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Research Article

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

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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 KGENYNTGVTPNLRPKAAEVVAFLNKEAIEAIADTMKK. We concluded that *Lactobacillus acidophillus* 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 Philipine, *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 hypertention 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. *Lactobacilus* and *Pediococcus* during fermentation [12]. 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 compestries*, *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 acidophylus*. 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 phospat (2 g/L), sodium acetate 3H2O (5 g/L), triammonium citrate (2 g/L), magnesium sulfate $7H_2O$ (0.2 g/L), and manganese sulfate $4H_2O$ (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 λ = 620 nm.

Lovastatin assay

Lovastatin content was determined by spectrophotometer (UV-Mini-1240, Shimadzu) and High Performance Liquid Chromatrography (HPLC, Agilent 1200 series). Five mililitre 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 flash and the volume was filled up with methanol. The absorbance was measured by spectrophotometer at λ = 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 periode, 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 Bransonic Ultrasonic (Branson 8510E-MTH). Supernatant was separated using C18 Colum (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 λ = 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 subtrate, 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 λ = 340 nm, which represented oxidation of NADPH by the catalytic subunit of HMG-CoA reductase in the presence of the subtrate HMG-CoA. One unit was defined as 1.0 µmole of NADPH converted to NADP+ per minute. Specific activity was defined as µmol/min/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 asetic 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 ($Na_2S_2O_3.5H_20$) 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 matcing 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) form *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 µg/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 acidophillus*

Screening of statin producing coumpound from the Indonesian *bekasam* extract revealed and identified Lactobacillus *acidophylus* [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 corellate 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.acidophillus* 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 smal 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).

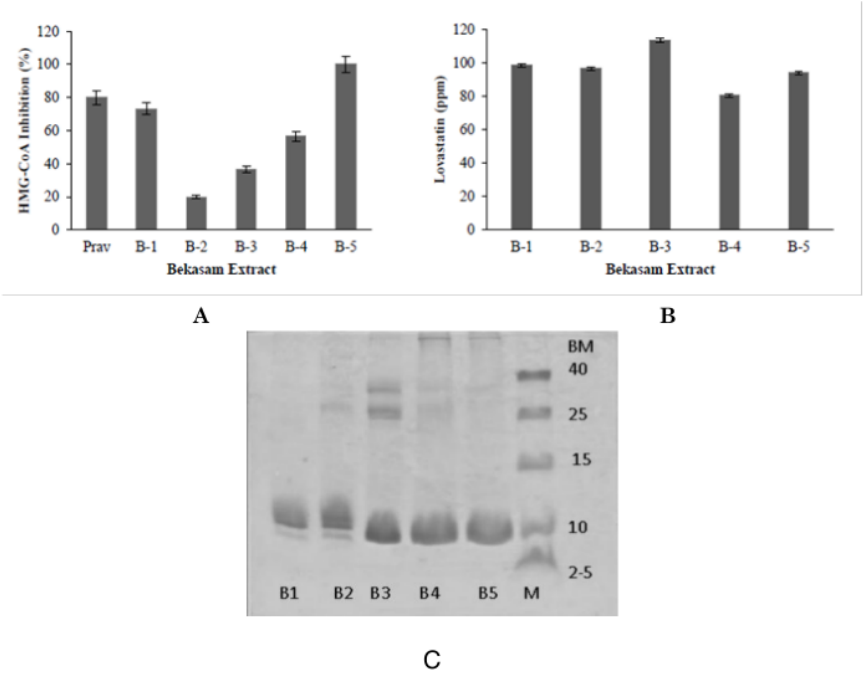


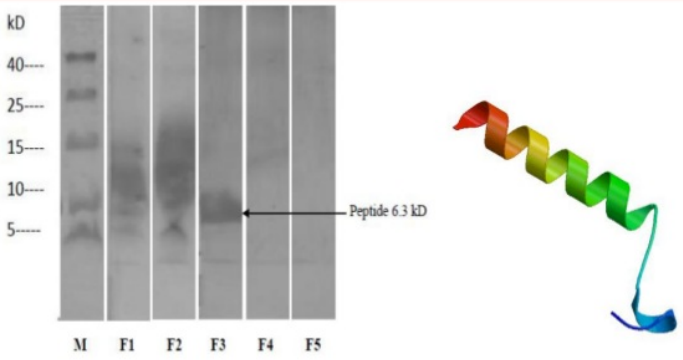
Fig. 1: Inhibition of HMG-CoA reductase 1) *bekasam* extract (A); Lovastatin content in *bekasam* extract (B) and Peptides profile of *bekasam* extract (C).

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



A B

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 (KGENYNTGVTPNLRPKAAEVVAFLNK**EAIEAIADTMKK). M**odelling 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 anlasyed furthur, 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

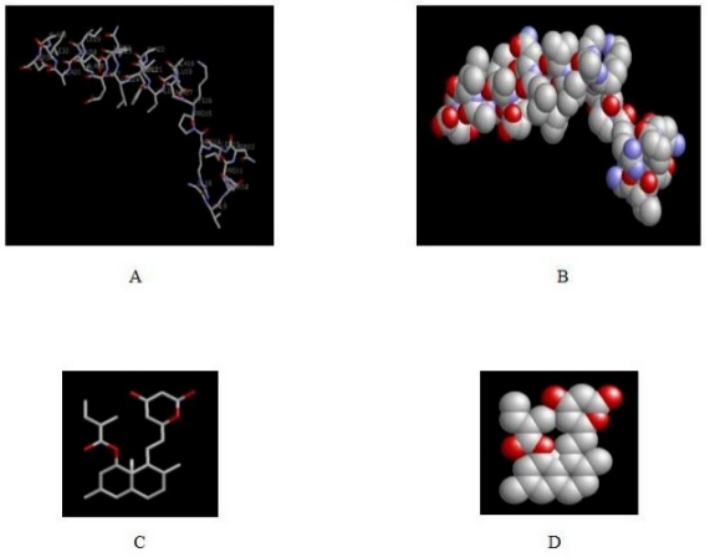


Fig.3. Modelling of peptide 6 kD (A-B) and lovastatin (C-D) were conducted using RasMol

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 furthur transformed into monacolin J by hydroxylation process involving cytochrom P-450 in the cell.

Screening for statin producing bacteria from *bekasam* extract revealed *Lactobacillus acidophylus* 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 simillar 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 substrade [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 subtances known as anti-microbes, 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 comfirmed 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 subtrate binding side, and inhibit convertion of HMG-CoA to mevalonate. Atom O in the carboxylate moeity of HMG-CoA subtrate is known to bind to the K735, S684, and K692 amino acid of the enzyme. Atom C in the methyl side of HMG-CoA subtrate binds to the L853 enzyme, atom O in the hydroxyl side HMG-CoA subtrate binds to D690 enzyme. Lactone side of statin possess similar structure as HMG-CoA subtrate and statin dimension is 2.05 Å similar to HMG-CoA subtrate [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 RPKAAEVVAFLNKEAIEAIADTMKK.

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 biosymthesis. The statin found in *bekasam*, lead us to screen for statin producing microorganism and revealed a *Lactobacillus acidophillus*. 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.

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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.

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