead fish (0.18% - 0.23%) [35]. In our study, these two factors may promote higher lovastatin and/or peptide inhibitor production and thus their higher HMGCoA inhibition activities in *bekasam* from *seluang* fish compare with *bekasam* from snakehead fish. Methionine is known as the best amino acid for lovastatin production, as it is involved in its biosynthesis [27]. Lovastatin biosynthetic pathway begins with the formation of monacolin-L from 9 molecules acetate and 1 methionine which is further transformed into monacolin J by hydroxylation process involving cytochrom P-450 in the cell.

Screening for statin producing bacteria from *bekasam* extract revealed *Lactobacillus acidophilus* as capable of producing lovastatin. *L. acidophilus* is a mesophyllic bacteria which grow at temperature 20 to 40 °C and optimally grow at 37 °C. They are normal flora in human gastrointestinal track. Lovastatin is a secondary metabolite which is usually produced by microorganism when they are at stationary phase. One of the function of lovastatin is for self defense and to increase their competitiveness over other microorganisms. *Lactobacillus acidophilus* grew more slowly at 25 °C, and this produced a little higher lovastatin in our study. Maximum lovastatin productivity in our study was at 25 °C, which is similar to that of *Aspergillus terreus* [43] and *Aspergillus niger* [38]. Optimum biosynthesis of lovastatin from *A. Terreus* was at 30 °C [39] and *Aspergillus niger* at 28 °C [40].

Lovastatin content in *bekasam* is 101.6 ppm, which is not as high compared with statins in red yeast *angkak* (175-254 ppm), a traditional fermentation product of *Monascus* on rice substrate [16, 44]. Thus, lovastatin produced by *L. Acidophilus* and statin presence in the

bekasam turned out to be not as high despite the high inhibition of HMGCoA reductase showed by *bekasam* and by extracts of *Lactobacillus acidophilus* (> 70% in comparison to the 100 % exhibited by the control drug pravastatin). This imply other bioactive compounds (beside lovastatin) which may also have been produced by *L. acidophilus* and presence in *bekasam*. Lactic acid bacteria is known capable of producing bioactive peptides such as bacteriocine substances known as antimicrobes, including *acidofilin*, *acidocin*, or *lactacin* [7]. *Lactobacillus plantarum* produced peptides Gly-Leu-Leu and Phe-OH-Pro as antifungal [9].

No report on HMG-CoA reductase inhibitor produced by Lactic Acid Bacteria (*Lactobacillus acidophilus*) so far. Japanese traditional fermented fish Narezushi and *heshiko* contain peptide fractions which inhibited HMG-CoA reductase [1-2, 23]. Kirana et al, 2005 [23] designed synthetic peptide (Ile-Ile-Ala-Glu-Lys) which act as cholesterol reducing agent by inhibiting solubilisation of cholesterol into micelles and cholesterol absorption. Gupta et al, 2005 [24] reported synthetic peptide (Leu-Arg-Lys-Leu-Arg-Lys-Arg-Leu-Leu-Arg) which rapidly reduced total plasma cholesterol (VLDL and LDL). Furthermore, Sharifof et al., 2011, [25] reported that lowering cholesterol by peptide (Leu-Arg-Lys-Leu-Arg-Lys-Arg-Leu-Leu-Arg) was due to its inhibition to lipid binding receptor in the apolipoprotein E. Peptide designed which act as competitive inhibitor for HMG-CoA reductase based on statin structure has been reported. Peptides Tyr-Val-Ala-Glu and Gly-Phe-Pro-Thr-Gly-Gly showed high ability to inhibit HMG-CoA reductase. The Tyr-Val-Ala-Glu peptide has 75% hydrophobic amino acid and Gly-Phe-Pro-Thr-Gly-Gly peptide has 67% hydrophilic amino acid [41, 42].

In our study, fraction 3 containing a 6 kD peptide show inhibition of HMG-CoA reductase up to 87.88% which is as effective inhibition activities to that shown by statin (no peptide) containing fraction 4 and 5 with 93.94% inhibition to HMG-CoA reductase. Although the correlation between peptide and lovastatin have not been reported previously, in this study we confirmed the presence of such bioactive peptide produced by *L. Acidophilus* with similar action to lovastatin, namely as inhibitor of

HMG-CoA reductase. This is the first report of a peptide act as inhibitor of HMG-CoA reductase originated from a lactic acid bacteria, namely : *L. Acidophilus*.

Mode of action of statin as HMG-CoA reductase inhibitor is by acting as competitive inhibitor, but inhibition mechanism of peptide on the enzyme is not known yet. Statins bind to the active side of enzyme, which is the HMG-CoA substrate binding side, and inhibit conversion of HMG-CoA to mevalonate. Atom O in the carboxylate moiety of HMG-CoA substrate is known to bind to the K735, S684, and K692 amino acid of the enzyme. Atom C in the methyl side of HMG-CoA substrate binds to the L553 enzyme, atom O in the hydroxyl side HMG-CoA substrate binds to D690 enzyme. Lactone side of statin possess similar structure as HMG-CoA substrate and statin dimension is 2.05 Å similar to HMG-CoA substrate [34], thereby create binding competition to active side the of enzyme (Fig3).

The peptide presence in fraction 3 with high HMGCoA inhibition activity possesses amino acid sequence of KGENYNTGVTPNL RPKAAEVVAFLNKEAIEAIAIDTMKK.

Homology analysis with other bioactive peptide excreted from *Lactobacillus sp.* revealed this peptide as novel unrelated with any bioactive peptide previously reported [33]. Comparison of the peptide from this *Lactobacillus acidophilus* with other bioactive peptides from foods, herbal and synthetic peptide known also as a cholesterol reduction agent, revealed differences of their sizes and amino acid sequences (Table 3). Modelling of the peptide was conducted using SWISS-MODEL (32) and RasMol Program, and the result is presented in Figure 2 B and 3. Our peptide of had much larger size than statin (the competitive inhibitor of HMG Co A reductase) and substrate HMGCoA, and may inhibit the enzyme not through competitive mechanism.

In conclusion, we had found that the traditional fermented food *bekasam* from Sumatra island in Indonesia, showed substantial inhibition to HMGCoA reductase, which is the key enzyme in cholesterol biosynthesis. The statin found in *bekasam*, lead us to screen for statin producing microorganism and revealed a *Lactobacillus acidophilus*. This bacterium produced not only statin but also a peptide with HMGCoA reductase inhibiting activity. This is the first

report of peptide from Lactic acid bacteria with such activity.

ACKNOWLEDGMENTS

This research was supported by Indonesian Ministry of of Education and Culture (Directorate General of Higher Education) through Competitive Research Grant Program 2015 (contract no. 113/UN9.3.1/LT/2015).

CONFLICT OF INTEREST

All authors are responsible for the content of this manuscript and have participated in planning, execution, data analysis and manuscript writing. We declare no conflict of interest regarding this content.

REFERENCES

1. Itou K , Akahane Y. Effect of extract from heshiko, a fermented mackerel product, on cholesterol metabolism in wistar rats. *Fish Sci* 2009; 75: 241-248.
2. Itou K, Akahane Y. Effect of extract from narezushi, a fermented mackerel product, on cholesterol metabolism in wistar rats. *Fish Sci* 2010; 76: 537-546.
3. Rhee SJ, Lee JF, Lee CH. Importance of lactic acid bacteria. *Microb Cell Fact* 2011; 10: 1-13.
4. Wikandari. Potention of indigenous LAB as producer ACE inhibitor in bekasam fermentation. Dissertation, Gadjah Mada University. 2011. DIS 080-H-2011, 162p. http://etd.repository.ugm.ac.id/index.php?mod=penelitian_detail&sub=PenelitianDetail&act=view&typ=html&buku_id=53183.
5. Desniar et al. The anti-microbial compound produced microorganism from bekasam. *J Akuatika* 2012; 3: 135-145.
6. Chen H, Hoover DG. Bacteriocins and their food applications. *COMPR REV FOOD SCI F* 2003; 2: 82-100.
7. Yamato M, Ozaki K, Ota F. Partial purification and characterization of the bacteriocin produced by *Lactobacillus acidophilus* YIT 0154. *Microbiol Res* 2003; 158: 169-172.
8. Lavermicocca P et al. Purification and characterization of novel antifungal compounds from the sourdough *Lactobacillus plantarum* strain 21B. *Appli Environ Microbiol* 2000; 66 : 4084-4090.

9. Muhialdin BJ, Hassan Z, Sadon SK. Biopreservation of food by lactic acid bacteria against spoilage fungi. *Ann Food Sci Technol* 2011; 12: 45-57.
10. Wikandari PR et al. Characteristic of proteolytic lactic acid bacteria from *bekasam*. *J Nat Indonesia* 2012; 14: 120-125.
11. Friensen, JA, Rodwel VW. The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. *Genome Biol* 2004; 5: 2481-2487.
12. Zhuge B et al. Bioconversion of lovastatin to a novel statin by *Amycolatopsis* sp. *Appl Microbiol Biotechnol* 2008; 79: 209-216.
13. Barrios-González J, Miranda RU. Biotechnological production and applications of statins. *Appl Microbiol Biotechnol* 2010; 85: 869-883.
14. Kato M. et al. The mechanism of the Cholesterol-lowering effect of water-insoluble fish protein in ovariectomised rats. *Br J Nutri* 2009; 102: 816-824.
15. Chen CH et al. Screening of compactin resistant microorganisms capable of converting compactin to pravastatin. *Curr Microbiol* 2006; 53:108-112.
16. Lachenmeier DW et al. NMR evaluation of total statin content and HMG-CoA reductase inhibitor in red yeast rice food supplements. *Chinese Med* 2012; 7: 1-7.
17. Park JW et al. Bioconversion of compactin into pravastatin by *Streptomyces* sp. *Biotechnol Lett* 2003; 25: 1827-1831.
18. Lin CL, Tang YL, Lin SM. Efficient bioconversion of compactin to pravastatin by the quinoline-degrading microorganism *Pseudonocardia carboxydvorans* isolated from petroleum-contaminated soil. *Bioresour Technol* 2011; 2: 10187-10193.
19. Peng Y, Yashphell J, Demain AL. Biotransformation of compactin to pravastatin by *Actinomadura* sp. 2966. *J Antibiot* 1997; 50: 1032-1035.
20. Singh DK, Banerjee S, Porter TD. Green and black tea extracts inhibit HMG-CoA reductase and activate AMP kinase to decrease cholesterol synthesis in hepatoma cells. *J Nutri Biochem* 2009; 20: 16-22.
21. Li CH. Purification and characterization of a novel cholesterol-lowering protein from the seeds of *Sena obtusifolia*. *Science in China Series C: Life Sci* 2008; 5: 1020-1024.
22. Liyanage R et al. Potato and soy peptide diets modulate lipid metabolism in rats. *Bioscie Biotechnol Biochem* 2008; 72: 950-2008.
23. Kirana C et al. Rapid screening for potential cholesterol-lowering peptides using naturally derived micelle preparation. *Aust J Dairy Technol* 2005; 60: 163-166.
24. Gupta H et al. Apolipoprotein E. Mimetic peptide dramatically lowers plasma cholesterol and restores endothelial function in watanable heritable hyperlipidemic rabbits. *J Am Heart Assoc* 2005; 14: 3112-3118.
25. Sharifof OF et al. 2011. Apolipoprotein E Mimetics and Cholesterol-Lowering Properties. *Am J Cardiovasc Drug* 11. 2011; 371-381.
26. Rinto et al. Isolation and Identification of Lactic Acid Bacteria that Produced HMG-CoA Reductase Enzyme Inhibitor from *Bekasam* as a Cholesterol Reduction Agent. *Agritech* 2015; 35: 309-314.
27. Osman ME et al. Optimization of some physical and chemical factors for lovastatin productivity by local strain of *Aspergillus terreus*. *Aust J Basic Appl Scie* 2011; 5: 718-732.
28. Shaligram S et al. Compactin production. Studies using *Penicillium brevicompactum* under solid-states fermentation condition. *Appl Biochem Bioethanol* 2009; 159: 505-520.
29. Abe Y et al. Molecular cloning and characterization of an ML-236B (compactin) biosynthetic gene cluster in *Penicillium citrinum*. *Mol Genet Genomics* 2002; 267: 636-646.
30. Giri A, Nasu M, Ohshima T. Bioactive properties of Japanese fermented fish paste, fish miso, using *koji* inoculated with *Aspergillus oryzae*. *Int J Nutr Food Scie* 2012; 1: 13-22.
31. Protein identification by MS report S150113RIAL_3502. 2015. [cited 2015 Feb 11]. Available from:<http://www.proteomics.com.au>.
32. Structural bioinformatics. [cited 2015 Feb 11]. Available from:http://www.expasy.org/structural_bioinformatics/tools/swissmodel 2015.
33. Pfeiler EA, Azcarate-Peril, Klaenhammer TR. Characterization of novel bile-induced operon encoding a two-component regulatory

- system in *Lactobacillus acidophilus*. J Bacteriol 2007; 189: 4624-4634.
34. Istvan ES et al. Palnitkar M, Buchanan SK, Deisenhofer J. Crystal structure of the catalytic portion of human HMG-CoA reductase: insights into regulation of activity and catalysis. The EMBO J 2000; 19: 819-830.
35. Mustafa A, Widodo MA, Kristianto Y. Albumin and zinc content of snakehead fish extract and its role in health. Int J Scie Technol 2012; 1: 1-8.
36. Liuhartana R, Harris H. Identification of processing technology and quality analysis seluang dry on the traditional processing. J Fisheries Sci 2011; 6: 16-20.
37. Screenivasan A, Natarajan MV. Sulphur and methionine content of some fresh water fishes of Madras. Fishery Technol 1966; 3: 81-82.
38. Rashid SA, Ibrahim D, Anyatha INP. Effect cultural condition on lovastatin production by *Aspergillus Niger* Sar 1 using combination of rice bran and brown rice as substrate. Int J Appl Biol Pharm Technol 2013; 4: 150-156.
39. Javed S et al. Screening of indigenously isolated fungi for lovastatin production and it's in vivo evaluation. Curr Pharm Biotechnol 2010; 15: 422-427.
40. Raghunath RA et al. Optimised production of lovastatin through solid state fermentation by endophytic fungi. Int J Pharma Bioscie 2012; 3: 562-570.
41. Park VV et al. Peptide design of competitive inhibitor for HMG-CoA reductase based on statin structure. Pept Scie 2006; 84: 586-594.
42. Park VV et al. Recognized sequence and conformation in design of linier peptides as a competitive inhibitor for HMG-CoA reductase. J Mol Recognit 2007; 20: 197-203.
43. Jahromi MF et al. Lovastatin Production by *Aspergillus terreus* using agro-biomass as substrate in solid state fermentation. J Biomed Biotechnol 2012; 1-11.
44. Danuri H. Optimizing angkak pigments and lovastatin production by *Monascus purpureus*. Hayati J Bioscie 2008; 15: 61-66.

Cite this article as:

Rinto, Ratih Dewanti, Sedarnawati Yasni, Maggy Thenawidjaja Suhartono. Novel HMG-CoA Reductase Inhibitor Peptide from *Lactobacillus acidophilus* Isolated from Indonesian fermented food Bekasam. J Pharm Chem Biol Sci 2017; 5(3): 195-204