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Mutational and biochemical analyses of the endolysin Lys_{gaY} encoded by the *Lactobacillus gasseri* JCM 1131^T phage φgaY

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Abstract

The Lys_{gaY} of *Lactobacillus gasseri* JCM 1131^T phage φgaY endolysin was purified to homogeneity using the *Escherichia coli*/His·Tag system. Zymographic and spectrophotometric assays showed that Lys_{gaY} lysed over 20 heated Gram-positive bacterial species as the substrates, including lactobacilli, lactococci, enterococci, micrococci, and staphylococci. The enzymatic activity had the pH and temperature optima of about 6.5 and 37°C, respectively. Amino-acid substitution analysis revealed that 13 residues of Lys_{gaY} were involved in cell-lytic activity: in the β/α_{gaY} domain, G10, D12, E33, D36, H60, Y61, D96, E98, V124, L132, and D198; in the $SH3b_{gaY}$ domain, Y272 and W284. In addition, deletion analysis demonstrated that the β/α_{gaY} domain of N-terminal 216 residues is the core enzyme portion, although the cell-lytic ability is lower than that of Lys_{gaY}. These mutational experiments suggested that β/α_{gaY} (in which two acidic residues of D12 and E98 likely act as catalytic residues) is responsible for cell-lytic activity, and $SH3b_{gaY}$ promotes β/α_{gaY} possibly through cell-wall binding function. The purified His-tagged $SH3b_{gaY}$ domain containing 94 residues from S217 to K310 (i) bound to Gram-positive bacteria susceptible to Lys_{gaY}, (ii) induced aggregation of exponentially growing cells of *L. gasseri* JCM 1131^T, *L. casei* IAM 1045, *Lactococcus lactis* C2, *L. lactis* MG 1363, and *Enterococcus hirae* IAM 1262 by forming thread-like chained cells, (iii) inhibited lytic activity of Lys_{gaY}, and (iv) impeded autolysis of *L. gasseri* JCM 1131^T in buffer systems. A truncated protein HΔ$SH3b_{gaY}$ lacking in N-terminal 21 residues (from S217 to E237) of $SH3b_{gaY}$ and an amino-acid substituted protein $HSH3b_{gaY}$G (W284G) lost the activities of $HSH3b_{gaY}$, showing that the N-terminal region and W284 probably play important roles in the $SH3b_{gaY}$ function(s).

Keywords: *Lactobacillus gasseri*; φgaY; Endolysin; Muramidase; SH3b

1. Introduction

Various phages and their cell-lysis genes have been hitherto reported from lactic acid bacteria (LAB) (see for a review, Kodaira and Taketo, 2005). Primarily, these cell-lysis proteins designated as an endolysin act in the phage propagation for extracellular release of mature virion particles *via* their degrading activities towards the peptidoglycan layer, and cause a threat to industrial fermentation (Brussow, 2001). In the manufacturing of fermented foods, these endolysins are also

Abbreviations: Amp, ampicillin; bp, base pair; h, hour(s); IPTG, isopropyl-β-D-thiogalacto-pyranoside; kDa, kilo dalton; LB, Luria–Bertani (medium); *lys*, gene encoding endolysin (Lys); PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; P_{lac}, promoter of *lacZ*′; gene; P_{T7}, promoter of T7; r, resistant; s, sensitive; SDS, sodium dodecyl sulfate; SH3b, bacterial Src homology 3; wt, wild type.

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expected to facilitate the release of bacterial intracellular enzymes for ripening and flavour development, and active against pathogenic and spoilage bacteria (Shearman et al., 1994; Vasala et al., 1995; Gasson, 1996).

From LAB phages, more than thirty endolysins have been so far reported, and they have been divided into three groups (Kodaira and Taketo, 2005): muramidase and endopeptidase families. Although some of these endolysins have been characterized biochemically, their molecular properties are still scarce.

In the muramidase family, several *Chalararopsis* (Ch)-type endolysins (designated as Lys in this study) have been reported from the *Lactococcus* phages φLC3 (Birkeland, 1994) and Tuc2009 (Arendt et al., 1994), and the *Lactobacillus* phages φadh (Henrich et al., 1995), LL-H (Vasala et al., 1995), mv1 (Boizet et al., 1990), mv4 (Dupont et al., 1993), and φg1e (Oki et al., 1996). Three-dimensional (3D) structures of these Lys proteins are as yet unavailable.

Previously, we cloned a new two-component lysis system (hol_{gaY}–lys_{gaY}) encoded by its prophage φgaY of *Lactobacillus gasseri* JCM 1131^T (Yokoi et al., 2005a). The lys_{gaY}-encoded protein (Lys_{gaY}) belongs to the Ch-type muramidase group, and the protein overproduced in *Escherichia coli* exhibited a broad cell-lytic spectrum towards Gram-positive bacterial strains.

A recent *in silico* study (Kodaira and Taketo, 2005) suggested that Lys species including Lys_{gaY} are modular proteins composed of three putative regions: (i) the N-terminal enzyme-catalytic region, (ii) the linker region containing lysine and proline residues, and (iii) the C-terminal cell-recognition region. In Lys_{gaY}, the N-terminal region of about 200 amino acids long (designated as β/α_{gaY}) likely forms an unusual $(\beta/\alpha)_5\beta_3$-barrel fold composed of eight β-strands and four α-helices (see Fig. 1). These β/α barrels belonging to GH25 contain several conserved acidic residues; *e.g.*, in β/α_{gaY}, D12–E33–D36–D96–E98–D198. In Cellosyl (or CPL1), two residues of D9 (10) and E100 (94) have been regarded as possible candidates for the catalytic residues.

Throughout the endolysins of the GH25 family, the enzyme-catalytic and cell-binding regions are separated by the linker regions, which are frequently rich in lysine and proline (the KP sequence). Vasala et al. (1995) have supposed that the KP sequences facilitate membrane association of phage endolysins, but their actual functions are still unknown. The Lys_{gaY} linker is about 10 amino acids long, and has two lysines and prolines.

In the C-terminus, Lys_{gaY} has one bacterial Src homology 3 motif ($SH3b_{gaY}$). $SH3b_{gaY}$ structurally resembles other SH3b motifs from endolysins and autolysins; *in silico* analysis suggested that these SH3b motifs have a common tertiary fold composed of eight secondary β-strands (β1 to β8) with conserved amino acids (Kodaira and Taketo, 2005). In lysostaphin (Lss) of *S. simulans*, the C-terminal SH3b region (92 residues) has been inferred to direct this enzyme to the

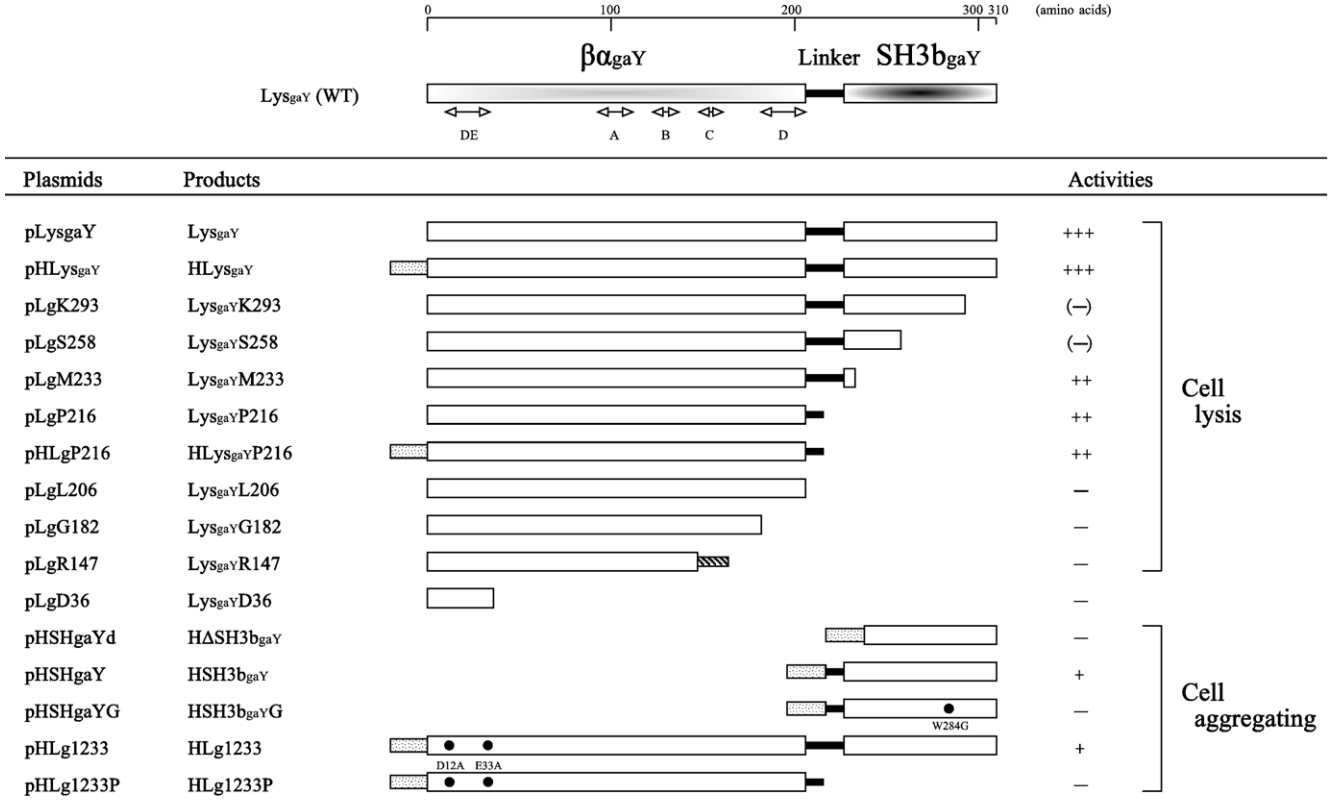

Fig. 1. Structure of the φgaY-encoded endolysin Lys_{gaY} and the deletion mutants. Lys_{gaY} of 310 amino-acid length is shown with the scale. The two putative domains, $\beta\alpha_{gaY}$ and $SH3b_{gaY}$, are shown with shadowed boxes. Five conserved motifs of DE, A, B, C, and D (Kodaira and Taketo, 2005) are shown with open allows. The putative linker region between $\beta\alpha_{gaY}$ and $SH3b_{gaY}$ is indicated by a thick line. Under Lys_{gaY}, its deletion mutants constructed in this study (Section 2.2) are delineated with their cell-lytic or cell-binding activities. The His ∗ Tag region is indicated with a dotted box.