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RESEARCH ARTICLE

ISOLATION AND CULTIVATION OF TRANSGLUTAMINASE-PRODUCING BACTERIA FROM SEAFOOD PROCESSING FACTORIES

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Abstract

Microbial transglutaminase (MTGase) is a transferase that catalyzes the acyl transfer reaction. MTGase has been applied to many protein containing foods to improve their textural property. Screening of MTGase producing microorganisms from various sources might lead to the discovery of novel MTGase with different characteristics. In this study, MTGase producing microorganisms were screened from soils, wastewater and floc-floating on wastewater from seafood processing factories by the filter paper disc (FPD) assay. The MTGase-positive colonies were confirmed by the hydroxamate assay in SPY medium. 95% of 312 FPD positive isolates showed the MTGase activity by hydroxamate assay in the SPY medium after cultivation at 37˚C for 36 h. Based on the assay, 15.86% of the tested colonies were considered as high MTGase activity colonies. The MTGase positive isolates, C1112, C1714 and C2361 were identified by 16s rDNA as neighbor of *Providencia stuartii*, *Providencia alcalifaciens* and *Enterobacter cloacae* with the similarity at 99.2, 97.3 and 99.9%, respectively. *Enterobacter* sp. C2361 and *Providencia* sp. C1112 were selected to cultivate in four media to compare the growth and MTGase productivity with *Streptoverticillium mobaraense* DSMZ 40847. The SPY medium was the most effective medium for production of MTGase by *Enterobacter* sp. C2361 and *Providencia* sp. C1112 with the activity of MTGase at 0.77±0.04 and 0.92±0.02 U/ml, respectively.

Key words: *Enterobacter* sp., microbial transglutaminase, MTGase, *Providencia* sp.

Introduction

Transglutaminase (TGase) is accounted as protein glutamine γ-glutamyl transferase (EC 2.3.2.13) (Motoki & Seguro, 1998). The TGase catalyzes the formation of isopeptide and leads to the cross-linking of protein via formation of ε-(γ-glutamyl) lysine linkages. In the absence of primary amine, water may replace it as the acyl acceptor to form glutamic acid (Motoki and Seguro, 1998). TGase could be found in various organisms including mammal, avian, fish, plant, invertebrates, and microorganisms (Ichinose *et al.*, 1990; Greenberg *et al.*, 1991; Duran *et al.*, 1998). TGase obtained from microorganisms, microbial TGase (MTGase) is independent to Ca^{2+} in catalytic activity (Ando *et al.*, 1989). Recently, MTGase has captured people interest due to its attractive potential application in food industries (Zhu *et al.*, 1996; Motoki and Seguro, 1998), immobilization of enzymes (Josten *et al.*, 1999) and textile industries (Cortez *et al.*, 2004). MTGase is an important enzyme on cell elongation, cell division, spore germination, formation of cilia and flagella of some microorganisms (Zhu *et al.*, 1996; Goffin and Ghuysen, 1998; Kobayashi *et al.*, 1998; De Souza *et al.*, 2009; Iancu *et al.*, 2009). In addition, MTGase could catalyze the transamidating of slime mold myosin thick filament (Hussain *et al.*, 2001). Therefore, discovery of novel MTGase with high activity still is of interest.

The MTGase could be produced by many microorganisms isolated from various environmental sources. The MTGase from *Streptoverticillium mobaraense* and some of

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Streptomyces was elucidated for scaling up to trade in food industry (Ando *et al.*, 1989). MTGase of *Streptoverticillium* was screened for the ability to produce transglutaminase using the hydroxamate assay (Ando *et al.*, 1989). These bacteria excreted MTGase, and one of the strains with the high MTGase activity was the variant of *Sv. mobaraense* (Washizu *et al.*, 1994). After that decade, MTGase was also reported in culture of *Streptomyces* sp. (Neilson, 1995). MTGase could be highly produced by *Bacillus substilis* (Kobayashi *et al.*, 1998), *Sv. cinnamoneum* (Duran *et al.*, 1998), *Sv. ladakanum* (Ashie and Lanier, 2000), *Streptomyces fradiae* (Liu *et al.*, 2007), *Strep. hygroscopicus* (Cui *et al.*, 2007), *Strep. lividans*, *Strep. platensis* (Lin *et al.*, 2006), *Pseudomonas putida, P. amyloderamosa, P. palleroni, Zygomonas mobilis,* and *Hafnea alvei* (Bech *et al.*, 2002).

Media compositions affected MTGase production by microorganisms (Zheng *et al.*, 2001; Zheng *et al.*, 2002a; Zheng *et al.*, 2002b). It is also important to reduce the cost of the medium as this may affect the overall process economics (Souza *et al.*, 2006). The carbon and nitrogen sources are main components affecting MTGase productivity. Of all nitrogen sources, peptone was the most promising for MTGase production by *Streptomyces* sp. P20 and *Sv. mobaraense* (Zhu *et al.*, 1996; Macedo *et al.*, 2007). The carbon sources for high production of MTGase by *Sv. mobaraense* were the mixture of potato starch, glucose and maltodextrin (Macedo *et al.*, 2007). The combination of sugar cane molasses and glycerol showed synergistic effect on the production of MTGase by *Sv. ladakanum* (Téllez-Luis *et al.*, 2004). The consumption of carbon sources in the cultivation of *Sv. ladakanum* was different for sugars (glucose, fructose and saccharose) and glycerol (Téllez-Luis *et al.*, 2004). On the other hands, using of glycerol and casein in the medium had the benefit for the production of MTGase by *S. ladakanum* (Téllez-Luis *et al.*, 2004). However, the cross-linking of peptides in the media could be catalyzed by MTGase. Therefore, free amino acids were probably limited for further growth and MTGase synthesis (Xie and Wang, 1994; Zhu *et al.*, 1996). It results in retardation of microbial growth and affects MTGase production. However, the previous works are focused on media for production of MTGase by *Sv. mobaraense*, *Sv. ladakanum*, and *Streptomyces* sp. Therefore, this study aimed to screen the medium formulation for cultivation of MTGase producing bacteria isolated from the seafood factories.

Materials and methods

Chemicals and media

Carbobenzoxy glutamine glycine (CBZ-Gln-Gly), hydroxylamine, glutathione (reduced form), trichloroacetic acid (TCA) and hydroxamic acid were purchased from Sigma Chemical Co. Ltd. (St. Louis, MO, USA). Iron (III) chloride, magnesium sulphate, potassium dihydrogen phosphate and dipotassium hydrogen phosphate were obtained from Fluka Chemical Co. Ltd. (Buchs, Switzerland). Nutrient broth, peptone, and yeast extract were purchased from Difco™ (Becton Dickinson and Company, Franklin Lakes, NJ, USA).

Bacterial strain

Sv. mobaraense strain DSM 40847, a positive MTGase-producing strain (Motoki and Seguro, 1998), was purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) (Braunschweig, Germany).

Samples

Seafood processing wastewater and soil samples were collected from 6 seafood (canning, frozen and surimi) processing factories situated in Songkhla province, Thailand. Soil samples were collected from 10-15 cm depth at four positions beside the wastewater treatment ponds. Floc-floating samples on the wastewater were drawn from the pond by a scoop. The samples were kept in sterilized bottles at 4˚C until used but not longer than 24 h.

Qualitative screening of MTGase-producing bacteria

The samples were diluted in 0.85% NaCl, spreaded on nutrient agar (NA) plates and incubated at 37˚C for 24 h. The plates with 20 or less colonies were

replica plated on the NA plate and were incubated at 37˚C for 24 h. One of the replica plates was used for direct detection of MTGase activity by the filter paper disc (FPD) assay (Bourneow *et al.*, 2009). The FPD was placed on each colony and 30 µl of the substrates mixture (37.5 mM CBZ-Gln-Gly, 125 mM hydroxylamine, and 12.5 mM glutathione in 200 mM citrate buffer, pH 6.0) was transferred on the FPD and incubated at 37˚C for 3 h. Thereafter, 10 µl of 5% $FeCl_3$ in 15% TCA was placed on the FPD and incubated at 37˚C for 1 h. The MTGase-positive colony was scored according to the color intensity and the corresponded colony on another plate was restreaked until the pure isolate was obtained.

Quantitative screening of MTGase-producing bacteria

The positive colonies on an agar plate by the FPD assay were quantified based on the extracellular MTGase activity in the culture broth. One loopful of the colony was inoculated into a test tube containing 5 ml of SPY medium (2.0% soluble starch, 2.0% peptone, 0.2% yeast extract, 0.2% Mg_2SO_4, 0.2% KH_2PO_4 and 0.2% K_2HPO_4, pH 6.5) and incubated on a shaker at 150 rpm and 37˚C for 36 h. The cell pellet was removed by centrifugation at 7,500 ×*g* for 10 min at 4˚C. The MTGase activity in the supernatant was analyzed using hydroxamate assay (Folk and Cole, 1965). One unit of MTGase activity was defined as the amount of enzyme producing 1 µmole of hydroxamic acid per min.

Identification of MTGase producing bacteria

The selected MTGase positive isolates were identified by DNA sequence. Firstly, genomic DNA was extracted using Geneaid® Genomic DNA Mini Kit following the manual instruction of the provider (Geneaid Biotech Ltd.). The 16S rDNA was PCR amplified using the 27F(5′-AGAGTTTGATCCTGGCTCAG-3′), 421F(5′-CAGCTCAAGAACCTCACATT-3′) and 1488R(5′-CGGTTACCTTGTTACGACTTCACC-3′) primers (Jadhav *et al.*, 2010). Amplifications were performed with the denaturation at 94°C for 5 min and subsequent by 35 cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec, and extension at 72°C for 2 min followed by final extension at 72°C for 10 min by using a GeneAMP PCR System 2400 thermocycler (Perkin Elmer). The presence of PCR products was determined by electrophoresis of 10 μL of the reaction product in a 1% agarose gel. The PCR products were subjected to the DNA sequencing service of the Central Scientific Equipment Facility, Mahidol University, Thailand. The percent similarity of the resulting DNA sequences was analyzed through BLAST (Altschul *et al.*, 1997) on www.ncbi.nlm.nih.gov/blast.

Selection of medium for MTGase synthesis

The selected isolates were activated in nutrient broth (NB) at 37˚C and shaking at 150 rpm for 12 h. Thereafter, 5% (v/v) of the starter (late logarithmic phase was at 12 h of cultivation in NB) was inoculated into 5 ml of various media which had been used for cultivation of some bacteria for production of MTGase (Table 1).

Table 1. *Media formulations used for production of MTGase*

Medium	Formulation	Reference
A	1.5% soluble starch, 1.5% glucose, 1.5% peptone, 0.4% yeast extract, 0.2% Mg_2SO_4, 0.2% K_2HPO_4 and 0.2% KH_2PO_4, pH 6.8	Macedo *et al.* 2007
B	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, pH 7.0	Zhu *et al.*, 1996
C	1.0% peptone, 0.5% yeast extract, 0.5% NaCl, pH 6.5	Nutrient broth
D	1.7% pancreatic digest of casein, 0.3% papaic digest of soybean meal, 0.5% NaCl, 0.25% K_2HPO_4 and 0.25% glucose, pH 7.3	Téllez-Luis *et al.*, 2004

In this study, Sv. *mobaraense* DSMZ 40847 was used to compare the effectiveness of the media on production of MTGase by the tested isolates which was incubated at 37˚C, shaking at 150 rpm for 60 h. The MTGase activity (Folk and Cole, 1965) and cell density (OD_{660}) were monitored. After that, the selected isolates were cultivated in 100 ml of the most effective medium in 250 ml conical flask

at 37°C, 150 rpm for 60 h. The samples were collected for monitoring of growth (total plate count) and MTGase activity (Folk and Cole, 1965).

Determination of cell density

The sample was centrifuged at 7,500×*g* for 10 min at 4°C to discard the supernatant. The cell pellet was washed twice using 0.85% NaCl. The cell pellet was resuspended with the same volume of 0.85% NaCl and then the cell density was measured at 660 nm.

Growth as viable count

The culture was diluted in 0.85 % by decimal dilution and then 100 µl of sample was spreaded on the NA plate and incubated at 37°C for 24 h.

Statistical analysis

The experiments were carried out in triplicate. Analysis of variance (ANOVA) was performed and means comparisons were run by Duncan's multiple range test. The regression parameters for validation of the FPD method were analyzed at $P<0.05$. Analysis was performed using a SPSS package (SPSS 10.0 for windows, SPSS Inc, Chicago, IL, USA).

Results and discussion

Screening of MTGase producing bacteria

MTGase-producing bacteria were screened and isolated from seafood processing factories in Songkhla province. The samples of soils, floating-floc and wastewater were collected from the treatment ponds of canned seafood, frozen seafood and surimi processing plants. The initial microbial load of samples varied from 5.11 to 7.70 log CFU/g. This variation was caused by the different nutrients in the treatment ponds and the treatment systems. By using FPD assay, 580 colonies were screened and 53.79% (312 colonies) was accounted as MTGase positive colonies; 90, 130 and 92 colonies were scored as 1, 2 and 3, respectively (Table 2).

Table 2. *Primary screening of MTGase-producing bacteria from seafood processing factories by FPD assay on nutrient agar plate*

Sample	Factory	Total plate count	Number of Isolates	Visual score level			
		log CFU/g		0	1	2	3
Soils	Surimi processing A	7.10±0.45	28	15	2	3	8
	Surimi processing B	7.35±0.01	28	12	3	11	2
Waste water	Surimi processing A	6.23±0.39	46	18	8	12	8
	Surimi processing B	5.39±0.08	42	23	2	5	12
	Frozen seafood processing A	5.11±0.32	36	12	2	13	9
	Frozen seafood processing B	5.47±0.01	45	27	2	12	4
	Tuna canning A	5.89±0.07	56	23	7	14	12
	Tuna canning B	5.40±0.11	51	24	4	12	11
Floating-floc	Surimi processing A	7.70±0.51	42	18	12	5	7
	Surimi processing B	6.57±0.02	41	12	23	0	6
	Frozen seafood processing A	7.45±0.20	29	12	3	12	2
	Frozen seafood processing B	6.59±0.03	54	23	4	23	4
	Tuna canning A	6.56±0.05	33	25	0	3	5
	Tuna canning B	6.62±0.03	49	24	18	5	2
Total			580	268	90	130	92

The positive-MTGase isolates by FPD assay were further quantified for the MTGase activity by the hydroxamate assay which was used for final selection of the isolates of interest. Among all of positive colonies, there were 12 isolates obtained as the dominant MTGase producers. The first three isolates with the highest MTGase activity were obtained from the isolates with the score of 3 tested by the FPD assay (Table 3). By the hydroxamate assay, the isolates C1714, C2361 and C1112 provided the MTGase activity of 0.66±0.02, 0.64±0.05 and 0.41±0.02 U/ml, respectively after

cultivation for 36 h in the SPY medium. The isolates C1714 and C1112 were obtained from wastewater of surimi processing, while the isolate C2361 was isolated from the floc-floating of the frozen seafood factory. The three isolates of interest were further subjected to the taxonomical analysis.

Table 3. *MTGase activity of the selected strains collected from various sources*

Ranking	Isolate code	FPD assay (Visual score level)	MTGase activity (U/ml*)	Source	Factory
1	C1714	3	0.66 ± 0.02^{c}	Waste water	Surimi
2	C2361	3	0.64 ± 0.04^{c}	Floc-floating	Frozen seafood
3	C1112	3	0.41 ± 0.02^{bc}	Waste water	Surimi
4	C2121	3	0.40 ± 0.07^{bc}	Waste water	Surimi
5	C2123	3	0.38 ± 0.04^{bc}	Waste water	Frozen seafood
6	B3107	3	0.29 ± 0.00^{b}	Waste water	Tuna canning
7	B3207	2	0.29 ± 0.04^{b}	Floc-floating	Tuna canning
8	A3303	2	0.24 ± 0.02^{b}	Waste water	Frozen seafood
9	C1713	2	0.24 ± 0.02^{b}	Waste water	Surimi
10	C1843	3	0.22 ± 0.08^{b}	Waste water	Surimi
11	C2101	2	0.22 ± 0.02^{b}	Waste water	Frozen seafood
12	B3111	2	0.20 ± 0.07^{b}	Waste water	Tuna canning
13	C1091	0	0.00 ± 0.02^{a}	Floc-floating	Surimi
14	C1101	0	0.00 ± 0.00^{a}	Floc-floating	Surimi
15	B3000	0	0.00 ± 0.00^{a}	Floc-floating	Tuna canning

The different superscripts in the same column denote the significant difference ($P<0.05$).
* ml of the culture after cultivated in SPY medium at 37˚C for 36 h

The number of MTGase-positive isolates was varying among samples depending on sample source, media use, incubation time, and assay methods. The MTGase-positive isolates by the FPD assay could be found in all sample sources. The isolates with MTGase activity higher than 0.20 U/ml were found in the wastewater and floating-floc samples, but not in the soil samples. The soil samples contained bacteria and a vast number of molds. The bacteria grew faster than mold which needs 3-7 days and the SPY medium might not be appropriate for the mold growth. Thus, there was not found mold producing- MTGase in this study.

Identification of selected isolates

The isolates C1112, C2361 and C1714 were identified by 16S rDNA sequencing analysis. The isolate C1112 (GenBank accession no. HQ888847.1) was 99.2% similar to *Providencia stuartii*. The isolate C2361 (GenBank accession no. HQ888848.1) showed 97.3% nearly to *Enterobacter cloacae*. *P. stuartii* and *E. cloacae* were classified as bacteria in the biosafety level 1 (Figure 1) (Li *et al.*, 2007). Therefore, the isolates C1112 and C2361 might be safe for further study. The isolate C1714 showed 99.9% similarity to *P. alcalifaciens* which was classified to be the biosafety level 2 that can cause violent disease in human. However, there is no report on MTGase from *Enterobacter* sp. and *Providencia* sp. The extracellular MTGase could be produced by a large number of microorganisms but difference in amount of the excreted MTGase. The higher MTGase activity could be found in the microorganisms that have to adapt themselves for living in their environments.

Four major families of bacteria were highly producing MTGase including *Streptomycetaceae, Micrococaceae, Bacillaceae* and *Pseudomonadaceae*. The MTGase-dominant bacteria were *Streptomyces lydicus* (1.30 U/ml), *Strep. sioyansis* (3.30 U/ml), *Strep. platensis* (1.40 U/ml), *Rothia dentocariosa* (0.90 U/ml), *Bacillus aneurinolyticus* (0.80 U/ml) and *Pseudomonas putida* (1.40 U/ml) (Bech *et al.*, 2001). The MTGase activity was varied depending on strain, culture medium and cultivating condition. Therefore, the culture medium should be elucidated for each strain.

Selection of medium for MTGase production

Four media were used to cultivate *Enterobacter* sp. C2361, *Providencia* sp. C1112 and *Sv. mobaraense* DSM 40847 for MTGase production. Among those, Medium B or the SPY medium gave the highest specific growth rate (μ_{max}) with 1.73, 1.82 and 1.51 h^{-1} for the growth of *Enterobacter* sp.C2361, *Providencia* sp.C1112 and *Sv. mobaraense* DSM 40847, respectively at 37°C, 150 rpm (Figure 2). Medium A contained two kinds of carbon source, glucose and starch, while Medium B had only soluble starch as the major carbon source which provided MTGase activity higher than Medium A. The result suggested that glucose could affect the growth and MTGase production by glucose inhibitory effect (Portilla-Rivera *et al.*, 2009).

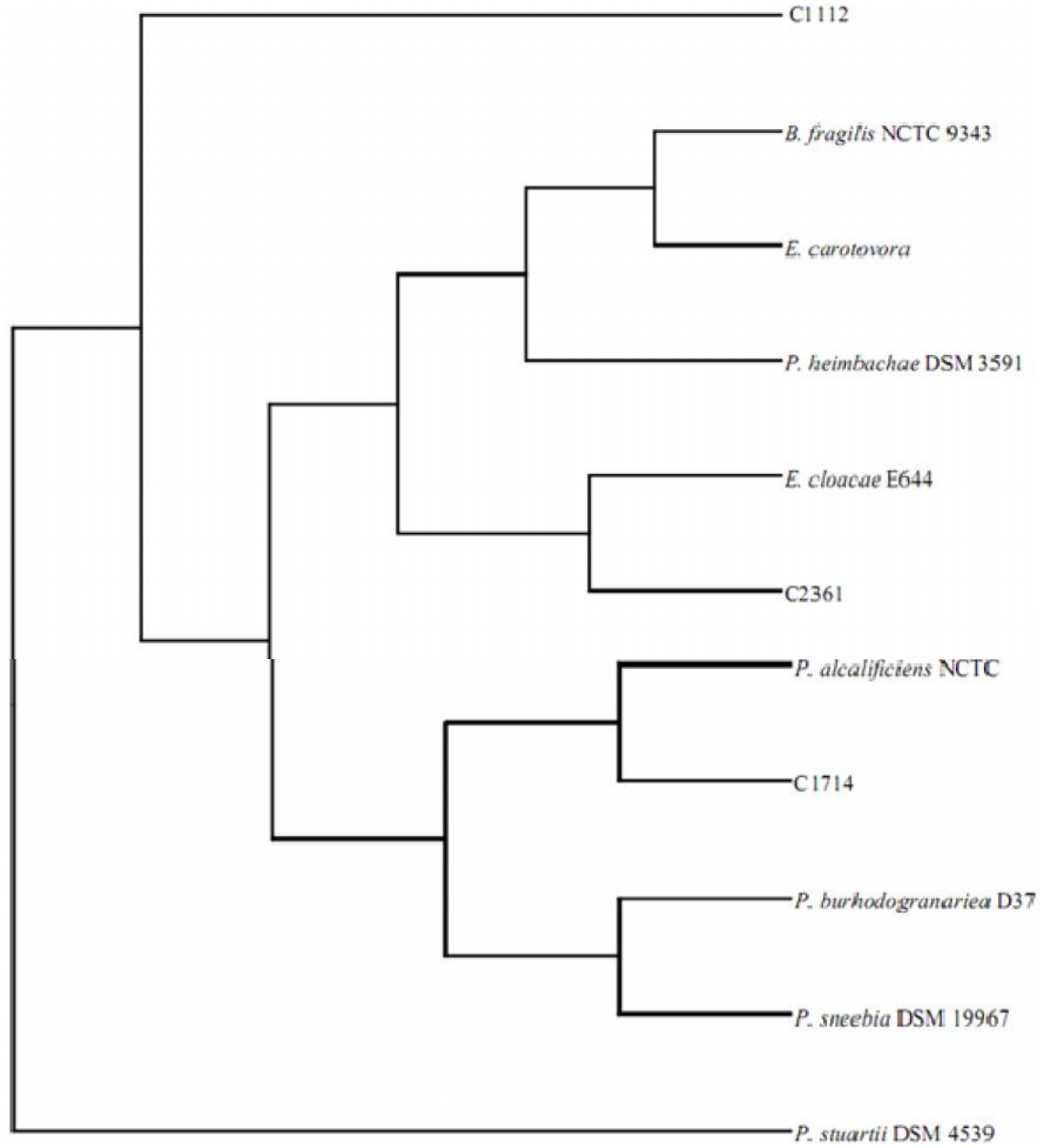

Figure 1. *Phylogenetic tree showing of neighbor link of C1112, C2361, C1714 and their neighbors based on the 16S ribosomal RNA, partial sequence*

Medium C showed the lowest MTGase activity compared to other media. It might be caused by lacking of carbon source. However, in the presence of peptone and yeast extract the tested strains were

able to grow. Medium D contained complex nitrogen sources that could induce the bacteria to excrete some proteases for hydrolysis various proteins in the culture (Kurbanoglu and Kurbanoglu, 2003). Medium B consisted of peptone as a basic nitrogen source which used as a precursor for biosynthesis of MTGase and other proteins for living. Soluble starch possessed both benefit to be used as a carbon source and prevent MTGase from some deteriorative factors. The monitoring of the growth of the tested strains by measuring the optical density could be interfered by soluble starch particles in the Medium A and B although washing step was done before measuring. However, in this study, the MTGase activity was the main purpose, thus, the effective medium for production of MTGase was judged according to the MTGase activity.

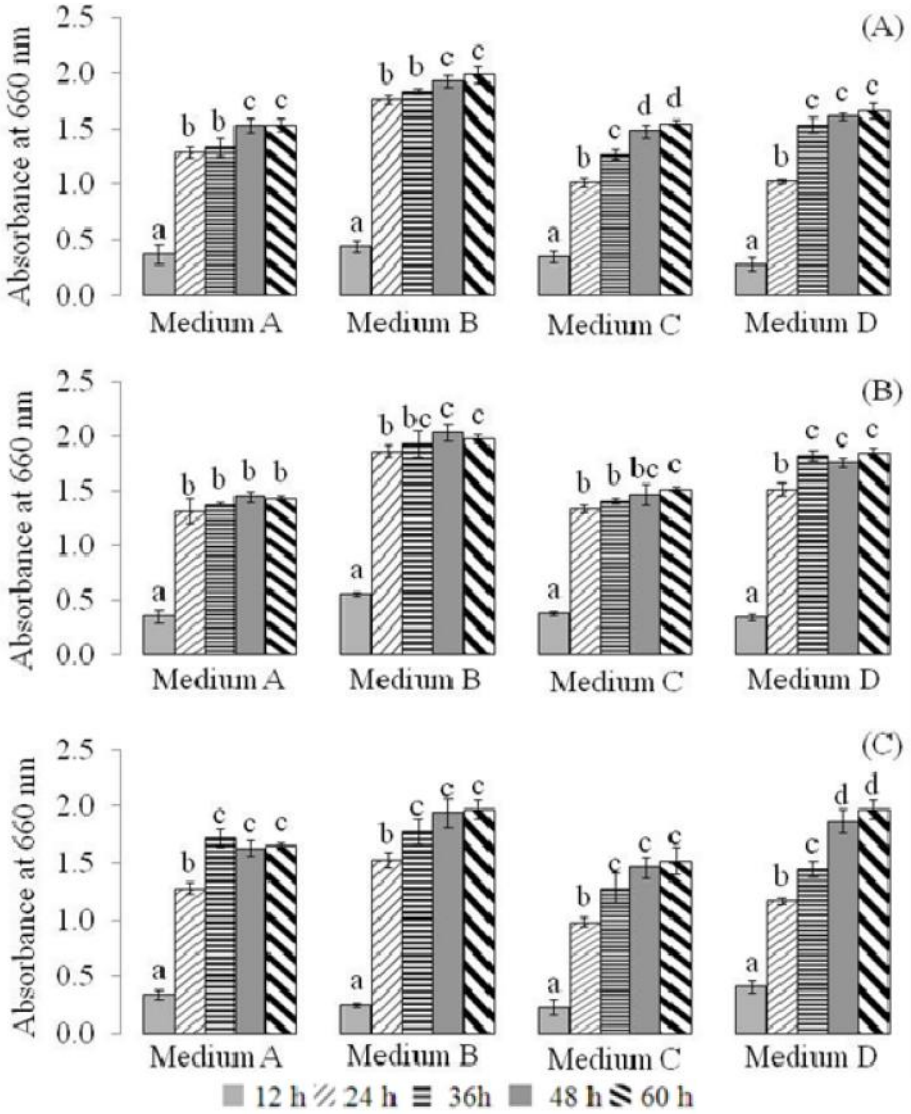

Figure 2. *Growth of Enterobacter sp.C2361 (A), Providencia sp.C1112 (B) and Sv. mobaraense (C) grown in 5 ml of various media at 37˚C and 150 rpm. Bar represents standard deviation from triplicate determinations. Different letters within the same parameter indicate the significant differences ($p<0.05$)*

MTGase was rapidly produced by the tested strains in Medium B with the highest activity of 0.87±0.02, 0.82±0.02 and 0.85±0.01 U/ml at 48, 24 and 48 h cultivation for *Enterobacter* sp. C2361, *Providencia* sp. C1112 and *Sv. mobaraense* DSM 40847, respectively (Figure 3). On the other hands, MTGase could be also produced by the tested strains cultivated in Medium A, D and C in the same way but sequentially lower in the MTGase activity,

respectively. The results suggested that the SPY medium (medium B) was the best medium to grow the selected strains for rapid MTGase production. *Sv. mobaraense* produced the highest amounts of MTGase in the SPY medium (Zhu *et al*., 1996).

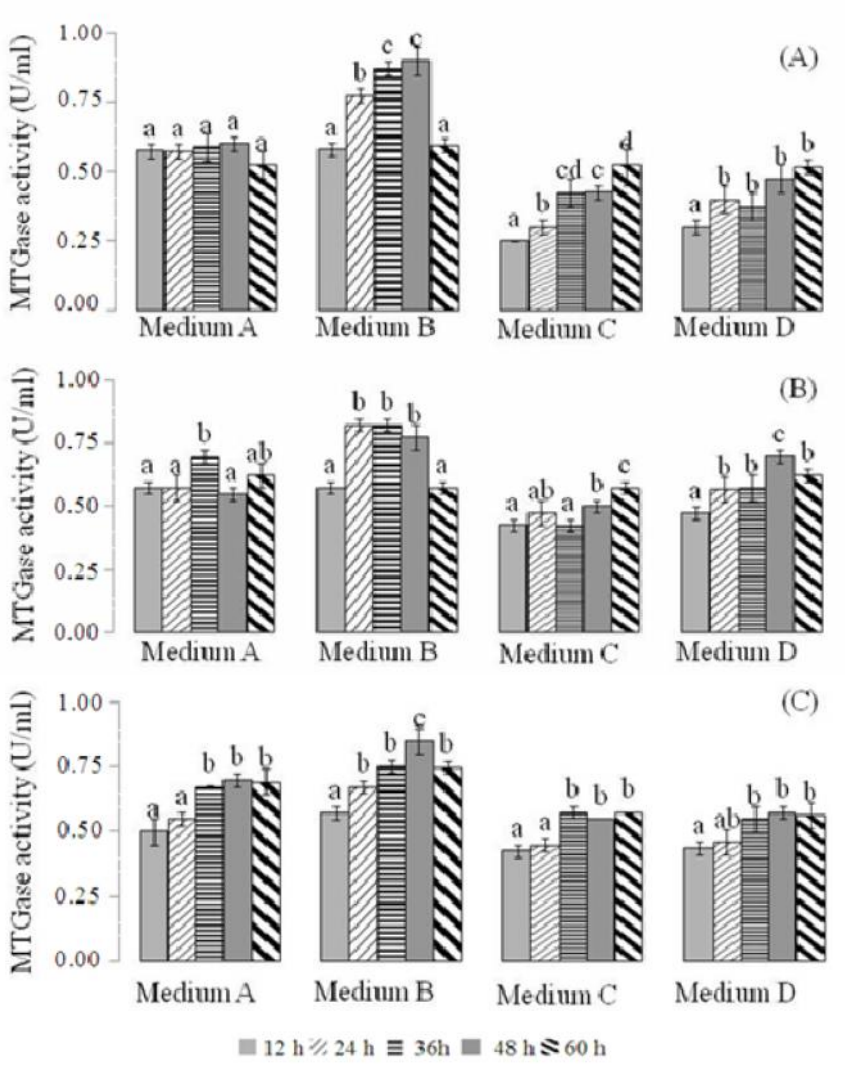

Figure 3. *MTGase activity of Enterobacter sp.C2361 (A), Providencia sp.C1112 (B) and Sv. mobaraense (C) grown in 5 ml of various media at 37˚C and 150 rpm. Bar represents standard deviation from triplicate determinations. Different letters within the same parameter indicate the significant differences (p<0.05)*

Time courses for growth and MTGase production

When grown in the SPY medium, *Enterobacter* sp. C2361, *Providencia* sp. C1112 and *Sv. mobaraense* DSM 40847 reached the stationary phase at 12-24 h (Figure 4). *Sv. mobaraense* DSMZ 40847 showed higher growth than *Enterobacter* sp. C2361 and *Providencia* sp. C1112. Whereas, the highest activities of MTGase excreted from *Enterobacter* sp. C2361, *Providencia* sp. C1112 and *Sv. mobaraense* DSM 40847 were 0.77±0.04, 0.92±0.01 and 0.90±0.02 U/ml at 24, 42 and 18 h of cultivation, respectively.

It is noted that *Providencia* sp. C1112 provided MTGase activity as high as *Sv. mobaraense* DSM 40847 excreted in the medium B. MTGase is a growth associated product which is important to bacterial growth.

In the middle of stationary phase, MTGase activity was decreased_maybe due to the feedback inhibition of MTGase production and by the released ammonia (Zhu *et al.*, 1996).

It is noted that although *Providencia* sp. C1112 showed the lowest growth, it provided the highest MTGase activity. In addition, some nitrogen sources might crosslinked by the excreted MTGase which could not be hydrolyzed by some proteases (Xie and Wang, 1994). It is noted that the MTGase activity in the screening of medium (Figure 3) was different from the step of time course study (Figure 4).

It could be affected by the volume of medium to the culture container.

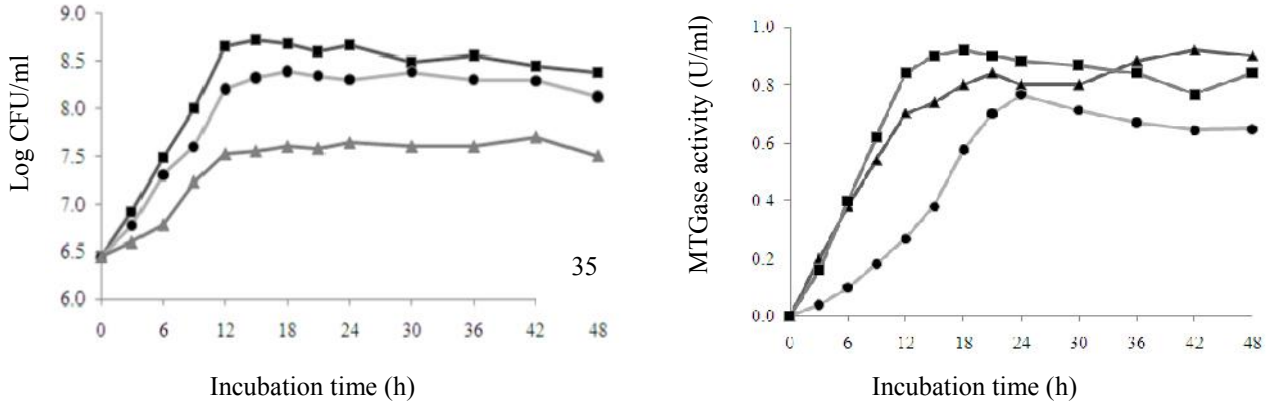

Figure 4. *Growth and MTGase activity of Enterobacter sp. C2361 (●), Providencia sp. C1112 (▲) and Sv. mobaraense (■) grown in 100 ml of SPY medium (medium B) at 37°C and 150 rpm*

Test tubes (15×160 mm) with 5 ml of medium were used in the step of media screening, while conical flasks (250 ml) with 100 ml of medium B were used in the time course study for the production of MTGase. Therefore, the rate of air exchange in the medium and cells could be different and affected the growth and MTGase production by the tested strains (Portilla-Rivera *et al.*, 2009).

There were various media used for production of MTGase from different bacteria. SPY medium was used to cultivate *Sv. mobaraense* DSMZ 40847 which showed the highest MTGase activity in a range of 1.80-3.40 U/ml (Zhu *et al.*, 1996; Zheng *et al.*, 2001; Zheng *et al.*, 2002b). However, the condition used for cultivation was different and took a longer time (36-60 h).

The MTGase of *Streptomyces* sp. gained the highest activity in a range of 0.05-0.20 U/ml in the medium with complex starch after cultivation for 7 days (Iancu *et al.*, 2009). Bahrim *et al.* (2010) reported that *Streptomyces* sp. produced a high level of MTGase activity at 0.43 U/ml in the medium containing glucose and peptone at 7 days of cultivation. In this study, *Enterobacter* sp. C2361 and *Providencia* sp. C1112 produced MTGase with high activity within 24-42 h. In addition, *Providencia* sp. C1112 had the MTGase activity comparable with *Sv. mobaraense* DSMZ 40847. The result suggested that the selected isolates were the potential source of MTGase.

Conclusions

This study presented the MTGase active strains isolated from wastewater and floc-floating of the wastewater from seafood factories. *Enterobacter* sp. C2361 and *Providencia* sp. C1112 were the dominant MTGase-producing bacteria. When cultivated in different media, the SPY medium was the most effective medium for production of MTGase by the tested strains. *Providencia* sp. C1112 possessed the MTGase activity comparable to *Sv. mobaraense* DSMZ 40847.

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