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Identification of Transglutaminase-Producing Bacterium Isolated From Seafood Processing Wastewater

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Abstract. Transglutaminase is a thiol enzyme that catalyzes protein reticulation and used in various food production processes to improve the functional properties of food material. In this study, a transglutaminase-producing bacterium, strain C2361, was isolated from seafood processing wastewater and subjected to identification. C2361 was a Gram-negative, non-spore-forming rod-shaped and facultative anaerobic bacterium. Phenotypic and chemotypic characteristics, including phylogenetic analyses, showed that the strain was a member of the genus *Enterobacter*. The 16S rRNA gene sequence similarities between *Enterobacter* sp. C2361 and *Enterobacter cloacae* subsp. *cloacae* ATCC 13047^T and *Enterobacter cloacae* subsp. *dissolvens* LMG 2683^T were 97.5 and 97.7%, respectively. Moreover, *Enterobacter* sp. C2361 showed a low DNA-DNA relatedness with the above-mentioned species. On the basis of the polyphasic evidences gathered in this study, C2361 should be classified as a novel species in the genus *Enterobacter*

Keywords: Transglutaminase-producing bacteria, Identification, *Enterobacter*, Novel species

1. Introduction

Transglutaminase (TGase) is accounted as protein glutamine γ-glutamyl transferase (EC 2.3.2.13) [1]. The TGase catalyzes the formation of isopeptide and leads to the crosslinking 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 [1]. TGase could be found in various organisms including mammal, avian, fish, plant, invertebrates and microoraganism [2, 3, 4]. In commercial food processing, transglutaminase is used to bond proteins together. Examples of foods made using transglutaminase include imitation crabmeat and fish balls. It is produced by *Streptoverticillium mobaraense* fermentation in commercial quantities or extracted from animal blood [5] and is used in a variety of processes, including the production of processed meat and fish products. Moreover, transglutaminase can be used as a binding agent to improve the texture of protein-rich foods such as surimi or ham [6].

In our efforts to screen for efficient TGase-producing bacteria, a strain which designated as C2361 was obtained. This study aimed to identify the TGase-producing bacterium, C2361. The results of an examination of the phenotypic and chemotypic characteristics of strain C2361 are described, along with the phylogenetic characteristics of the strain.

2. Materials and Methods

2.1. Strains

Wastewater samples were collected from treatment ponds of seafood processing factories in Songkhla, Thailand. Bacteria were isolated by means of the usual dilution plating techniques on nutrient agar (NA)

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and incubated at 37°C for 24 hours. The appearance colonies were determined for TGase activity by a filter paper dish (FPD) assay based on the color reaction as described by Folk and Cole [7]. A MTGase-producing bacterium designated as C2361 was further purified by repeated transfers of separate colonies on NA plate. Unless otherwise stated, bacteria were subcultured onto NA and incubated overnight at 37°C.

2.2. References Strains

Enterobacter cloacae subsp. *cloacae* KCTC 2361^T and *Enterobacter cloacae* subsp. *dissolvens* JCM 6049^T were obtained from Korean Collection for Type Cultures (KCTC) and Japan Collection of Microoganisms (JCM), respectively. All strains were cultivated and tested under the same conditions of the unknown bacterium for comparative studies.

2.3. Phenotypic Characterization

The cell morphology was determined from cells grown in nutrient broth (NB) for 2 days. The Gram reaction was determined by the method of Hucker and Conn [8]. Basic biochemical characteristics of strain C2361 were determined by using API 20E and API 50CH Kits (bioMérieux) according to the manufacturer's instructions. Tests for utilization of citrate, growth on MacConkey agar and anaerobic growth were also performed.

2.4. Chemotypic Characterization

DNA was extracted and purified from their whole cells by the phenol method [9]. The base composition of DNA was determined by the method of Tamaoka and Komagata [10], with the modification that DNA was hydrolyzed and the result nucleotides were analyzed by reversed–phase HPLC.

2.5. Phylogenetic Characterization

The phylogenetic position of strain C2361 was studied by the standard analysis based on the 16S rRNA gene sequence. 16S rRNA gene sequencing was carried out as described by Sumpavapol *et al*. [11]. The 16S rRNA gene sequence obtained (1429 bases) was deposited in the DDBJ databases under the accession numbers HQ888848 and was aligned to reference 16S rRNA gene sequences available in the GenBank/EMBL/DDBJ databases by using the program CLUSTAL_X (version 1.81) [12]. Gaps and ambiguous bases were eliminated from the calculations and the distance matrices for the aligned sequences were calculated by the two-parameter method [13]. A neighbour-joining phylogenetic tree was constructed as described by Saitou and Nei [14] using the program MEGA (version 2.1) [15]. The confidence values of individual branches in the phylogenetic tree were determined by using the bootstrap analysis [16] based on 1000 samplings.

2.6. DNA-DNA Relatedness

DNA hybridization was carried out by photobiotin-labeling method with microdilution plate as described by Ezaki *et al*. [17].

3. Results and Discussion

3.1. Phenotypic and Chemotypic Characteristics

Strain C2361 was Gram-negative, rod shape and facultative anaerobic with ability to utilized the citrate and growth well on MacConkey agar. Strain C2361 could be classified as a member of genus *Enterobacter* and was differentiated from other related species in the genus *Enterobacter* by means of some phenotypic and chemotypic characteristics as shown in Table 1.

3.2. Phylogenetic Characteristic and DNA-DNA Relatedness

The result from 16S rRNA gene sequencing analysis indicated that the strain C2361 belongs to the genus *Enterobacter* and grouped most closely with a cluster containing *E. cloacae* subsp. *dissolvens* LMG 2683^T and *E. cloacae* subsp. *cloacae* ATCC 13047^T as shown in Fig 1. The similarity between strain C2361 and *E. cloacae* subsp. *dissolvens* LMG 2683^T and *E. cloacae* subsp. *cloacae* ATCC 13047^T were 97.46 and 97.65%, respectively. Stackebrandt and Goebel (1994) reported that the unknown strains may represent the novel

species when the 16S rRNA gene sequence similarities between them and any recognized species were fewer than 97% [19].

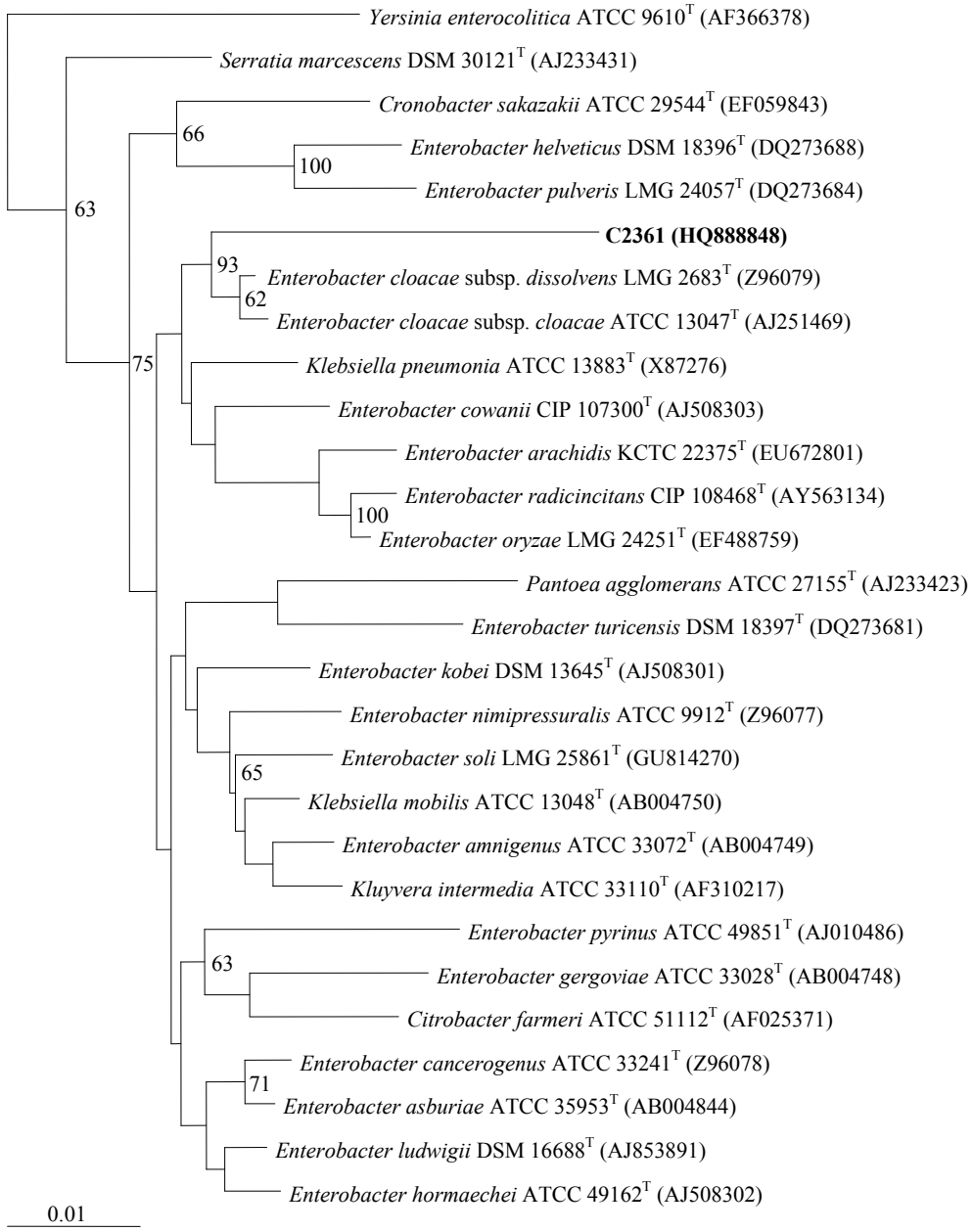

Fig. 1: Neighbour-joining tree comprising 16S rRNA gene sequences of strain C2361, recognized *Enterobacter* species and related taxa. Bootstap values (expressed as percentages of 1000 replications) greater than 60% are shown at the branch points. Bar, 0.01 substitution per nucleotide position.

Moreover, the DNA hybridization results revealed that strain C2361 and *E. cloacae* subsp. *dissolvens* JCM 6049^T and *E. cloacae* subsp. *cloacae* ATCC KCTC2361^T showed a DNA-DNA relatedness of 47.53 and 48.78%, respectively. The generally accepted limit for species delination indicated that the DNA-DNA relatedness between the strains of the different genospecies was falled below 70% [20]. Thus, the strain C2361 should be classified as a novel species in the genus *Enterobacter*.

4. Conclusion

Phenotypic, chemotypic and genotypic characteristics gathered in this study indicated that the strain C2361 should be classified as a novel species in the genus *Enterobacter.*

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Table 1 Differential characteristics of strain C2361 and related *Enterobacter* species

Characteristic	C2361	*E. cloacae* subsp. *dissolvens* JCM 6049^T	*E. cloacae* subsp. *cloacae* KCTC 2361^T
Ortho-nitrophenyl-β-galactoside	+	+	-
Aesculin hydrolysis	+	+	-
Carbon source utilization			
Arbutin	+	+	-
Gentiobiose	+	-	-
D-Fructose	-	-	+
D-Galactose	+	+	-
D-Glucose	+	-	+
D-Lyxose	+	+	-
D-Mannitol	-	-	+
D-Mannose	+	-	+
D-Ribose	+	-	+
D-Turanose	+	-	-
L-Arabinose	+	-	+
L-Rhamnose	+	-	+
G+C content (mol%)	53	55*	53*

Data were obtained in this study unless otherwise indicated.
+, positive reaction; -, negative reaction.
*Data from Hoffmann *et al*. [18]

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