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Cloning of the gene coding for transglutaminase from *Streptomyces platensis* and its expression in *Streptomyces lividans*☆

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Abstract

A gene, *mtgA*, encoding transglutaminase (TGase) in *Streptomyces platensis* M5218 was cloned and expressed in *Streptomyces lividans*. The *mtgA* gene consisted of an open reading frame of 1254 nucleotides encoding a protein of 418 amino acids with a calculated molecular weight of 46,511 Da. The deduced amino acid sequence shows 69.3–77.7% identity to TGases from *Streptoverticillium* spp., but exhibits less than 35% identity with TGases of *Bacillus subtilis* and eukaryotic origins. The putative active site, YGCVG, conserved in *Streptoverticillium* TGases is also present in MtgA. SDS-PAGE and immunoblotting analyses revealed that an intensively stained protein band with a size corresponding to that of mature TGase was present in the culture supernatant of the recombinant strain. The result suggests that the recombinant MtgA may be highly expressed and correctly processed during secretion in the transformed *S. lividans* JT46.

Keywords: Production; Secretion; *Streptomyces lividans*; *Streptomyces platensis*; Transglutaminase; Taxonomy

1. Introduction

Transglutaminase (TGase; protein-glutamine:amine γ-glutamyltransferase, EC 2.3.2.13) catalyzes cross-linking between the γ-carboxyamide group of glutamine and the ε-amino group of lysine or other primary amine, resulting in the formation of an isopeptide bond either within or between polypeptide chains and the covalent incorporation of polyamine into proteins [1]. TGases are widely distributed in various organisms, ranging from bacteria to mammal [2–4]. The enzymatic reaction catalyzed by TGases of vertebrates and some invertebrates require Ca^{2+} to expose a cysteine residue in the active site domain [5]. Mammalian TGases are involved in a wide variety of physiological processes [6]. Recent evidence suggests that they may also play a role in neurodegenerative disease [7].

There were few studies on microbial TGases. TGase activity has been found mainly in the genus *Streptoverticillium* spp. [4,8–10] and the genus *Bacillus* spp. [11]. The TGases of *Streptoverticillium* spp. are found extracellularly, while the *Bacillus* spp. enzymes are localized on spores. The enzymes from *Streptoverticillium* sp. S-8112 [4,10,12], *Sv. mobaraense* DSMZ strain [13], *Sv. ladakanum* [14], *Sv. cinnamoneum* CBS 683.68 [9], and *Bacillus subtilis* [15] have been purified and characterized. The bacterial enzymes are Ca^{2+}-independent. Genes for TGases of *Streptoverticillium* sp. S-8112 [10,16], *Sv. cinnamoneum* CBS 683.68 [9], *Sv. mobaraense* DSMZ strain [17], *Sv. ladakanum* B1 [18], and that of *B. subtilis* [19] have been cloned and sequenced. Little homology was found between the TGases from *Streptoverticillium* spp. and *B. subtilis*.

Production of TGase using recombinant *S. lividans* [16,18] and *E. coli* [20] has been reported. However, the expression levels are too low for any practical application. A more efficient production system for TGase is desirable. Recently, we have screened about 300 Actinomycete strains isolated in Taiwan for TGase activity. A strain M5218 with high TGase activity was

Abbreviations: TGase, transglutaminase; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; ISP, International Streptomyces Project; rDNA, ribosomal DNA

☆ The GenBank accession number of the sequence reported in this paper is AY555726.

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obtained. In this study, we identified the isolate as a strain of *Streptomyces platensis*. The TGase gene of *S. platensis* M5218 was cloned, sequenced and expressed in *S. lividans*. The expression level of the recombinant TGase may have potential for industrial-scale production.

2. Materials and methods

2.1. Taxonomic studies

The strain M5218 was isolated from a soil sample collected in Ping-Ton, Taiwan. The cell wall of M5218 was analyzed as described by Komagata and Suzuki [21]. Morphological observation was performed with a light microscope on cultures grown at 30 °C for up to 14 days on yeast extract malt agar (ISP 2), oatmeal agar (ISP 3), inorganic salts starch agar (ISP 4), and glycerol asparagine agar (ISP 5). The cultural and physiological characteristics of the strain were analyzed according to the methods of Shirling and Gottlieb [22] and Gordon [23]. The total DNA of M5218 was extracted according to the previously described procedure [24]. The first 447-bp fragment of the 16 S rRNA gene was amplified and sequenced by using the MicroSeqTM 500 16 S rRNA Gene Kit (Perkin-Elmer Applied Biosystems Division, Foster City, CA) according to Kim et al. [25] with minor modification. The PCR reaction conditions were as follows: (1) an initial denaturation step consisting of 95 °C for 10 min; (2) 35 reaction cycles, with each cycle consisting of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min and 45 s; and (3) a final extension step consisting of 72 °C for 7 min. The GenBank accession number of the amplified sequence is DQ144073.

2.2. Bacterial strains, plasmids, and culture condition

The bacterial strains and plasmids used in this study are listed in Table 1. *Streptomyces* cultures were grown on R2YE agar [26] or in liquid medium containing 1% glucose, 2% trypton peptone, 0.2% KH_2PO_4, 0.2% $MgSO_4 \cdot 7H_2O$, 0.2% yeast extract, and 0.3% glycine (medium I). A loop of fresh spore suspension was inoculated into 50 ml of medium I and cultivated at 30 °C and 220 rpm for 2–5 days.

2.3. Enzyme assay

S. platensis M5218 and *S. lividans* JT46 [29] transformants were cultivated at 30 °C for 2–5 days and the cell-free supernatants were assayed for TGase activity. TGase activity was measured by a colorimetric hydroxamate procedure using *N*-carbobenzoxy-L-glutaminyl-glycine [27]. The reaction proceeded for 10 min at 37 °C. A calibration curve was prepared using L-glutamic acid-γ-monohydroxamic acid. One unit (U) of enzyme activity was defined as the amount of enzyme that catalyzed the formation of 1 μmol of hydroxamic acid per minute.

2.4. DNA manipulation

Molecular methods for *Streptomyces* were as described by Hopwood et al. [28]. *S. lividans* JT46 transformants carrying pIJ702 [30] or pIJ702-derived plasmids were selected on a plate containing 20 μg/ml thiostrepton and 40 μg/ml tyrosine. When the transformants were grown in liquid medium, 5 μg/ml thiostrepton was added. Other molecular methods used in this work were as described by Sambrook et al. [31].

2.5. Construction and screening of the genomic library of S. platensis

Chromosomal DNA of *S. platensis* M5218 was partially digested with *Sau*3A and size-fractionated on an agarose gel. DNA fragments with sizes ranging from 2 to 5 kb were isolated from the gel, inserted into the *Bgl*II site of pIJ702, and transformed into *S. lividans* JT46. The transformants were cultivated at 30 °C for 3 days and the culture supernatants were screened for TGase activity by the colorimetric hydroxamate procedure [27].

2.6. DNA sequencing and analysis

DNA sequencing of the TGase gene of the strain M5218 was performed by the dideoxy chain termination method [32] with a Bigdye™ terminator RR mix (Applied Biosystems) for the ABI PRISM™ model 310 Automated Sequencer. The sequence was determined in both directions using M13 forward and reverse primers. Gaps in the sequence were determined by the use of appropriate primers. Nucleotide and protein sequence comparisons were performed using the University of Wisconsin Genetics Computer Group, GCG packages [33].

2.7. Western blot analysis

The native and recombinant TGases were analyzed using a 10% gel by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) [34]. Gel sliced mature TGase of *Sv. ladakanum* B1 [18] was used to produce rabbit anti-TGase IgG. TGase was detected by Western blot analysis [35] using rabbit anti-TGase IgG as the first antibody and goat anti-rabbit IgG conjugated with peroxidase as the second antibody.

3. Results

3.1. Identification of the strain M5218

The cell wall of M5218 contained LL-diaminopimelic acid, glucose, and ribose. Thus, it belongs to cell wall of chemotype IC as described by Becker et al. [36]. The aerial mycelium of M5218 formed spiral spore chains with more than 20 spores per chain. The spores were ellipsoidal with a smooth surface. M5218 grew and sporulated well on ISP 2–4 media but moderate on ISP 5 medium. No soluble pigment was produced during growth on ISP media. The physiological characteristics of M5218 tested were identical with those of *S. platensis*^{T} BCRC 11898 (a type strain of *S. platensis*) except in two features. Starch was decomposed by M5218 but not by *S. platensis*^{T} BCRC

Table 1
Hosts and plasmids used in this study

Strain and plasmid	Relevant characteristics	Source or reference
Strains		
S. platensis M5218	A strain with transglutaminase activity	This study
S. lividans JT46	A thiostrepton-sensitive strain with a genotype of *pro-2 str-6 rec-46* SLP2$^-$ SLP3$^-$	[29]
E. coli DH5α	*DeoR endA1 gyrA96 hsdR179*($r_k^- m_k^+$) *recA1 relA1 supE44 thi-1*Δ(*lacZYA-argFV169*) *φ80δ*	Clontech
Plasmids		
pIJ702	A derivative of pIJ101, containing *melC* promoter and *tsr*	[30]
pAE053	A 2.9 kb DNA fragment containing the region that encodes transglutaminase activity, cloned into *Bgl*II site of pIJ702	This study