Squalen Bull. of Mar. and Fish. Postharvest and Biotech. 11 (1) 2016, 13-21

www.bbp4b.litbang.kkp.go.id/squalen-bulletin

Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology

ISSN: 2089-5690
e-ISSN: 2406-9272

SCREENING OF INDONESIAN *Streptomyces* sp. CAPABLE OF SECRETING TRANSGLUTAMINASE (MTGase) AND OPTIMIZATION OF MTGase PRODUCTION USING DIFFERENT GROWTH MEDIA

Yusro Nuri Fawzya[1*], Dewi Seswita Zilda[1], Seprianto[2], Hana Nurullita Prestisia[2], Puspita Lisdiyanti[3] and Noer Khasanah[4]

[1] Research and Development Center for Marine and Fisheries Product Competitiveness and Biotechnology, Jalan KS. Tubun Petamburan VI, Slipi, Central Jakarta 10260, Indonesia
[2] Graduate student of Bogor Agricultural University
[3] The Research Center for Biotechnology, Indonesian Institute of Sciences
[4] Departement of Fishery, Faculty of Agricultural, Gadjah Mada University

Article history:
Received: 1 February 2016; Revised: 6 April 2016; Accepted: 20 April 2016

Abstract

Transglutaminase (TGase), an enzyme that catalyzes the formation of inter- and intra-molecular ε-(l-glutamyl) lysine (GL) crosslinks, plays an important role in surimi-based products production. The development and the diversification of surimi-based products have recently been getting popular in Indonesia. These surimi-based products can be made from various types of fish. These products generally exhibit good gel strength properties, depending on the fish type and the processing method used. Transglutaminase plays an important role in generating such properties. Fish's endogenous TGase reduces quickly after it is caught and is almost completely destroyed by freezing it, applying exogenous TGase may improve fish's gel forming ability. Microbial transglutaminase (MTGase) can potentially be used to increase gel properties. In this research, a total of 228 *Streptomyces* strains from marine and terrestrial environments were screened and selected based on their ability to produce MTGase. Strain TTA 02 SDS 14 exhibited the highest activity; and therefore, it was selected for further study. The 16S-rRNA gene analysis showed that it shared 99% similarity to *S. thioluteus.* In order to optimize MTGase activity, enzyme production was carried out using six different formulas media, designated as media A, B, C, D, E, and F. The result shows that the highest MTGase activity was observed in medium B that contains pepton (1.5%), $MgSO_4.7H_20$ (0.1%), KH_2PO_4 (0.5%), Na_2HPO_4 (0.5%), soybean powder (2%), potato starch (2%), and glucose (1.5%). The MTGase activity reached the highest level (1.45 U/ml) after 4 days of incubation

Keywords: screening, transglutaminase, *Streptomyces,* media composition

1. Introduction

Microbial transglutaminase (MTGase) is one of the most popular enzymes widely used in food industry. This enzyme, known as a *meat glue*, has the ability to form covalent bonds between glutamine and lysine; thereby improving food products' physical properties, including the appearance and the strength of dough, firmness, elasticity, and water-binding capacity (Kieliszek & Misiewicz, 2014). In fish processing industry, MTGase can be used to improve functional properties, particularly the texture of minced meat or surimi-based product (Tammatinna et al., 2007; Norziah et al., 2009).The quality of surimi or minced fish-based products is particularly affected by fish freshness and the type of fish, which are related to its gel forming ability. The use of MTGase has opened opportunities to process other fish, including cultured fished to generate surimi-based products (Chasanah & Fawzya, 2015) as MTGase is able to improve the gelling capabilities of some Indonesian minced freshwater fish.

Most MTGases are produced by the members of *Streptomyces* genus, exemplified by *Streptomyces lydicus* (Faergemand et al., 1997), *Streptoverticillium cinnamoneum* subsp. *cinnamoneum* (Duran et al., 1998), *Streptoverticillium mobaraense* (Kikuchi et al.,

*Corresponding author.
E-mail: nurifawzya@gmail.com

DOI: http://dx.doi.org/10.15578/squalen.v11i1.195

2003), *Streptoverticillium ladakanum* (Tellez-Luis et al., 2004), *Streptomyces netropsis* (Yu et al., 2008), and *Streptoverticillium hygroscopicus* (Aidaroos et al., 2011). It has also been reported to be found in *Bacillus subtilis* spores (Liu et al., 2014).

Increasing fish production in Indonesia has triggered the development of fisheries product processing industries, including those generating surimi-based products and processed minced fish products. This circumstances would increase the demand of MTGase which is used as an aid in fish based product processing. Therefore, studies on MTGase production using local TGase-microorganisms based on local media are expected to decrease the dependency of MTGase import from Japan (personal communication). This research is aimed at screening and selecting the TGase-producing *Streptomyces* on several different medias to obtain the most suitable medium for large-scale MTGase production.

2. Material and Methods

2.1. Streptomyces Isolates

Streptomyces isolates were obtained from two different Culture Collections, i.e. from Hydrology Laboratory, Department of Fisheries, Faculty of Agriculture, Gadjah Mada University (50 isolates) and from Research Centre for Biotechnology, Indonesian Science Institute (LIPI) (178 isolates). 50 isolates from the 1st group were isolated from marine environment (25 isolates) and soil (25 isolates). The 2nd group contains of 178 isolates, isolated from various environments, i.e. soil, peat soil, sediment, and litter.

2.2. Chemicals and Media

The chemicals used for standard and substrates for enzyme activity assay were purchased from Sigma Chemical Co. Ltd. Other chemicals and media were used for microbial cultivation and enzyme production, for the identification of selected Streptomyces, and for assaying enzyme activity.

2.3. Screening of Transglutaminase Producing Streptomyces

Samples from Gadjah Mada University were firstly screened qualitatively using filter paper disc (FPD) method developed by Bourneow et al. (2012) with slight modification. The positive colonies were then quantitatively assayed for their enzyme activity (Grossowicz et al., 1950) with slight modification. Steps for the qualitative test are as follows: FPD was put on the colony of the isolates grown on *International Streptomyces Project-2 (*ISP2) solid media that contains 0.4% glucose, 0.4% yeast extract, 1% malt extract, and 1.5% agar; then, 30 μl of substrates (mixed of 37.5 mM CBZ-Gln-Gly, 125 mM hydroxylamin, and 12.5 mM glutathion in 200 mM citrate buffer, pH 6.0) was dropped on it. Incubation was conducted at 37 °C for 3 hours. Then, 10 μl of $FeCl_3$ 5% in TCA 15% was dropped on FPD, and incubated at the same temperature for an hour. The positive colonies that produce MTGase showed brown color intensity of the FPD.

The screening for samples from LIPI was conducted quantitatively by determining the MTGase activity of the isolates produced in ISP2 liquid medium (Grossowicz et al., 1950). One loop of each colony on solid ISP-2 medium was inoculated into 100 ml erlenmeyer flasks that contain 15 ml ISP medium, and was incubated in a shaker incubator at 30 °C and 125 rpm for 5 days. The culture was then centrifuged; and the cell-free supernatant as crude MTGase was assayed through colorimetric procedure based on hydroxamate formation from N-carbobenzoxy-L-glutaminilglycine (CBZ-glutaminylglycine). *Streptoverticillium ladakanum* NRRL 3191 from Agricultural Research Service, United State Department of Agriculture was used as the positive control. One unit of MTGase activity was defined as the amount of enzyme causing the formation of one μmol of hydroxamic acid per min at 37 °C. L-glutamic acid y-monohydroxamate in a series of concentration was used as the standard curve.

Quantitatively positive samples were confirmed by PCR-detection for their partial MTGase gene. Amplification was done using degenerate primer PTGase4 (5'-TACGGCTGCGTCGGTGTCAC-3') and PTGase5 (5'-GACGGTCGTGATTGCCTCC-3') designed based on the conserved regions among TGase sequences obtained from GenBank. *Streptoverticillium ladakanum* NRRL 3191 was used as positive control. Genomic DNA from each isolate was extracted using Genomic DNA mini Kit (Geneaid), following the manual instruction from its manufacturer. After the amplification process that was carried out in a Gene Amp® PCR System 9700 (Applied Biosystem), the PCR product of the targeted gene was visualized on 1% agarose gel using a BIORAD UV-transluminator and was documented by Gel –Doc apparatus (Biometra).

2.4. Identification of MTGase Producing Isolates

The selected positive MTGase isolate was identified based on 16S-rRNA sequen gene. The amplification was conducted using primer of 27F (5'-AGAGGTTGATCCTGGCTCAG-3') and 1492R (5'-GTTTACCTTGTTACGACTT-3') (Lane et al., 1991),

followed by DNA sequencing. The sequenced data was analyzed at the National Centre for Biotechnology Information (NCBI) server (*http://www.ncbi.nlm.nih.gov*), using Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990). The result was then used to construct a phylogenetic tree using MEGA 6.0 (Tamura et al., 2013).

2.5. Optimization of MTGase Production from Streptomyces

Optimization was carried out to determine both the pH and the temperature of MTGase production in ISP-2 medium and to select the medium with high activity. One loop of fresh isolate cultivated in the ISP-2 solid was inoculated into a starter medium by referring to Tellez Luis et al. (2004), and was incubated at 30 °C and 150 rpm for 5 days. The starter was then transferred into production medium (10%) with the same composition as the starter, which was adjusted for 3 pH values, i.e. 6, 7, and 8. The culture was then incubated at 25, 30, and 35 °C and 150 rpm for 5 days. Furthermore, the crude enzymes were determined daily, based on Grossowicz et al. (1950). The condition that produces the highest MTGase activity was then applied to produce MTGase in six different media by referring to several journals as follows:

Medium A (Optimized medium of Tellez Luiz et al., 2004): Peptone 1.05%; Glycerol 5.05%, Yeast extract 0.25%; Na-caseinate 2%; $MgSO_4$ $7H_2O$ 0.05%; KH_2PO_4 0.2%; and Na_2HPO_4 0.5%.

Medium B (Optimized medium of Bahrim et al. 2010 with slightly modification): Peptone 1.5%, $MgSO_4$ $7H_2O$ 0.1%; KH_2PO_4 0.5%; and Na_2HPO_4 0.5%, soybean powder 2%, potato starch 2%, and glucose 1.5%.

Medium C (Initial medium of Tellez-Luiz *et al.,* 2004): Peptone 1.05%; Glycerol 3.12%, Yeast extract 0.25%; Na-caseinate 3.84%; $MgSO_4$ $7H_2O$ 0.05%; KH_2PO_4 0.2%; and Na_2HPO_4 0.5%, and soluble starch 2%.

Medium D (Optimized medium of Macedo et al., 2007): Peptone 1%; Yeast extract 0.2%; $MgSO_4$ $7H_2O$ 0.2%; KH_2PO_4 0.4%; and Na_2HPO_4 0.5%, soybean powder 2.5%, potato starch 2%, and glucose 0.2%.

Medium E (Aidaroos et al., 2011): Peptone 2%; Yeast extract 0.5%; $MgSO_4$ $7H_2O$ 0.2%; KH_2PO_4 0.2% and K_2HPO_4 0.2%, ammonium sulfate 2%, soluble starch 2%, and $CaCl_2$ 0.1%.

Medium F (Bourneouw et al., 2012): Peptone 2%; Yeast extract 0.2%; $MgSO_4$ $7H_2O$ 0.2%; KH_2PO_4 0.2%; and K_2HPO_4 0.2%, and soluble starch 2%.

First, the starter of the selected *Streptomyces sp.* was prepared using each medium. Then, each of them was transferred (10%) to a 50 ml medium in 200 ml flask for MTGase production. Incubation was done at 125 rpm for 5 days in a shaking incubator, and sampling was carried out daily. Each sample was centrifuged at 4 °C, 8000 g for 15 minutes, and the MTGase activity of the supernatant was determined.

3. Results and Discussion

3.1. Screening of MTGase Producing Streptomyces

In this research work, 50 *Streptomyces* isolates provided by Gadjah Mada University (UGM) were qualitatively screened for their ability to produce transglutaminase. The screening result showed that 9 isolates growing on solid media exhibit MTGase activity. However, the MTGase activity was not

Table 1. List of *Streptomyces* isolates that exhibited MTGase activity qualitatively

No	Isolate code	Source of isolate
1	G-2 DC (24)	Soil
2	C21NE-ID_2	Soil
3	7-2 DC $(4)_1$	Soil
4	7-2 DC $(4)_2$	Soil
5	7-2 S $(26)_1$	Soil
6	CG-1 II A ISP	Marine
7	TSB 9 AIA	Marine
8	DO-2 II B AIA	Marine
9	DO-2 II A AIA	Marine

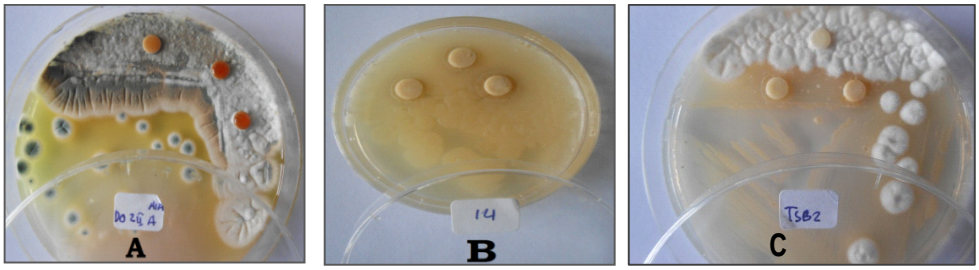


Figure 1. Qualitative test for MTGase-producing *Streptomyces* by filter paper disc (FPD) method: (A) positive, showed by brown colour; (B and C) negative.

detected in those cultivating in liquid medium for 3-7 days (Table 1 and Figure 1).

On the other hand, the qualitative screening of 178 *Streptomyces* isolates provided by Indonesian Science Institute (LIPI) showed that 10 of them exhibit relatively high MTGase activity in the range of 0.23 to 3.89 U/ml after being incubated in liquid medium for 3-7 days (Table 2). The highest activity was observed on isolate TTA 02 SDS 14 derived from Mount Tambora Savana, Bima, West Nusa Tenggara. However, the activity is slightly lower compared to the reference strain, *Streptoverticillium ladakanum* NRRL 3191 (5.85 U/ml). This reference strain had been used in the previous works at CRDMFPCB for local media development in MTGase production as well as for the production of restructured fish meat (Zilda et al., 2011; Fawzya et al., 2011).

PCR-amplification was conducted to confirm the presence of partial transglutaminase gene on 10 positive isolates. Among the 10 isolates, only TTA 02 SDS 14 exhibited the targeted PCR product of around 470 bp, similar to the size shown by positive control *S. ladakanum* (Figure 2). Therefore, TTA 02 SDS 14 isolate was selected for identification and further studies. The analysis of partial MTGase gene sequence showed that it shares 93% similarity (at the nucleotide sequence) with that from *Streptomyces cinnamoneous* as reported by Duran et al. (1998).

3.2. Identification of Streptomyces TTA 02 SDS 14 Based on Sequence Analysis of 16S rRNA

Analysis of 16S-rRNA *Streptomyces* TTA 02 gene showed that it belongs to Actinobacteria family. Furthermore, blast search showed that it is closely related (99% similarity) with *Streptomyces thioluteus,* as presented in Figure 3 (GenBank accession no. HQ853022.1). *S.thioluteus* was firstly reported by Okami in 1952 and is also known as "*Streptomyces thioluteum*" Okami 1952 and "*Verticillomyces thioluteus*" Shinobu, 1965 (LPSN, 2016). This strain was previously reported to produce aureothin, a nitro

Table 2. *Streptomyces* isolates which exhibited MTGase activity quantitatively

Isolates	MTGase activity U/ml
***S. ladakanum* NRRL3191 (control +)**	**5.85**
GKRL 5	1.08
GKRL 11	0.39
GKRL 7	0.23
TSA 03 SDS 12	1.07
TTA 02 SDS 14	**3.89**
TCA 01 SDS 17	0.48
CL1 04 RC2	0.46
ID 04 561	0.99
ID 04 509	0.34
ID 04 677	0.3

Note: Isolate producing the highest MTGase activity, and was selected for further work.

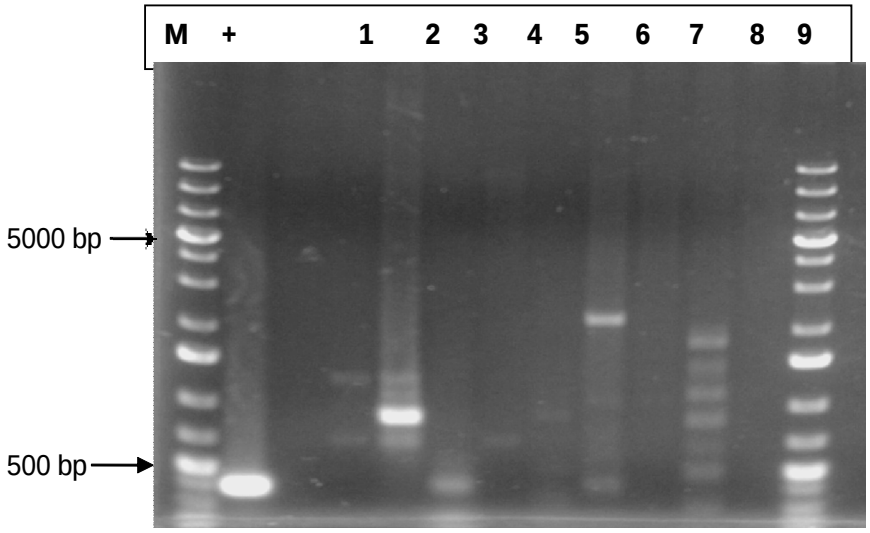


Figure 2. The PCR product of 10 isolates amplified using a MTGase-targeting primer pair.

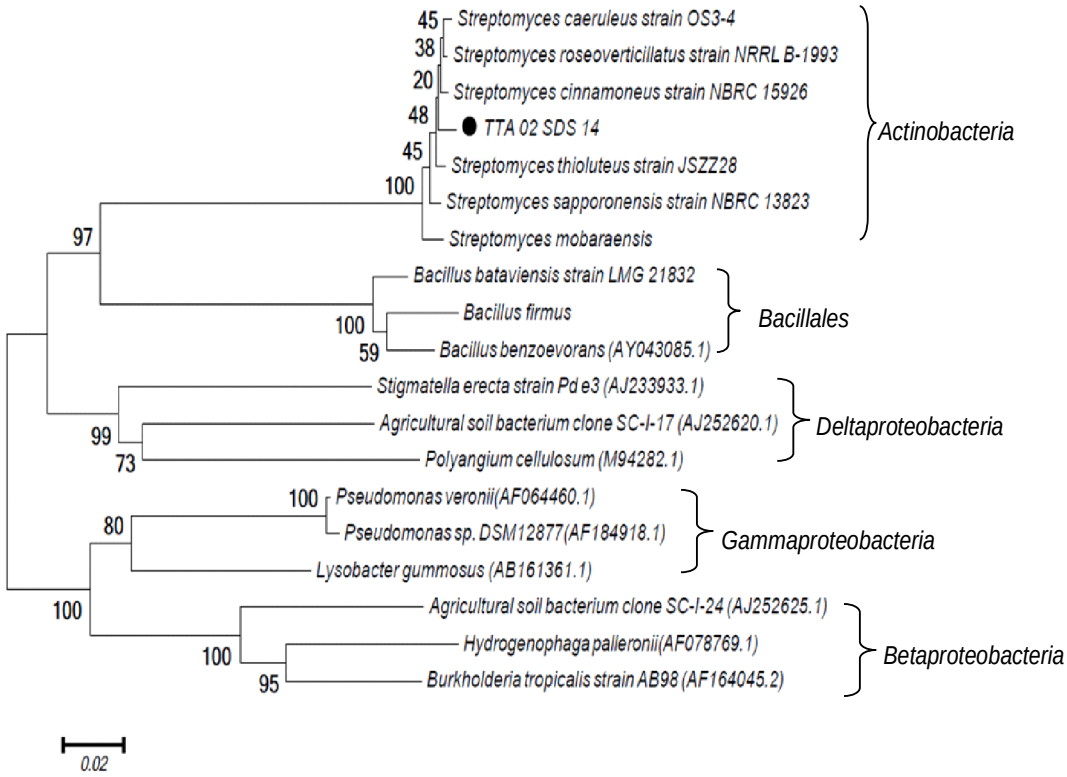
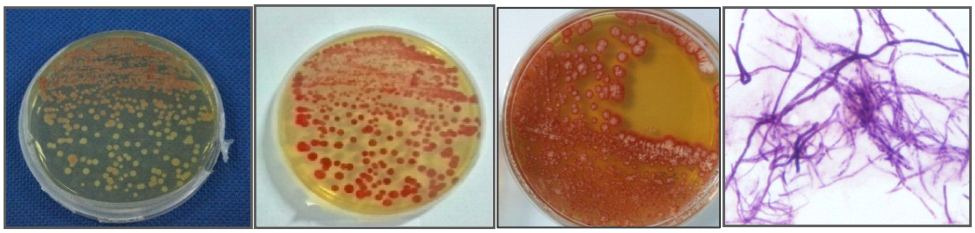


Figure 3. Phylogenetic analysis of the 16S-rRNA gene of TTA 02 SDS14 showing its relationship with representative members of various main bacterial groups.

group-containing antibiotics and aureothricin (Hirata et al., 1961 in Kou-San Ju & Parales, 2010; Whitman, 2012). However, the information about MTGase produced by this isolate has not been reported. Among many bacteria that are capable of producing MTGase, the members of *Streptomyces* exhibit significantly higher yield of MTGases compared to other bacterial groups. High activity of MTGases were particularly found in *S. platensis* (1.0 U/ml) with increased activity of up to 5.7 U/ml after being cloned in *S. lividans* (Lin et al., 2006), and up to *5.3* U/ml was found in a recombinant MTGase of *Yarrowia lipolytica* from

*Streptomyces hygroscopicus (*Liu et al., 2015*)*. Transglutaminase activity varies depending on strain, culture medium, and cultivating condition. These strains have been used for industrial MTGase producer (Zhang et al., 2009).

TTA 02 SDS 14 isolate grew well on ISP 2 medium, characterized by red pigment on the colony at 2-3 days incubation, and subsequently with white aerial mycelium observed at 8 days of incubation. The strain exhibited morphological characteristics typical to Streptomyces genus. Whitman (2012) stated that *Streptomyces thioluteus* grows well on several media, such as potato-glucose agar, inorganic salt-starch agar, glucose asparagine agar (ISP5 medium with 1% glucose replacing glycerol), yeast extract-malt extract agar, etc. On ISP5 medium, the strain produced yellow to brown yellow pigment and yellowish poor aerial mycelium. Meanwhile, brown color with yellowish aerial mycelium was observed on the TTA 02 SDS 14 colonies cultivated on yeast extract-malt extract agar. However, there has been no previous report on its growth on the ISP2 medium containing glucose, yeast extract, and malt extract. Gram staining showed that the isolate is Gram positive, non motil, and filamentous. It formed cylindrical shapes that stick with each other like a chain, and produced hyphae (Figure 4).



a b c d

Figure 4. Phenotype of TTA 02 SDS 14 on solid ISP2 medium identified in this work as *Streptomyces thioluteus* at different incubation times (a: 3 days; b: 4 days; c: 8 days). The cell morphology magnified at 100x (d).

3.3. Optimization of MTGase Production from Streptomyces thioluteus TTA 02 SDS 14

There are several environmental factors that affect cell growth and product formation, including temperature, pH, nutrient composition, dissolved oxygen, etc. The observation on the effect of temperature and pH on MTGase production during TTA 02 SDS 14 cultivation showed that the highest activity (0,115 ± 0.003 U/ml) was found on the cultivation condition at 25 °C and pH 6 for 4 days (Figure 5). At 30°C, the MTGase activity was still high, up to 5-days incubation. However, the optimal activity at this temperature is lower than that at the temperature of 25 °C. Members of *Streptomyces* mostly grow at 10-37 °C temperature (Deeble et al., 2005 in Hasani et al., 2014), except a few species that belong to thermophile, which show good growth at 45-55 °C. They also need less moisture than other bacterial genera, and are very sensitive to wet conditions, like muddy soil.

Idris et al. (2010) stated that *Actinomycetes* commonly grow at pH 5.5 to 9.5. Meanwhile, Kontro et al. (2005) found that *Actynomycetes* grow well in broad pH range, depending greatly on nutrients. Rich medium with high organic content allows growth over ~~a~~ wide pH range. Regarding MTGase production, Meiying et al. (2002) found that the two-step fermentation of *S. mobaraense* by shifting the pH from 7.0 to 6.5 results in higher MTGase activity than fermentation at a constant pH.

3.4. MTGase activity of Streptomyces thioluteus TTA 02 SDS 14 Cultivated in six Different Media

The effect of media composition on the MTGase activity produced by *Streptomyces thioluteus* TTA 02 SDS 14 is presented in Figure 6. Among six media used to cultivate *Streptomyces sp.* TTA 02 SDS 14 for MTGase production, medium B developed by Bahrim et al. (2010) resulted in the highest activity at 4 days of incubation. The MTGase activity reached 0.145 ± 0.014 U/ml (Figure 5). The other media, except medium F, showed the highest MTGase activity at 4 days of incubation. Medium F reported by Bourneouw et al. (2012) achieved the highest MTGase activity at 3 days of incubation. Medium B is particularly rich of carbon and nitrogen sources that contribute to the high growth of the isolate. Further, this influenced the high yield and activity of MTGase. Medium B contains peptone and soybean powder as its nitrogen source as well as glucose and potato starch as its carbon

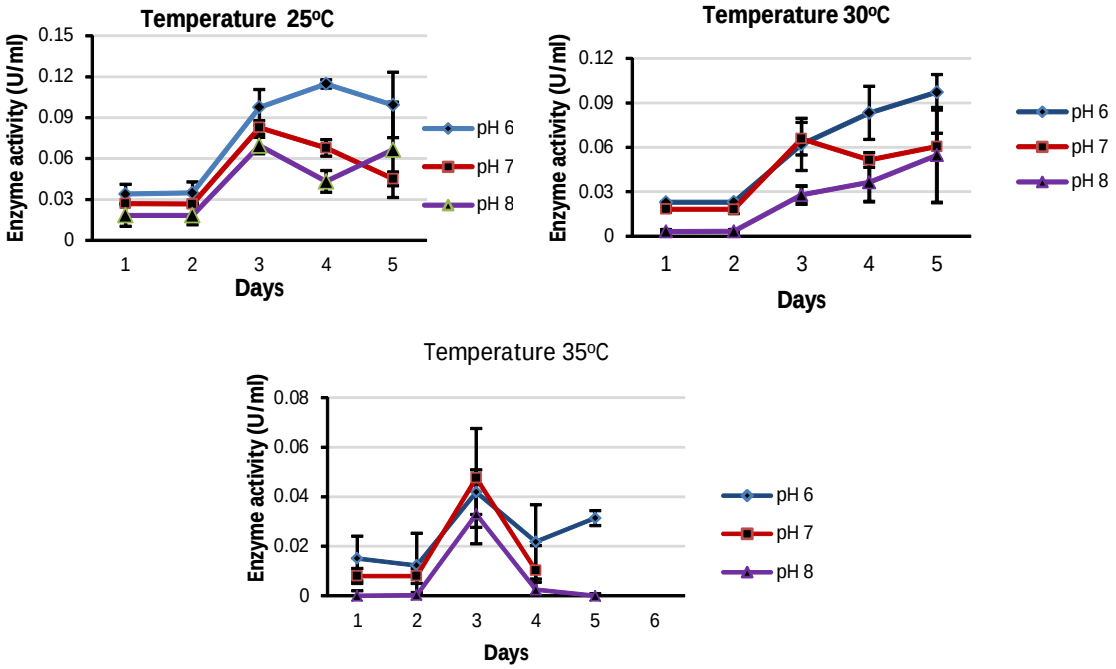
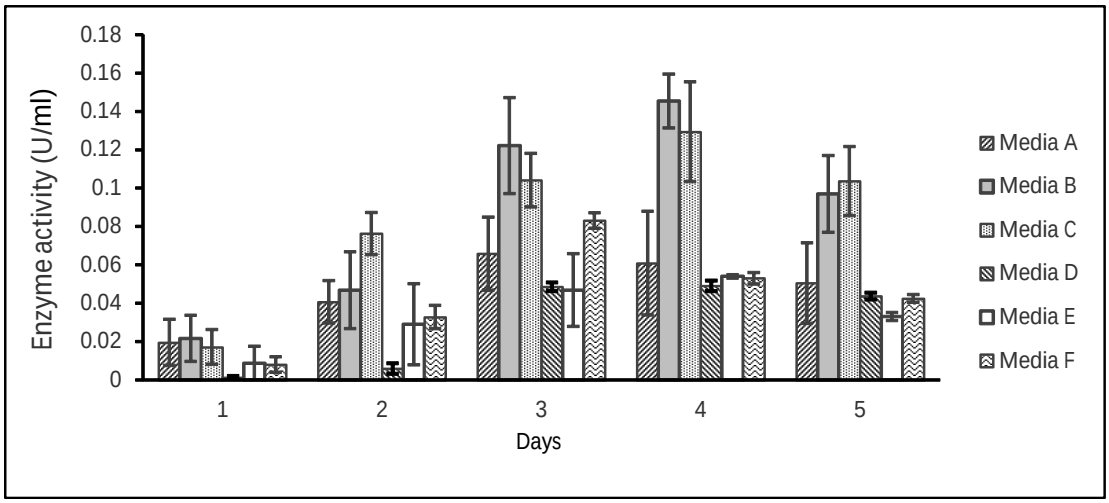


Figure 5. MTGase production of *Streptomyces thioluteus* TTA 02 SDS 14 at various pH and temperatures.



Note: A: Optimization Tellez-Luiz et al., 2004; B: Optimization Bahrim et al. 2010 with slightly modification; C: Initial Tellez-Luiz et al., 2004, D: Optimization Macedo et al., 2007, E: Aidaroos et al., 2011, F: Bourneouw et al., 2012.

Figure 6. Effect of media composition on MTGase activity of *Streptomyces thioluteus* TTA 02 SDS 14.

source, in which the total concentration is relatively higher than that in other media. Similar result was also reported by Yan et al. (2005) and Bahrim et al. (2010). The effect of medium composition on cell growth and product formation was also observed in microbial natural colorants (Palanichamy et al., 2011; Santos-Ebinuma et al., 2013). Other factors that may affect microbial growth and their metabolite products are salt types or trace elements and their concentrations and process conditions, including pH, temperature, dissolved oxygen, etc. Zhang et al. (2009) found that transglutaminase secretion is also associated with *Streptomcyes* differentiation.

MTGase activation mechanism in *Streptomyces* has been investigated by Pasternack et al. (1998). *Streptomyces* transglutaminase is initially secreted as a pro-transglutaminase that could subsequently be activated by several exogenous proteases. Furthermore, Zotzel et al. (2003) found that endogenous protease could also activate *S. mobaerensis* MTGase. According to Zhang et al. (2009), transglutaminase is involved in *Streptomyces*

differentiation, in which it is secreted and activated during the differentiation event. As revealed in this study, the TTA 02 SDS 14 cultivated in liquid ISP2 medium for 5 days exhibited higher MTGase activity compared to that cultivated in the same medium for shorter days of incubation (data was not shown). At 5 days of incubation, cell differentiation on solid media is indicated by the formation of aerial mycelium that may appear floccose (Ambarwati et al., 2012).

4. Conclusion

Among the ten Indonesia *Streptomyces* isolates selected based on their ability to produce MTGase quantitatively, the isolate TTA 02 SDS 14 derived from Mount Tambora Savana in Bima, West Nusa Tenggara, exhibited the highest MTGase activity. This activity was verified at genetic level through partial PCR-amplification of its MTGase gene. The 16S rRNA gene analysis showed its identity as *Streptomyces thioluteus*. Cultivation of this MTGase-producing isolate in a liquid medium developed by Tellez-Luiz et al. (2004) resulted in optimal MTGase production at 25 oC and pH 6. Among six different media used in MTGase production, medium B developed by Bahrim *et al* (2010) showed the highest activity. This medium contains pepton (1.5%), $MgSO_4.7H_20$ (0.1%), KH_2PO4 (0.5%), Na_2HPO_4 (0.5%), soybean powder (2%), potato starch (2%), and glucose (1.5%). Using medium B, the MTGase activity reached the highest level (1.45 U/ml) at 4 days of incubation.

Acknowledgement

The authors thank Yudi Kusnadi for his assistance in the qualitative screening of UGM's isolates. The authors also appreciate Dr. Agus R. Uria for improving the English language.

References

Aidaroos HI, Du G, & Chen J. (2011). Microbial fed-batch production of transglutaminase using ammonium sulphate and calcium chloride by *Streptomyces hygroscopicus*. *Biotechnol. Bioinforment. Bioeng*. 1(2),173–178.

Ambarwati, A., Sembiring, L., & Soegihardjo, C. (2012). Antibiotic produced by streptomycetes associated with rhizosphere of purple nut sedge (*Cyperus rotundus L*.) in Surakarta, Indonesia. *African Journal of Microbiology Research*, 6(1), 52-57.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990). Basic local alignment search tool. *J. Mol. Biol.* 215, 403-410.

Bahrim, G., Lancu, C., Butu, N., & Negoita, T.G. (2010). Production of a novel microbial transglutaminase using *Streptomyces sp.* polar strains. *Romanian Biotechnol. Lett.* 15(2), 5197-5203.

Bourneow, C., Benjakul, S., & H-Kittikun A. (2012). Hydroxamate-based colorimetric method for direct screening of transglutaminase-producing bacteria. *World J. Microb Biot*. 28, 2273–2277.

Chasanah, E. & Fawzya, Y.N. (2015). *Production and Application of Microbial Transglutaminase to Improve Gelling Capabilities of Some Indonesian Minced Fish.* In Seafood Science: Advances in Chemistry, Technology and Applications (Ed. Kim). CRC Press. Boca Raton. 577pp.

Lin, Yi-Shin; Chao, Mei-Li; Liu, Chang-Hsiesh; Tseng, M.; Lin, Shie-Jea; & Chu. Wen-Shen. (2006). Transglutaminase. US Patent No. 7179628 B2.

Duran, R., Junqua, M., Schmitter, J.M., Gancet, C., & Goulas P. (1998). Purification, characterisation, and gene cloning of transglutaminase from *Streptoverticillium cinnamoneum* CBS 683.68. Biochimie 80, 313–319.

Færgemand, M., J. Otte, & K.B. Qvist. (1997). Enzymatic cross-linking of whey proteins by a Ca^{2+}-independent microbial transglutaminase from *Streptomyces lydicus. Food Hydrocolloids* 11(1), 19-25.

Fawzya,Y.N., Puruhita, TKA., Gunawan & Patantis, G. (2011). Effect of salt and transglutaminase on the physical and sensory of restructured *Priacanthus macracanthus. Journal of Marine and Fisheries Post Harvest and Biotechnology* (In Indonesian), 4(1), 69-78.

Grossowicz, N., Wainfan, E., Borek, E., & Waelsch, H. (1950). The enzymatic formation of hydroxamic acids from glutamine. *J. Biol. Chem.,* 187, 111-25.

Hasani, A., Kariminik, A., & Issazadeh, K. (2014). Streptomycetes: Characteristics and their antimicrobial activities. *International Journal of Advanced Biological and Biomedical Research* 2(1), 63-75.

Idris, A., Saed, K. & Hung, Yung-Tse. (2010). Biotreatment of Sludge and Reuse. In *Environmental Bioengineering* (Eds. Wang, L.K., Tay, Joo-Hwa, Tay, S.T.L. and Hung, Yung-Tse). Humana Press. Springer Science, New York. p165-189.

Kieliszek, M. & Misiewicz, A. (2014). Microbial transglutaminase and its application in the food industry. A review. Folia Microbiol (Praha); 59(3), 241–250. DOI:10.1007/s12223-013-0287-x.

Kikuchi, Y., Date, M., Yokoyama, Kei-ichi, Umezawa, Y., & Matsui, H. (2003). Secretion of Active-Form *Streptoverticillium mobaraense* Transglutaminase by *Corynebacterium glutamicum*: Processing of the Pro-Transglutaminase by a Cosecreted Subtilisin-Like Protease from *Streptomyces albogriseolus. Appl. Environ. Microbiol.* 69 (1), 358-366. DOI: 10.1128/AEM.69.1.358-366.2003.

Kontro ,M., Lignell, U., Hirvonen, M.R., & Nevalainen, A. (2005). pH effects on 10 Streptomyces spp. growth and sporulation depend on nutrients. *Lett. Appl. Microbiol.* 41(1), 32-8.

Kou-San, Ju & Parales, R.E. (2010). Nitroaromatic Compounds, from Synthesis to Biodegradation. Microbiol Mol Biol Rev. 74(2),250–272. DOI: 10.1128/ MMBR.00006-10.

Lane, D.J. (1991). 16/23S-rRNA sequencing: In Stackebrandt, E. and Goodfellow, M. (Eds). *Nucleic acid techniques in bacterial systematics*. John Wiley and Sons, Chichester, New York (US), Brisbane, Toronto and singapore. 115-175.

Lin, Y.S., Chao, M. L., Liu, C.H., Tseng, M., & Chu, W.S. (2006). Cloning of the gene coding for transglutaminase from *Streptomyces platensis* and its expression in *Streptomyces lividans*. Process Biochem. 41, 519–524. DOI:10.1016/j.procbio.2005. 09.009.

Liu, S., Wan, D., Wang, M., Madzak, C., Du, G. & Chen, J. (2015). Overproduction of pro-transglutaminase from *Streptomyces hygroscopicus* in *Yarrowia lipolytica* and its biochemical characterization. *BMC Biotechnology* 15:75. DOI:10.1186/s12896-015-0193-1.

Liu Y., Lin, S., Zhang, X., Liu, X., Wang, J., & Lu, F. (2014). A novel approach for improving the yield of *Bacillus subtilis* transglutaminase in heterologous strains. *J Ind Microbiol Biotechnol.* 41(8),1227-35. DOI: 10.1007/s10295-014-1468-6.

LPSN. (2016). List of Prokaryotik Names with Standing in Nomenclature.*http://www.bacterio.net/streptomycesc.html.*Accessed in April 2016.

Macedo, J. A., Sette, L. D., & Sato, H. H. (2007). Optimization of medium composition for transglutaminase production by a Brazilian soil *Streptomyces* sp. *Electronic Journal of Biotechnology*, *10*(4), doi:10.2225/vol10-issue4-fulltext-10.

Meiying, Z., Guocheng, D., & Jian, C. (2002). pH control strategy of batch microbial transglutaminase production with Streptoverticillium mobaraense. *Enzyme and Microbial Technology* 31, 477–481.

Norziah, M.H., Al-Hassan, A., Khairulnizam, A.B., Mordi, M.N., & Norita, M. (2009). Characterization of Fish Gelatin From Surimi Processing Wastes Thermal Analysis and Effect of Transglutaminase on Gel Properties. *Food Hydrocolloids* 23(6), 1610-1616.

Palanichamy, V., Hundet,A., Mitra, B. & Reddy, N. (2011). Optimization of cultivation parameters for growth and pigment production by *Streptomyces* spp. Isolated from marine sediment and rhizosphere soil. *International Journal of Plant, Animal and Environmental Sciences* 1(3), 158-170. Available online at *www.ijpaes.com.*

Pasternack, R., Dorsch, S., Otterbach, J., Robenek, I., Wolf, S., & Funchsbauer, H.L. (1998). Bacterial pro-transglutaminase from *Streptoverticillium mobaraense*, purification, characterization and sequence of the zymogen. *European Journal of Biochemistry, 257*, 570–576. DOI:10.1046/j.1432-1327.1998.2570570.x.

Santos-Ebinuma, V.C., Teixeira, M.F., & Pessoa, A. J.r. (2013). Submerged culture conditions for the production of alternative natural colorants by a new isolated *Penicillium purpurogenum* DPUA 1275. *J Microbiol. Biotechnol*. 23(6), 802-810.

Tammatinna, A., Benjakul, S., Visessanguan, W., & Tanaka, M. (2007). Gelling properties of white shrimp (*Penaeus vannamei*) meat as influenced by setting condition and microbial transglutaminase. *LWT-Food Technology* 40, 1489–1497.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Mol. Biol. and Evol*. 30, 2725-2729.

Téllez-Luis, S.J., Ramírez, J.A., & Vázquez, M. (2004). Production of transglutaminase by *Streptoverticillium ladakanum* NRRL-3191 using glycerol as carbon source. *Food Technol. Biotechnol. 42,* 75–81.

Whitman, W.B. (2012). *Bergey's Manual of Systematic Bacteriology* 2nd ed., Volume 5: The Actinobacteria part A and B. Springer. New York. 2031pp. DOI 10.1007/978-0-387-68233-4.

Yan, G., Du,Y., Li,J., Chen, J., & Zhong. (2005). Enhancement of microbial transglutaminase production by Streptoverticillium mobaraense: Application of a two-stage agitation speed control strategy. *Process Biochem.* 40, 963-968.

Yu, Y.J., Wu S.C., Chan H.H., Chen Y.C., Chen Z.Y., & Yang M.T. (2008). Overproduction of soluble recombinant transglutaminase from *Streptomyces netropsis* in *Escherichia coli*. *Appl Microbiol Biotechnol*. 81, 523–532. DOI: 10.1007/s00253-008-1688-7.

Zhang, D., Zhu, Y. & Chen, J. (2009). Microbial Transglutaminase Production: Understanding the Mechanism. *Biotechnology and Genetic Engineering Reviews*, 26:1, 205-222, DOI: 10.5661/bger-26-205

Zilda, D.S., Patantis, G., Kristiana, I., & Fawzya, Y.N. (2011). Utilization of liquid waste of tofu and cassava starch for production of transglutaminase from *Streptoverticillium ladakanum* (In Indonesian). Paper presented at National Seminar on Marine and Fisheries Product Processing and Biotechnology at Jakarta, 30 August 2011.

Zotzel, J., Pasternack, R., Pelzer, C., Ziegert, D., Mainusch, M., & Fuchsbauer, H.L. (2003). Activated transglutaminase from Streptomyces mobaraensis is processed by a tripeptidyl aminopeptidase in the final step. *Eur. J. Biochem.* 270(20), 4149-55.