J. Microbiol. Biotechnol. (2000), **10**(2), 187–194

JOURNAL OF
MICROBIOLOGY
AND
BIOTECHNOLOGY

Production and Characterization of a Novel Microbial Transglutaminase from *Actinomadura* sp. T-2

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Received: October 13, 1999

Abstract An actinomycetes strain, T-2, which produces transglutaminase (EC 2.3.2.13), was isolated from soil and identified as belonging to the *Actinomadura* sp., based on taxonomic studies. The conditions for the transglutaminase production and its enzymatic properties were investigated. The optimum components for the transglutaminase production were 2% glucose, 1% polypeptone and soytone, and 0.1% $MnCl_2$. The optimum pH and temperature of the enzyme reaction were pH 8.0 and 45°C, respectively. The enzyme was stable within the pH range of 5.0–9.0 and 30°C–45°C. The novel enzyme required no calcium ions for its activity. This enzyme polymerized various proteins such as casien, soy protein, hemoglobin, egg white, gelatin, and soybean milk.

Key words: Microbial transglutaminase, *Actinomadura* sp., gelation, glutamyl-lysine crosslink

Transglutaminase (*R*-glutaminyl-peptide: amine γ-glutamyl-transferase, EC 2, 3, 2, 13) is an enzyme that catalyzes an acyl transfer reaction between a γ-carboxyamide group of the glutamine residue in a peptide chain and a primary amine [2, 4]. Transglutaminase (TGase) catalyzes the intramolecular or intermolecular crosslinking of certain proteins by γ-glutamyl-ε-lysine side chain bridges. TGase has been found in mammals, vertebrates, invertebrates, molluscs, plants, and microorganisms [13]. The enzymic activity of eukaryotic tissue TGase (type II) is regulated by Ca^{2+} and GTP [19], while coagulation factor XIII and epidermal TGase (type III) are soluble pro-enzymes which have to be activated by a proteolytic cleavage [10, 24].

In microbial sources, TGase activity has been found only in the actinomycetes from the genus *Streptoverticillium* [15], and extracellular TGase has been isolated from *Streptoverticillim* S-8112, a variant of *Streptoverticillim mobaraense* [1, 9], and *Streptoverticillium mobaraense* [5]. Intracellular TGase was also found in *Bacillus subtilis* [20] and in the spherules of *Physarum polycephalum* [12]. A remarkable characteristic of this microbial TGase (MTGase) is its Ca^{2+}-independent catalytic property. These TGase-catalyzed reactions can be used to modify the functional properities of food proteins [16, 27]. TGase has been used to catalyze the crosslinking of a number of proteins, such as whey proteins, soya proteins, gluten, myosin, and actomyosin. The modification of food proteins by TGase may lead to textured products, help to protect lysines in food proteins from various chemical reactions, encapsulate lipids and/or lipid soluble materials, form heat- and water-resistant films, avoid heat treatment of gelation, improve elasticity and water-holding capacity, modify the solubility and functional properties, and produce food proteins of higher nutritive value through the crosslinking of different proteins containing complementary limiting essential amino acids [11, 14, 16]. The effects of the MTGase from *Streptoverticillium mobaraense* and *Streptoverticillium* sp. on protein gels have been extensively studied [17, 18, 21, 23]. Protein modification by enzymes, especially by MTGase, whose mass production can be achieved by fermentation from cheap substrates, is also one of the most promising alternatives in developing novel protein foods. Modification of strains by genetic engineering is also one of the alternatives. However, in Western countries, there is an increasing tendency among consumers not to approve the application of genetically engineered organisms to food and food ingredients. In this respect, we have screened microorganisms which produce MTGase for the purpose of mass production. In this study, a novel MTGase that produces actinomycetes was isolated and the conditions of its production and its characteristics were investigated.

Materials and Methods

Screening Method

The isolation of rare actinomycetes was achieved by means of a Humic acid-vitamin (HV) agar [6, 7] from

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various soil samples. The isolates were stored on a yeast extract-malt extract agar slant. To isolate the TGase-producing strains, the isolated strains were cultured for 5 days at 30°C on a basal medium composed of 2% polypeptone (Difco Co., U.S.A.), 2% soluble starch (Sigma Co., St. Louis, U.S.A.), 0.2% yeast extract (Difco Co., U.S.A.), 0.2% K_2HPO_4, 0.1% $MgSO_4 \cdot 7H_2O$, and 1.5% agar, at pH 7.0. The TGase produced was detected using a colony staining method on an agar plate. Filter papers dipped into 0.2 M N-carbobenzoxy(CBZ)-L-glutaminyl-glycine (Sigma Co., St. Louis, U.S.A.), 0.1 M calcium chloride, 2.0 M hydroxylamine, and 0.02 M EDTA, were placed on the colonies grown on the agar plate and incubated at 37°C. After 2 h incubation, 20 µl of 15% trichloroacetate, 0.1 N HCl, and 2.5 N HCl in 5% ferrous chloride were added to the filter papers. The MTGase activity of the test colonies was indicated by a red color, due to the formation of γ-glutamyl hydroxamate from CBZ-L-glutaminylglycin [3]. The selected strain T-2 was used throughout this study as the extracellular MTGase-producing strain.

Culture Conditions

The composition of the basal medium for the MTGase production was 2% polypeptone, 2% soluble starch, 0.2% yeast extract, 0.2% K_2HPO_4, and 0.1% $MgSO_4 \cdot 7H_2O$, pH 7.0. For the preculture preparation, 20 ml of the medium in a 100-ml Erlenmyer flask was inoculated from 0.1 ml of prepared spore suspension (10^9 spores/ml) and cultured at 28°C for 36 h on a reciprocating shaker (120 strokes/min). The main culture was grown in 70-ml portions of the medium in 500-ml Erlenmyer flasks. Each flask was inoculated with 2.1 ml of the preculture, and incubated at 28°C for 5 days on a rotary shaker at 150 rpm. The culture filtrate was used as a crude MTGase enzyme.

Identification of Strain T-2

The characterization and identification of strain T-2 were carried out according to "Bergey's Manual of Systematic Bacteriology" [15] and an identification experiment for actinomycetes [5]. For an evaluation of the cultural characteristics, the strain was incubated for 14–28 days at 28°C. The spore chain morphology of 14-day cultures on the yeast-malt extract agar (ISP medium 2) was examined by light and scanning electron microscopy [15].

MTGase Activity

The enzyme activity was measured using a colorimetric hydroxamate procedure with N-carbobenzoxyl-L-glutaminyl-glycin [3]. A calibration curve was made using L-glutamic acid-γ-monohydroxamate (Sigma Co., St. Louis, U.S.A.). One unit of MTGase was defined as the amount of enzyme to form 1 µmol L-glutamic acid-γ-monohydroxamate/min at 37°C.

Conditions of MTGase Production

To determine optimal conditions for MTGase production, 2% of various carbon sources (shown in Table 2), 2% of various nitrogen sources (shown in Table 4), and 0.1% of various inorganic salts (shown in Table 6) were added to the basal medium. The MTGase production was measured 3, 4, and 5 days after the incubation, respectively.

Effect of pH on Enzyme Activity and Stability

The pH dependence of the MTGase activity was measured using a 0.1 M Tris-acetate buffer (pH 4.0–11.0) system. The enzyme reaction was performed in each buffer at 37°C for 10 min. The pH-stability of the MTGase was measured by incubating the enzyme prior to the enzyme assay in the same buffers (pH 4.0–11.0) at 4°C for 10 min, 30 min, and 50 min. Then, each enzyme solution was adjusted to pH 6.0, and the remaining activity was measured at 37°C for 10 min.

Effect of Temperature on Enzyme Activity and Thermostability

The optimum temperature for MTGase activity was measured between 25–60°C by incubation of each reaction mixture in a 1.0 M Tris-acetate buffer (pH 6.0) for 10 min. A thermal stability measurement for the enzyme was performed by incubating the enzyme solution prior to the enzyme assay within a temperature range of 30–60°C for 10 min, 30 min, and 50 min. After quickly cooling the enzyme solution in an ice-water bath (4°C), the remaining activity was measured at 37°C for 10 min.

Gel Formation by MTGase

Protein solutions (100 mg/ml) were prepared in a 0.1 M Tris-HCl buffer (pH 7.6). Aliquots of 4.0 ml from the protein solutions and 0.3 ml (1.5 U/ml) of concentrated crude MTGase in 80% $(NH_4)_2SO_4$ were mixed with or without DTT (10 mM) and put into a small test tube, followed by incubation at 45°C for 60 min. The protein gel formation was confirmed by standing the test tubes on their heads as shown in Fig. 7.

Results and Discussion

Screening for MTGase-Producing Actinomycetes

About 250 strains of actinomycetes, isolated from soil collected from various locations at Keimyung University in South Korea, were investigated for their hydroxamate-forming activity through a plate assay. Four strains showed MTGase activity(data not shown), however, the T-2 strain exhibited the strongest enzyme activity and was, therefore, selected for this study.

Identification of T-2 Strain

The cultural characteristics of strain T-2 grown on various media at 28°C for 14–28 days are shown in Table 1. Good

Table 1. Morphological and physiological characteristics of strain T-2.

Characteristics	T-2
Colony morphology on ISP[a] 2 and 4	
Periphery	Limited, limited
Surface	Wrinkle, flat
Aerial mycelium	Abundant, thin
Spore mass color	Ivory-white
Spore chains on ISP 2	Pseudosporangia, spirals (short chain)
Color of colony on ISP 5	
Substrate mycelium	White
Soluble pigment	None
Temperature range for growth	10–37°C
Optimum temperature	27–37°C
Liquefaction of gelatin	-
Coagulation of skim milk	-
Hydrolysis of starch	-
Hydrolysis of cellulose	-
Tolerance of NaCl	10%
Melanin pigment on ISP 7 and 1	None, None
Carbon utilization	
D-Fructose	++
D-Glucose	++
Inositol	++
D-Mannitol	±
Raffinose	+
L-Rhamnose	±
Sucrose	++
D-Xylose	+

[a]ISP medium. No. 1, Tryptone-yeast agar, No. 2. Yeast-malt agar; No. 4, Inorganic salt-starch agar, No. 5. Glycerol-asparagine agar, No. 7. Tyrosine agar.

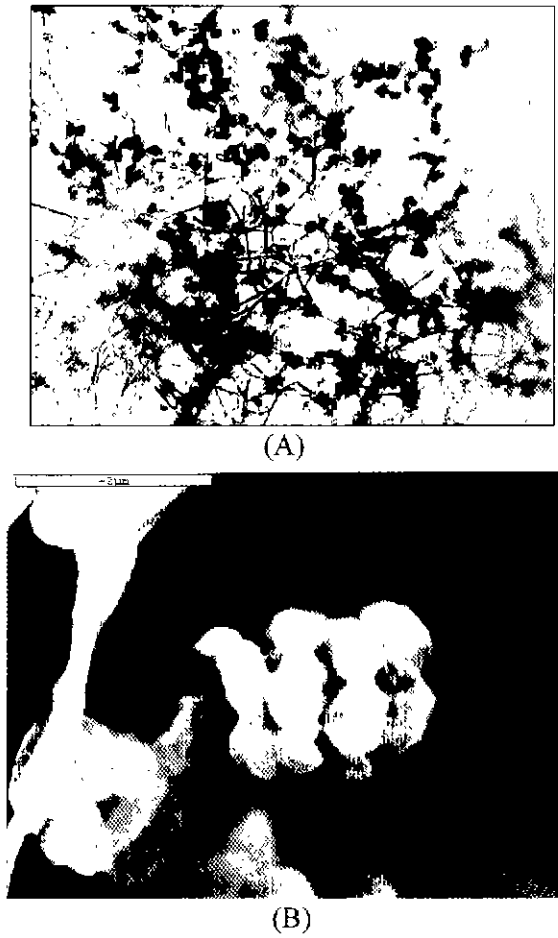

Fig. 1. Light micrograph (A, ×600) and scanning electron micrograph (B, ×7,500) of the T-2 strain
Cultivation was performed with ISP medium No 2 at 28°C for 14 days.

growth was observed on the yeast-malt extract agar (ISP. No. 2) and inorganic salts-starch agar (ISP. No. 4). Melanoid pigments and other soluble pigments were not produced on the tyrosine agar (ISP. No. 7) and tryptone-yeast extract agar (ISP. No. 1). The strain grew well within a temperature range of 10°C to 37°C, but not above 45°C, and did not produce spores at 10°C on the yeast-malt extract agar. The liquefaction of gelatin, coagulation of skim milk, and hydrolysis of starch and cellulose were all negative. The strain grew well within a range of 4 to 10% NaCl, but not above 13%. The strain utilized D-glucose, inositol, raffinose, sucrose, and D-xylose for growth, however, D-mannitol and L-rhamnose revealed weak growth. By microscopic examination, the strain showed the characteristic morphology of aerial mycelium. The spore chains displayed spirals forming so-called pseudosporangia (Fig. 1A) and spirals of four to six turns (Fig. 1B). The spore surface was smooth. These results matched the characteristics of this strain with those of the genus *Actinomadura*. The physiological and morphological characteristics, with an exception of the spore surface, were classified as those of a strain belonging to *Actinomadura spiralis*. Accordingly, this microorganism was designated as *Actinomadura* sp. T-2, although the actual species was not determined.

Cultural Conditions for MTGase Production

Studies on the conditions conducive to MTGase production showed that the presence of carbon sources, nitrogen sources, and salts in the various microorganisms were important [12, 26, 27]. As the MTGase produced may crosslink peptide and/or amino acid, suitable nitrogen sources for MTGase production were studied in a fed-batch fermentation process [26]. The time course of the MTGase production was examined using the basal medium. As shown in Fig. 2, the kinetics of the MTGase production during incubation revealed that the growth reached the stationary phase after 5 days of incubation, and the maximum level of the enzyme activity was observed after

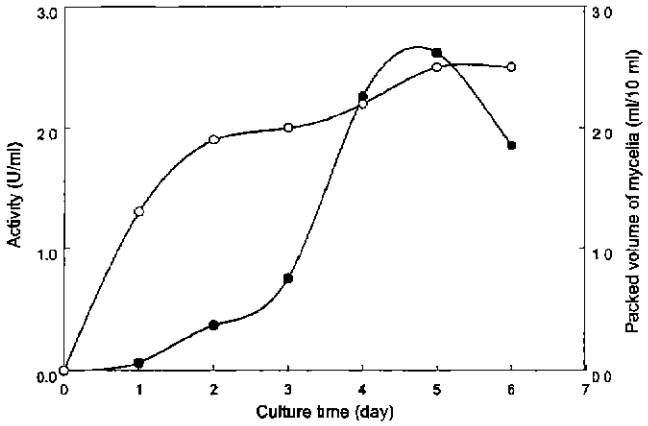

Fig. 2. Time course of growth and MTGase production in a culture of *Actinomadura* sp. T-2.

Cultures were grown in a basal medium with 2% polypeptone, 2% soluble starch, 0.2% yeast extract, 0.2% K_2HPO_4, and 0.1% $MgSO_4 \cdot 7H_2O$ at 28°C ○, growth; ●, MTGase activity.

5 days of incubation, which produced 2.26 U/m. In order to determine the optimal conditions of the MTGase production with the strain T-2, optimum carbon sources, nitrogen sources, and inorganic salts were examined. To study the effect of carbon sources, 20 g/l of each carbon source (shown in Table 2) was added to the basal medium in place of soluble starch. As shown in Table 2, the highest MTGase production was observed after 4 and 5 days of incubation in the medium containing glucose, whereas a lower level of activity was observed in glycerol. The glucose concentration was further tested to find out the optimum concentration needed to enhance MTGase production. The highest level of MTGase activity occurred with 20 g glucose/l (Table 3). These results differed from the others in which the carbon source used in the production medium was soluble starch [1, 26] and glycerol

Table 2. Effect of carbon sources on microbial transglutaminase (MTGase) production.

Carbon source (20 g/l)	Incubation time (days)			
	2	3	4	5
	(U/ml)			
Dextrin	0.68	0.58	0.49	0.21
Galactose	0.29	0.81	0.49	0.86
Glucose	0.96	1.42	4.53	4.22
Glycerol	0.68	1.73	2.10	1.63
Lactose	0.50	0.25	0.07	0.06
Maltose	0.11	0.36	0.30	0.30
Sodium acetate	0.06	0.07	0.07	0.07
Soluble starch	0.49	0.80	0.58	2.51
Sucrose	0.62	0.62	0.60	0.56

Cultivation was carried out aerobically at 28°C in a medium containing 2% peptone, 0.2% yeast extract, 0.2% K_2HPO_4, and 0.1% $MgSO_4 \cdot 7H_2O$, at pH 7.0.

Table 3. Effect of glucose concentration on MTGase production.

Glucose (g/l)	Incubation time (days)		
	3	4	5
	(U/ml)		
10	1.54	2.80	2.25
20	1.51	4.38	5.05
30	1.88	2.44	3.27
40	1.68	2.28	1.41
50	0.68	1.39	0.99

[25], and yet were similar in that glycerol and glucose showed high production of intracellular MTGase in *Physarum polycephalum* [12]. To determine the effect of nitrogen sources on the production of MTGase, a high concentration of nitrogen sources was used because the MTGase produced may crosslink peptides [26]. A concentration of 20 g of each nitrogen source/l was added to the basal medium containing glucose (20 g/l). As shown in Table 4, only organic nitrogen sources such as soytone, beef extract, polypeptone, and yeast extract showed a high production of MTGase 5 days after the incubation. Consequently, the effects of various combinations of these sources were carried out. The results are shown in Table 5. A combination of polypeptone plus soytone (each 10 g/l) revealed the highest MTGase production of all the combinations. However, 6 days after the incubation, the MTGase activity significantly decreased. Since the combination of polypeptone and soytone gave a high MTGase production, the effects of these concentrations were tested. A concentration of total 10 g/l of each polypeptone (5 g/l) and soytone (5 g/l), showed high MTGase production after 5 days of incubation (Table 5). Zhu *et al.*

Table 4. Effect of nitrogen sources on MTGase production.

Nitrogen source (20 g/l)	Incubation time (days)	
	3	5
Organic	(U/ml)	
Beef extract	2.67	4.37
Casamino acid	0.28	1.40
Casitone	0.76	1.76
Malt extract	0.17	1.33
Polypeptone	2.51	4.76
Soytone	2.79	4.48
Urea	0.09	1.14
Yeast extract	1.54	4.57
Inorganic		
Ammonium chloride	0.14	0.06
Ammonium sulfate	0.09	0.12
Sodium nitrate	0.09	0.09

Cultivation was carried out at 28°C in a medium containing 2.0% glucose, 0.2% K_2HPO_4, and 0.1% $MgSO_4 \cdot 7H_2O$, at pH 7.0.

Table 5. Effect of addition of two nitrogen sources on enzyme production.

Nitrogen sources (20 g/l)[a]	Incubation time (days)			
	3	4	5	6
	(U/ml)			
Polypeptone+Bacto-soytone	2.20	3.95	4.63	1.56
Polypeptone+Beef extract	1.43	2.60	3.33	-
Polypeptone+Yeast extract	2.45	3.23	3.50	2.68
Beef extract+Bacto-soytone	1.49	2.73	3.76	-
Yeast extract+Bacto-soytone	1.28	2.71	3.89	2.16
Yeast extract+Beef extract	1.52	2.25	4.32	2.36
Polypeptone and Bacto-soytone (g/l)				
5		2.06	3.80	
10		4.53	4.84	
20		3.87	4.41	
30		3.76	4.09	
40		1.11	1.43	
50		1.21	1.76	

[a]10 g/l of each nitrogen source was added to the medium.

[26] studied the development of initial and feeding media for the batch-fed production of MTGase from *Streptoverticillium mobaraense*. They reported that the yeast extract is essential for the growth and production of MTGase, and $(NH_4)_2SO_4$ is effective in MTGase production, because of its inhibition crosslinking by the MTGase produced. The effect of switching the organic and inorganic nitrogen sources was not investigated in this study. In general, during the fermentation procedure for the production of MTGase, necessary minerals and trace elements such as phosphate, magnesium, potassium, iron, copper, zinc, and vitamins were used [1, 15]. Therefore, the influence of inorganic salts on the MTGase production was tested. Each inorganic salt (0.1%) was added to the basal medium containing 2% glucose, and 1% polypeptone and soytone. After 4 and 5 days of incubation, the highest MTGase production was observed in the medium containing manganese, however, the production was much less in the medium containing iron and zinc (Table 6). The MTGase showed a maximum activity with a supply of 0.1% (w/v) manganese, and then exhibited a decreasing trend as the manganese concentrations were increased (Table 6). Even though the relationship between Mn^{2+} ions and MTGase synthesis is still unknown, Mn^{2+} ions are suggested to contribute to the stabilization of the MTGase produced in a cell broth. From these production conditions tested, the optimum components for the production medium were found to be 2% glucose, 1% polypeptone and soytone, and 0.1% $MnCl_2$. In the production of transglutaminase derived from microorganims, the fermentation broth had an enzyme activity ranging from 0.28 U/ml to 2.5 U/ml, depending upon the strain used [27], whereas the culture broth of strain T-2 had high enzyme activities ranging from about 4.0 U/ml to 5.0 U/ml.

Table 6. Effect of inorganic salts on MTGase production.

Inorganic salts (0.1%)	Incubation time (days)		
	3	4	5
		(U/ml)	
$CaCl_2$	0.56	2.38	3.34
$FeCl_3$	0.83	1.08	0.80
$FeSO_4$	0.57	0.81	0.86
$MgSO_4$	1.40	4.48	4.19
$MnCl_2$	1.57	4.44	4.96
$ZnSO_4$	0.56	0.91	1.28
None	0.58	4.00	4.22
$MnCl_2$ (%)			
0.1		4.64	4.80
0.2		3.79	3.81
0.3		3.55	3.17
0.4		1.23	2.13
0.5		0.17	0.32

Cultivation was carried out at 28°C in a medium containing 2% glucose, 0.5% polypeptone, and 0.5% Bacto-soytone, at pH 7.0.

Optimal pH and Temperature, and Thermal and pH Stabilities

The optimum pH of the MTGase in a 0.1 M Tris-acetate buffer at 37°C was 8.0 (Fig. 3). The enzyme was stable at pH 5.0–9.0 after 10 min incubation at 4°C, in which more than 80% of the activity was retained (Fig. 4). The optimum temperature of the MTGase was at 45°C (Fig. 5). The enzyme was stable below 45°C and lost 40% and 90% of its activity when it was incubated at 50°C and 60°C for 10 min, respectively (Fig. 6). The optimum pH (8.0) and temperature (45°C) of this enzyme were different from those of *Streptoverticillium* sp. (pH 6–7 and 50°C) [1, 25].

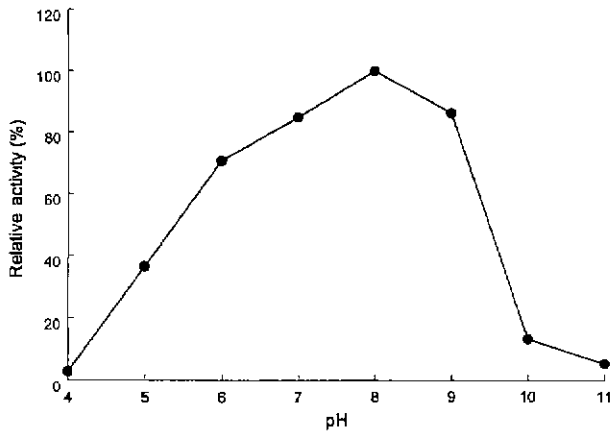

Fig. 3. Effect of pH on MTGase activity.
MTGase activity was measured at various pHs for 10 min at 37°C. Buffer used was 0.1 M Tris-acetate (pH 4.0–11.0).

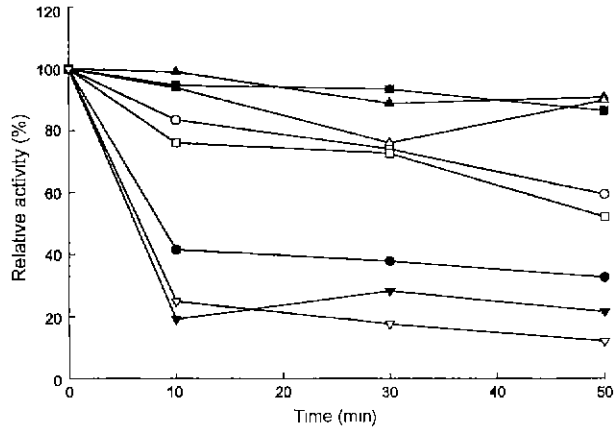

Fig. 4. pH stability of MTGase.
MTGase was incubated at various pHs for 10 min, 30 min, and 50 min at 4°C, and the remaining activity was measured at 37°C.
pH 4.0, -●-; pH 5.0, -○-; pH 6.0, -▲-; pH 7.0, -△-; pH 8.0, -■-; pH 9.0, -□-, pH 10.0, -▼-; pH 11.0, -▽-

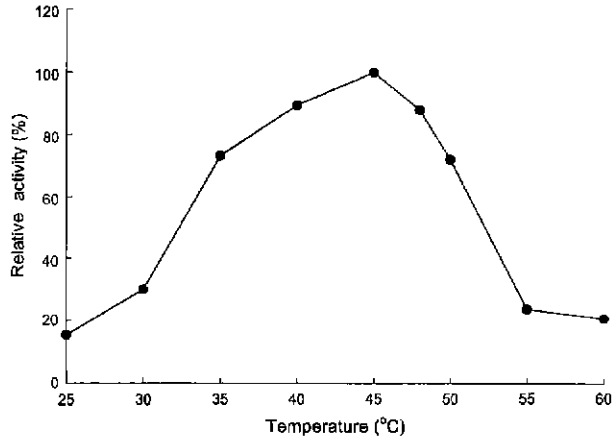

Fig. 5. Effect of temperature on MTGase activity.
MTGase activity was measured at various temperatures (25°C-60°C) for 10 min in a 1.0 M Tris-acetate buffer (pH 6.0).

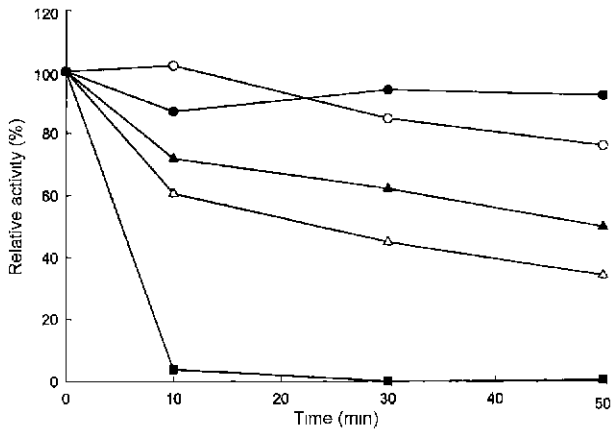

Fig. 6. Temperature stability of MTGase.
MTGase was incubated at various temperatures for 10 min, 30 min, and 50 min in a 1.0 M Tris-acetate buffer (pH 6.0) and the remaining activity was measured at 37°C. 30°C, -●-; 40°C, -○-, 45°C, -▲-, 50°C, -△-, 60°C, -■-.

The pH stability (5.0-9.0) of the enzyme was similiar to that of *Streptoverticillium* sp. [1]. The thermal stability (60% activity at 50°C for 10 min) was lower than that of *Streptoverticillium* sp. (74% activity at 50°C). From these results, therefore, the MTGase of the isolated strain T-2 was considered to be different from the other known MTGase from *Streptoverticillium* sp.

Influence of Calcium Ions

The important feature of MTGase is that it is Ca^{2+}-independent [1], however, the TGases from animals require Ca^{2+} as a cofactor for the transfer reaction [13]. Therefore, the influence of calcium ions on the MTGase activity was tested. The relative activity in the presence or absence of calcium ions and EDTA is shown in Table 7. The enzyme acted in both the presence and the absence of calcium ions, and the activity was also not inhibited by EDTA. Thus, this enzyme can be defined as a Ca^{2+}-independent enzyme, similar to the MTGase of *Streptoverticillium* sp.

Gelation of Protein Solution by MTGase

The application of MTGase has been tested in a variety of food processes [18, 21, 22, 27], and the foods have shown improved elasticity, texture, taste, and flavor. In this study,

Table 7. Influence of Ca^{2+} and EDTA on MTGase activity.

Concentration	Relative activity (%)
None	100
1 mM $CaCl_2$	103.7
1 mM EDTA	96.7
1 mM $CaCl_2$+1 mM EDTA	112.9
5 mM $CaCl_2$	95.4
5 mM $CaCl_2$+1 mM EDTA	99.3

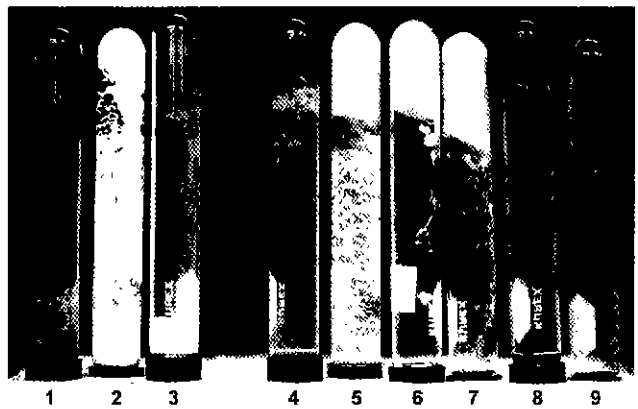

Fig. 7. Gelation of protein solution by MTGase.
Each protein solution (100 mg/ml) was incubated with 0.3 ml of concentrated crude MTGase in 80% $(NH_4)_2SO_4$ at 45°C for 60 min. 1, albumin (control, -DTT, +MTGase) 2, casein (control, -MTGase); 3, concentrated commercial milk (control, -MTGase); 4, albumin (+10 mM DTT, +MTGase); 5, casein (+MTGase); 6, concentrated commercial milk (+MTGase); 7, concentrated commercial soybean milk (+MTGase); 8, hemoglobin (+MTGase); 8, gelatin (+MTGase).

Table 8. Gelation activity of MTGase on various proteins.

Protein (100 mg/ml)	TGase (-DTT)	TGase (+DTT)
Albumin	×	◎
Casein	◎	◎
Egg white	○	○
Egg yolk	×	×
Gelatin	◎	◎
Hemoglobin	◎	◎
Soybean milk	◎	◎
Soy protein	◎	◎
Strong flour	○	○
Weak flour	○	○

◎, Very good; ○, Good; ×, None.
DTT, 10 mM addition.

the gelation ability of the novel MTGase in various proteins was investigated. The protein solutions served as self-supporting gels with the addition of MTGase (4–9 in Fig. 7), and did not form gelation unless MTGase was added (2–3 in Fig. 7). Since a conformational change by the reduction of disulfide bonds was needed for the substrate to polymerize [17], the gelation of the protein solutions was tested with or without DTT (Table 8). Most proteins were polymerized (except egg yolk), however, albumin only gelated when DTT was added to the reaction mixture (1 and 4 in Fig. 7). Sakamoto *et al.* [21] reported on the polymerization and gelation of various proteins containing egg yolk with the MTGase from *Streptoverticillium* sp. Based on the results of the gelation test, the substrate specificity for polymerization seemed to be different from that of the MTGase from *Streptoverticillium* sp.

CONCLUSIONS

The novel MTGase in the culture filtrate of *Actinomadura* sp. T-2 had high enzyme activity under optimum production conditions. The broad pH range for the activity suggests a strong possibility of applying this enzyme to different types of foods. In particular, the gelation of various proteins demonstrates its high potential for application to food processes. The isolated *Actinomadura* sp. T-2 may be a good resource for the hyperproduction of MTGase. Further studies on the purification and enzymatic characteristics of this enzyme are currently being conducted.

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