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REVIEW

MICROBIAL TRANSGLUTAMINASE: SOURCE, PRODUCTION AND ITS ROLE TO IMPROVE SURIMI PROPERTIES

Microbial Transglutaminase: Sumber, Produksi dan Peranannya untuk Memperbaiki Sifat Surimi

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ABSTRACT

Transglutaminases (EC 2.3.2.13) have attracted a wide interest from both scientific and applied points of view due to their capacity to cross-link protein substrates. Obtaining transglutaminases derived from animals are extremely high cost process, which has hampered its wider application until the discovery of transglutaminase produced by microorganisms. In the early 1990, since microbial transglutaminase have been found, many transglutaminase-producing microbial strains have been isolated and the enzyme production processes have been optimized. This resulted in the increased uses of transglutaminases in the food industries. In the fisheries industry, MTGase has successfully been used to improve the mechanical properties of surimi from various fishes.

Keywords: Microbial transglutaminase (MTGase), surimi

ABSTRAK

Transglutaminases (EC 2.3.2.13) merupakan enzim yang diminati secara luas baik dari segi ilmiah maupun untuk aplikasi karena kemampuannya dalam membentuk ikatan silang substrat protein. Transglutaminase yang berasal dari hewan mempunyai harga sangat tinggi yang menjadi kendala untuk aplikasi lebih luas. Sejak awal 1990-an setelah ditemukannya *Streptomyces mobaraensis, s*udah banyak dilaporkan mikroorganisme yang mampu menghasilkan enzim ini dan proses optimasi produksinya juga sudah banyak dilakukan. Dampaknya adalah meningkatnya aplikasi enzim ini pada industry makanan. Dalam industri perikanan MTGase telah digunakan untuk meningkatkan sifat mekanik surimi yang dihasilkan dari berbagai jenis ikan.

Kata Kunci: Microbial transglutaminase (MTGase), surimi

1. Introduction

Transglutaminases (TGase: protein-glutaminase γ-glutamyltransferase, EC 2.3.2.13) comprise a class of enzymes catalyzing the substitution of the amide ammonia with another amine at the γ-position in glutamine residues, normally an 1-amino group from a suited lysine residue (Dadabay and Pike, 1989). The establishment of 1-(γ-glutamyl) lysine isopeptide bonds produce both intra- and inter molecular cross-linking of proteins, which lead to polymerization. (Figure 1).

Until the end of the 1980s, guinea pig liver was the only source for commercial transglutaminase.The price for the enzyme was extremely high due to the rare source and complicated downstream procedure, which hampered its wide range of applications in food processing (Zhu & Tramper, 2008).The production of transglutaminase from microorganisms have received increasing interest after *Streptomyces mobaraensis* was reported to produce transglutaminase (Ando et al., 1989). Since then, screening of new strains capable of producing tranglutaminase have been carried out (Neilson, 1995; Kobayashi et al., 1998; Liu et al., 2007) and effors to enhance enzyme yield and production has been conducted through various methods (Sommer et al., 2011; Kittikun et al., 2011; Guera-Rodriguez & Vázquez, 2013a, 2013b; Guera-Rodriguez et al., 2012a, 2012b). ,

Recently, Microbial Transglutamine (MTGase) has been used for food processing and has been shown to improve the flavour, appearance andtexture of various protein-based foods.Surimi is the minced fish meat that has been washed to remove fat, water-soluble

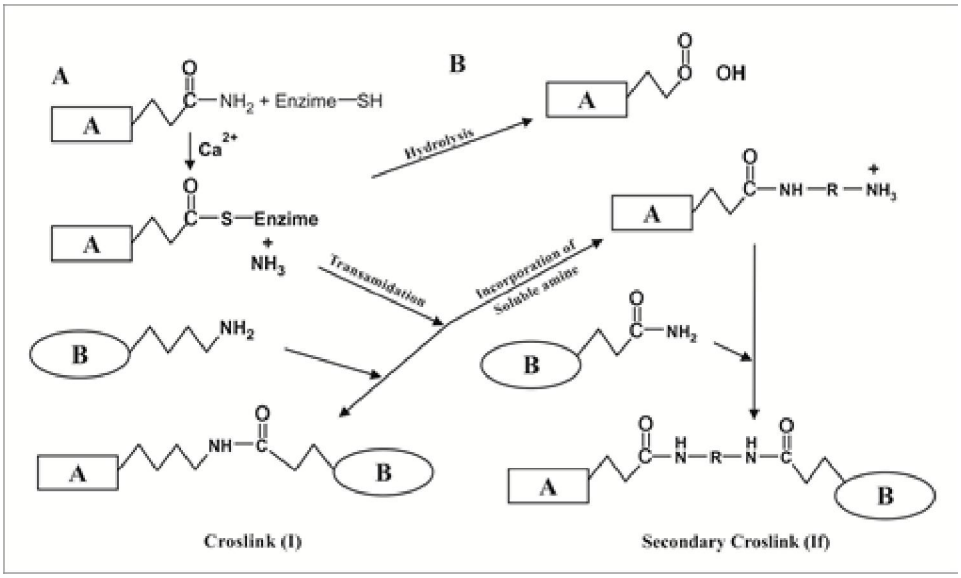


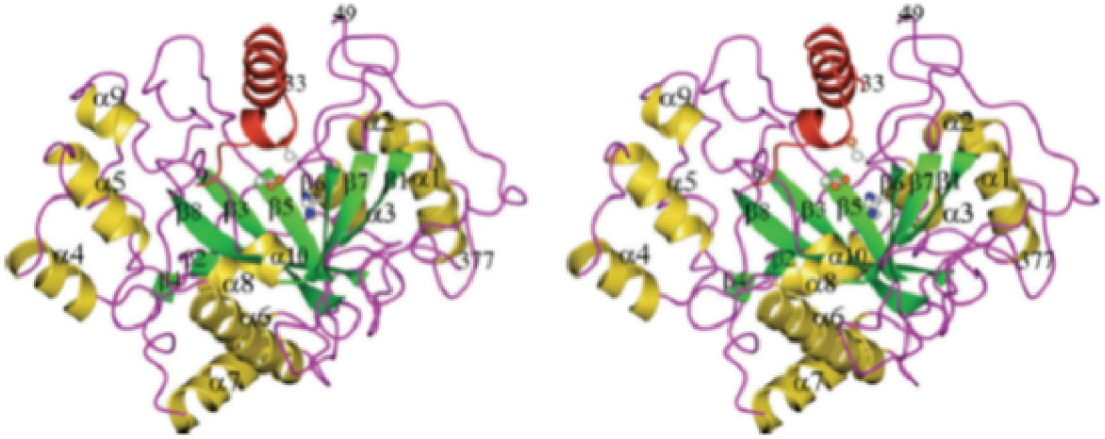
Figure 1. Reactions catalysed by TGases. (A) TGase forms a covalent intermediumte between the active-site thiol residue and a glutamine residue releasing of ammonia. (B) thioester undergoes either hydrolysis (an unfavourable reaction), releasing glutamic acid in the substrate protein, or (C) an acyl transfer to a primary amine resulting 1) a simple amine-isopeptidyl adduct, 2)Polymerization form cross-link II 3) in the second instance a direct glutamyl-lysine protein cross-link is produced (cross-link I). (Griffin et al., 2002).

proteins and undesirable muscle components such as blood and pigments. This process result in the concentrated myofibrillar proteins, which directly contribute to gelation (Iwata et al., 2001). Surimi has been used as the primary ingredient in a variety of processed foods such as kamaboko, chikuwa, fish sausages, fish balls, etc. The prime factor to determine the quality of surimi is the mechanical properties. Many approaches for improving the texture of surimi-based products have been therefore proposed and implemented i.e addition of transglutaminase. We will describe the sources of MTGase , the optimization of its production, and its application to improve the quality of surimi.

Source of Microbial Transglutaminase

TGase identified in all of mammalian members require calcium as a cofactor (Kumazawa et al., 1997). Although MTGase are a variant of the transglutaminase family, but their calcium independent catalysis are distinct by virtue of the isopeptide bond formation (Aluko & Yada, 1999; Pastemack et al., 1998). MTGaseis 38-kDa enzyme which is expressed as a zymogen and activated through N-terminal cleavage (Pastemack et al., 1998; Kanaji et al., 1993; Zotzel et al., 2003). Very little sequence similarity was shown by MTGase isolated from *Streptomyces mobaraensis*to any mammalian TGase, whose molecular weights are greater than 70 kDa (Piredda et al., 2003). Although sharing a similar function as its mammalian counterparts but MTGase exhibits more robust catalytic properties, including high stability throughout a broad range of pH and temperatures in comparison with other TGase (Shi et al., 2011). The crystal structure of MTGase from *Streptomyces mobaraensis* is presented in Figure 2.

After the discovery of *S.mobaraensis,* further screening effort has led to the isolation of other MTGase-producing *Streptomyces*, such as *Streptomyces* sp. (Neilson, 1995), *Streptoverticillium cinnamoneum* (Duran et al., 1998), *Streptoverticillium ladakanum* (Ashie & Lanier, 2000), *Streptomyces fradiae* (Liu et al., 2007), *Streptomyces. Hygroscopicus* (Cui et al., 2007), *Strepmyces lividans and Streptomyces platensis* (Lin et al., 2006). *Pseudomonas* species was also reported to produce transglutaminase. Some notable examples include *Pseudomonas putida, Pseudomonas amyloderamosa and Pseudomonas palleroni,* (Bech et al., 2001). Other MTGase microbial producers include *Bacillus substilis* (Kobayashi et al., 1998), *Zygomonas mobilis* and *Hafnea alvei* (Bech et al., 2002). Kittikun et al. (2012) reported *Enterobacter* sp. C2361 and *Providencia* sp. C1112 as active MTGase-producing strains isolated from waste water and flock-floating.



Gambar 2. Stereo view of the overall structure of the MTGase zymogen generated with program CCP4MG. The visible portion of prosequence (residues 9-33) of the zymogen has been colored red. Reprinted from: Crystal Structure and Inhibition Studies of Transglutaminase from *Streptomyces mobaraense*. Yang, M. T., Chang, C. H., Wang, J. M., Wu, T. K., Wang, Y. K., Chang, C. Y., & Li, T. T. 2011. Journal of Biological Chemistry. 286, 7301-7307.

Using molecular approach, Patantis et al. (2008) screened 98 bacterial isolates from culture collection hosted at Research and Development Center for Marine and Fisheries Product Processing and Biotechnology (RDCMFPPB) and Institute of Indonesian Science (LIPI). A pair of primers used was PTGase 4 (5'-TACGGCTGCGTCGGTGTCAC-3') dan PTGase 5 (5'-GACGGTCGTGATTGCCTCC-3') with *Streptoverticillium ladakanum* as the positive control. The result showed that there are 3 isolates exhibiting PCR-amplification of 400-bp fragments, which are considered as the fragments of transglutamniase.

Over-production of MTGase have been examined using various host strains. For example, MTGase production was heterologously expressed as intracellular inclusion body or pro-MTGase using *Escherichia coli*, which required additional processing by a subtilisin-like protease to produce the mature form of recombinant MTGase (Yokoyama et al., 2000; Yang et al., 2009). *Corynebacterium glutamicum* was reported as the host for efficient MTGase production. However, it was also secreted as pro-MTGase (Kikuchi et al., 2003). The Active form of MTGase from *Streptomyces albogriseolus* was successfully produced by constructing a strain of *C. glutamicum* that co-expressed pro-MTGase and SAM-P45 (Date et al., 2003).

Ogino et al. (2004) constructed a heterologous over-production system for the secretory phospholipase D using *S. lividans* as the expression host. They assumed that this vector would be suitable for the production of active-form MTGase because the vector used in this system contained putative promoter and terminator regions, and the signal peptide sequence (pld signal) derived from the *S. cinnamoneus* phospholipase D gene. MTGase from *S. mobaraensis* was reported to have 416 residues, including a predicted pre-region of 32 residues, pro-region of 54 residues, and mature region of 330 residues (Washizu et al., 1994). Pro-domain of transglutaminase from *S. cinnamoneus*, which shows 62.2% identity to that of the transglutaminase from *S. mobaraensis*, was fused to the N-terminus of MTGase, resulting in the successful production of active-form MTGase from *C. glutamicum* (Date et al., 2003).

Noda et al. (2013) successfully produced MTGase using a genetically modified strain of *S. lividans*. The pld-signal and prepro-domain of *S. cinnamoneus* transglutaminase contributed to the effective secretion of MTGase. This is the first report demonstrating the efficient production of mature active-form MTGase using *S. lividans*.

Optimization of Production

MTGase,in particular produced by *Streptomyces mobaraensis,* has been widely used as catalyst in various industry due to its rather broad substrate specificity for acyl acceptors and its independence of Ca2+, which are distinct from animal transglutaminases (Tanaka, 2004). The studies on transglutamaninase production from *Streptoverticillium* and *Streptomyces* had been carried out to obtain economical optimal methods (Motoki & Seguro, 1998; Ando et al., 1989). Production of MTGase industrially was conducted by extracting the

enzyme from culture medium.The enzyme free culture is separated from remain medium component and transferred into a product in powder form.

Since it was published by Ando et al. (1989), the medium composition for MTGase productions from *Streptomyces* has been almost the same which contains yeast extract, peptone, sodium phosphate, potassium phosphate, magnesium sulphate and a carbon source (Portilla- Rivera et al., 2009; Bahrim et al., 2010; Aidaroos et al., 2011; Zheng et al., 2001).The common carbon sources used are glucose (Bahrim et al., 2010), starch (Zheng et al., 2001), solubilized starch (Kittikun et al., 2012; Bourneow et al., 2012) anddextrins (Guerra-Rodríguez & Vazquez, 2013b). However, to be economically acceptable for industrial-scale, the source of culture medium should not only abundant but also cheap.The formulation of culture medium was crucially importance due to its effect on yield and volumetric productivity (Bahrim et al., 2010; Portilla-Rivera et al., 2009).The cost for a microbial enzyme production represented almost 30% by fermentation medium (Portilla-Rivera et al., 2009).The medium commonly used for transglutaminase production from *Streptomyces*are not economically attractive. It contains expensive for medium components i.e. peptone, yeast extract, and many mineral salts.

Some studies that using cheaper medium by utilizing wastes from the agro-industry as carbon source have been carried out for the production of MTGase such as acid hydrolysates of sorghum straw (Tellez-Luis et al., 2004a), acid hydrolysates of wheat straw (Guerra-Rodríguez et al., 2012[b]), sugar cane molasses (Portilla-Rivera et al., 2009) and enzymatic or acid hydrolysates of potato (Guerra-Rodríguez & Vazquez, 2013b; Guerra-Rodríguez et al.,2012a,b). Non-commercial potatoes that are considered as agricultural wastes can be used as medium for the microbial production of MTGase after being hydrolized with acid or enzyme. The acid hydrolysis of potato is aimed at obtaining the degradation products, such as furfural, 5-(hydroxymethyl)-2-furaldehyde and acetic acid that remain in the glucose solutions. These by-products are known as inhibitors of microbial growth, which are lethal at the certain concentrations (Guerra-Rodríguez et al., 2012b; Wang et al., 2012; Zeni et al., 2011). Enzymatic hydrolysis of potato will generates glucose solutions that can be consumed by microorganisms without further treatments. In the enzymatic hydrolysis there are no microbial growth inhibitors exist (Delgado et al., 2009; Vazquez et al., 2009). However, the hydrolysis processes are time and enzyme consuming.

Guera-Rodríguez & Vázquez (2013b) accomplished their previous research to eliminate the hydrolysis process. The potatoes were dried at 60°C before being ground. The potato powder dissolved in water along with skim milk and glycerol was used as the growth medium. Using the same strain, *S. ladakanum* NRRL 3191, the activity of transglutaminase obtained with this medium was increased by more than 300% compared with those obtained from hydrolysates of sorghum straw (Tellez-Luis et al., 2004a), sugar cane molasses with glycerol (Portilla-Rivera et al., 2009), with glycerol alone as carbon source (Tellez-Luis et al., 2004b) and with enzymatic hydrolysates of potato supplemented with yeast extract, corn steep liquor and casein (Rodríguez & Vazquez, 2013a). The fermentation using dried potatoes, skim milk and glycerol was also faster (72 h) (Guera-Rodríguez & Vázquez ,2013b) than those mentioned above (about 96h)(Tellez-Luis et al., 2004a; Portilla-Rivera et al., 2009; Tellez-Luis et al., 2004b; Rodríguez & Vazquez, 2013a).

Kittikun et al. (2012) has prepared the alternative raw materials for fermentation medium with low cost using surimi waste water (SWW). This waste could have a negative impact on the environment but on the other hand it still contains high nutrients and minerals that can be used as medium component.The protein in SWW can be recovered and utilized to produce value-added product while environmental problems can be resolved. The protein hydrolisate from SWW can replace the expensive nutrients in culture medium such as peptone, tryptone, meat extract or yeast extract as a source of nitrogen (proteins, peptides, and amino acids) and organic phosphate (Afonso & Borguez, 2002; Bourtoom et al., 2009).Kittikun et al. (2012) used fish protein hydrolisate (FPH) obtained from hydrolyzed SWW to produced MTGase from *Enterobacter* sp. C2361,*Providencia* sp. C1112 and *S. mobaraensis*as as control. The FPH was used to replace pepton in SPY medium (2.0% soluble starch, 2.0% peptone, 0.2% yeast extract, 0.2% Mg_2SO_4, 0.2% K_2HPO_4 and 0.2% KH_2PO_4). The result showed that the strains entered the stationary phase longer (42 h) in SPY medium than FPH (36 h) as well reaching maximum activity (48 h in SPY and 18 h in FPH).

Application of MTGase on Fish Meat Processing

MTGase can be applied to the raw material either in dry or rehydrated form depending especially on the properties of the raw material to which it is added. The temperature affects the reaction where higher temperatures require less time for the reaction between transglutaminase and free protein groups. Transglutaminase is active optimally in the range of

temperature from 50 to 55°C (stable at the range of 0–60°C) and pH from 6 to 7 (stable at pH 5–9). The enzyme can be inactivated easily by setting temperature at above 60°C with the duration of inactivation depends on the type of food stuffs (Vácha et al., 2006).

Restructuration of raw meats were previously carried out using NaCl and sodium tripolyphosphate, which resulted undesirable flavour changes as well as increasing sodium intake (Kuraishi et al., 2000; Muguruma et al., 2003). Many research works had been conducted to investigated the effects of MTGase on various meats, such as pork (Hong &Chin, 2012; Pietrasik & Li-chan, 2002), beef (Castro-Briones et al., 2009, Martínez et al., 2010, and Pietrasik, 2002), chicken (Abdulatef et al., 2009; Tseng, 2000; Sun, 2011), and fish (Uresti et al., 2006, Cardoso, 2007, Cardoso et al., 2010; Moreno et al., 2010). Some researchers had reported that the addition of exogenous MTGasecan improves drastically the textural characteristics (elasticity and firmness), the mechanical strength, and water-holding capacity of protein-based product, such as restructured steaks, sausages,hot dogs doner and other reconstructed fish and meat products (Kuraishi et al., 2000; Motoki & Seguro, 1998; Ramirez et al., 2000). The treatment with MTGase would be expected to affect the ability of proteins to aggregate (Fernandez-Diaz et al., 2001; Herrero et al., 2008). Significant decrease in α-helix content and increase in α-sheets after addition of MTGase can be observed under microscope (Herrero et al., 2008).

In fisheries, transglutaminase is currently used to improve the mechanical properties of fish product (surimi, fillets, separate). Overall surimi products in the Southeast Asian region are estimated to be 315,800 metric tons in 2005 (Pangsorn et al., 2007). Surimi can be used to prepare a variety of processed foods such as kamaboko, kani (crab) kamaboko, chikuwa, satsuma-age, fish sausages, fish balls, etc. High-quality surimi yields flexible gel with white color. Although the quality of surimi products depends mainly on their gelling properties, but the product contained healthy and natural ingredient also possess added value for the consumer.The quality of fish mince or surimiand the types of ingredients used will affected

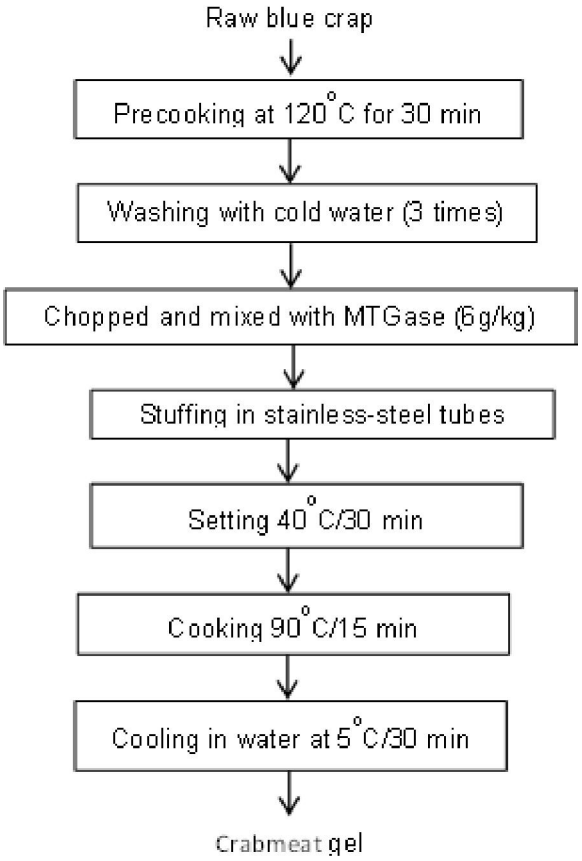


Figure 3. Flowchart of Crabmeat Gels Production (Martinez et al., 2014).

the gel properties. Technological functions of the ingredients will determine textural properties. They also have antimicrobial and antioxidant and/or nutritional function.

MTGase has successfully been used in surimi to strengthen the gel, as reported by Martinez et al. (2014). Addition of MTGase and precooking treatment at 120° C for 30 min allowed interaction with MTGase and improve the mechanical properties of blue crab (*Callinectes sapidus*) gels. The flowchart of crabmeat gel process is presented in Figure 3. Benjakul et al. (2008) reported that the addition of MTGase from *S. mobaraense* in mince from lizard fish effectively increased the breaking force and deformation of gels. MTGase was also reported to increase the hardness and water holding capacity of common carp meat (Vácha et al., 2006).

Gel properties of threadfin bream (*Nemipterus bleekeri*) surimi added with fish gelatin in combination with MTGase were studied by Kaewudom et al. (2013).This study showed that addition of fish gelatin up to 10% in conjunction with 1.2 units MTGase/g surimi was recommended to obtain surimi with grade AA. Surimi gel network became finer and denser with the addition of 0.1% MTGase, as compared with the control gel (without MTGase).More compact and denser gel network was yielded by myofibrillar proteins which more effectively form the cross-linking in the presence of MTGase. Finer gel and structure which more ordered as a result of MTGase addition

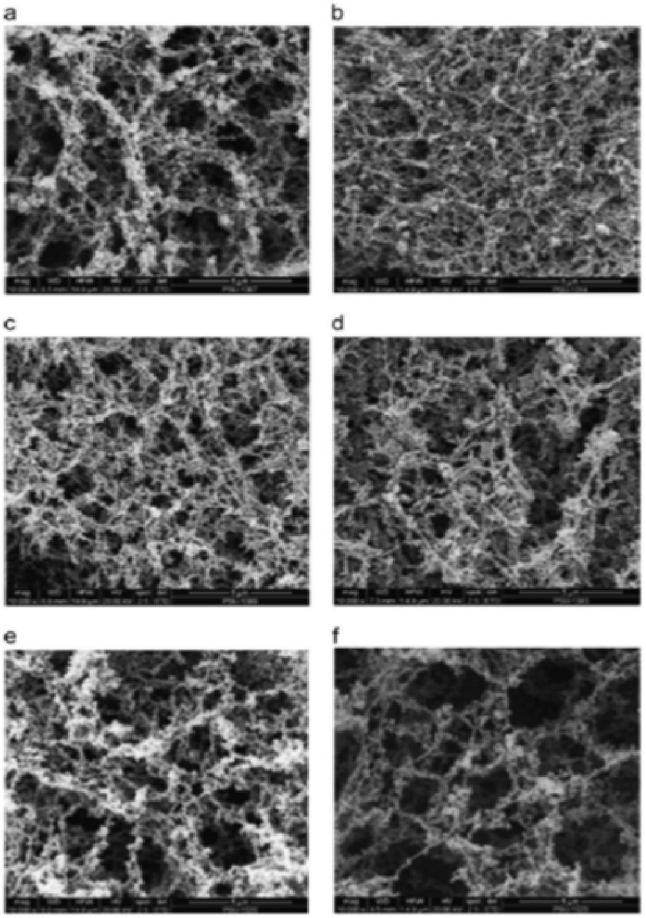


Figure.4. Electron microscopic images of surimi gel added with fish gelatin (FG) at different levels and MTGase at a level of 1.2 units g”1 surimi (magnification:10,000_): (a)surimi gel without gelatin and MTGase;(b)surimi gel added with MTGase 1.2 unitsg”1 surimi (without gelatin);(c)surimi gel added with 5% gelatin and MTGase1.2units g”1 surimi;(d)surimi gel added with10% gelatin and MTGase1.2 units g”1 surimi;(e)surimi gel added with15% gelatin and MTGase1.2unitsg”1 surimi; and (f) surimi gel added with 20% gelatin and MTGase1.2 unitsg”1 surimi Reprinted from : “Properties of surimi gel as influenced by fish gelatinand microbial transglutaminase” Kaewudom, P.,Benjakul, S. & Kijroongrojana, K. 2013..*Food Biosci*.1 : 39 –47.

correlated with higher breaking force and deformation as well as the lowered expressible moisture content. Microstructures of surimi gels added with fish gelatin at various levels in the presence of 1.2 units MTGaseg^{-1} surimi are illustrated in Figure 3 (Kaewudom et al., 2013).

Chenarat & Benjakul, (2013) reported the same result where in the presence of MTGase, indian mackerel fish protein isolates could undergo the crosslinking more effectively. Addition of MTGase yielded gel which was slightly more compact, with a denser gel network and smaller voids. Benjakul et al. (2008) also reported the agreement result that MTGase addition was able to improve the gel matrix of lizardfish surimi which became more compact and filamentous.Thus, alkaline solubilisation process and MTGase addition affected the gelling properties of Indian mackerel mince (Chenarat & Benjakul, 2013)

Researchers at Research and Development Center for Marine and Fishery Product Processing and Biotechnology (RDCMFPPB) Jakarta have used MTGase produced by *Streptoverticillium ladakanum* to improve the properties of restructured meat made from *Priacanthus macracanthus* (Mata Goyang) and *Euthynnus*spp (Tongkol). The experiment results showed that gel strength of restructured fish made from *Euthynnus* spp. increasing up to 22 times compared to control (Fawzya et al., 2011a). The gel strength, springiness and cohesiveness of restructured fishmeat made from *Priacanthus macracanthus* increased with the addition of 1% MTGase along with 1% NaCl and 1% sodium caseinat (Fawzya et al., 2011b).

Prospect and Constraints for MTGase Development in Indonesia

MTGase-based industry has promising prospects to be developed in Indonesia. The cheap raw material such as potatoes and cassava are abundantly available. In addition, the waste of fishery product processing can be utilized for enzyme production. Although many researches on screening of microorganisms, optimization of production and application of transglutaminase have been conducted and published, so far there is no report on transglutaminase-producing microorganisms of Indonesia origin, therefore the production and application of this enzyme still limited. So far the commercially available MTGase for food industry was produced only by Ajinomoto (TG-Activa). Therefore, research on transglutaminases, starting from screening of microbial producers towards the optimization of enzyme production and application, have intensively been done by us in Research and Development Center for Marine and Fishery Product Processing since 2009. Discovery of novel transglutaminases isolated from Indonesian microorganisms and further research and development towards cost-effective MTGase production will open great opportunities to develop domestic industry, particularly in fish processing.

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