Computational analysis of peptidoglycan hydrolase genes in *Pediococcus acidilactici* D3 genome

Bansal Poonam¹, Singh Jasbir² and Dhanda Suman²*

1. Department of Biotechnology, Maharishi Markandeshwar (Deemed to be University), Mullana Ambala 133 207, Haryana, INDIA
2. Department of Biochemistry, Kurukshetra University, Kurukshetra, Haryana, INDIA
*dhanda.suman999@gmail.com

Abstract

Peptidoglycan hydrolases (PGH) hydrolyze the covalent bond in peptidoglycans. PGHs are valuable tools for biotechnologists with many applications in medicine, food industry and for recovery of intracellular products from bacteria. Due to their ability to hydrolyze bacterial cell walls, PGHs might be promising in combating multidrug resistant pathogens. Genome of *Pediococcus acidilactici* D3 was analysed for genes encoding for PGHs. D3 genome encodes for 9 PGHs that might possess different activity. Their physiochemical properties and structure were predicted using computational tools.

Functional annotation, physiochemical properties and predicted structures will increase the understanding of PGHs of *P. acidilactici* D3 in the light of earlier studied PGHs by domain swapping, construction of chimeras and production of engineered lysins with diverse applications.


Introduction

Cell wall is a vital structure for survival of bacteria as it preserves cells integrity from internal osmotic pressure¹². Peptidoglycan (PG), a major component of cell wall of gram-positive bacteria, is comprised of repeating disaccharide of N-acetylmuramic acid and (β-1,4)-N-acetylglucosamine (MurNAc-GlcNAc) connected to peptide stem through MurNAc⁹. Peptidoglycan hydrolase or autolysins are a group of enzymes that hydrolyse PG. PGHs have been classified further depending upon their catalytic domains². PGHs play major role in different processes viz. autolysis, sporulation and germination, daughter cell separation, biofilm formation, cell-wall turnover and allogalactose in genetic transformation⁹. They also have impact on immune response, in down regulation of inflammatory response through induction of anti-inflammatory mechanisms mediated by immunocompetent cells and secreted cytokines.

Inactivation of these enzymes resulted in change in immunomodulatory properties⁹. Many pathogens are gram positive bacteria including some *Enterococcal* strains. PGHs are antimicrobial and can antagonize pathogens. Infections caused by these pathogens become uncontrollable when they acquire antibiotic resistance. PGHs seem to be efficient alternative to antibiotics¹¹.

Lactic acid bacteria (LAB) are harmless and conferred GRAS status by FDA. LAB are known to possess PGHs activity. PGHs cause autolysis. Lysis of LAB results in release of cytoplasmic enzymes i.e. lipases, peptidases and many other. This is important in improving organoleptic properties and texture of fermented food as LAB which are used as starter culture. PGHs are also involved in host-microbe interaction and confer probiotic properties to bacteria. *Pediococcus acidilactici* is a facultative anaerobe.

Study of PGHs is of great significance in developing strains resistant to autolysis under stress conditions and for better understanding of contribution of released muramyl peptides as probiotic immunomodulators and antipathogenic enzymes.

PGHs seem to be an interesting approach to fight against multidrug resistant pathogens and in future could be a tremendous tool for treating diseases caused by gram positive microbes. PGHs of *P. acidilactici* NCDC 252 genome were recently purified, in silico and physiochemically characterized⁹. This study encouraged the *in silico* study of PGH encoding genes of *P. acidilactici* D3.

Material and Methods

Sequence Retrieval: 27 contigs sequence of *Pediococcus acidilactici* D3 were retrieved from NCBI (National Center for Biotechnology Information) (www.ncbi.nlm.nih.gov/). Sequences encoding for different PGHs activities were retrieved from Uniprot.

Similarity check: Extent of contig similarity with genes sequence was checked using tBLASTn which (www.ncbi.nlm.nih.gov/) compares a protein’s query sequence against a nucleotide sequence database which is dynamically translated in all reading frames.

Computational analysis: Subcellular localization of proteins was determined by PSORTb v.3.0 (www.psort.org/psortb/). Presence and localization of signal peptide was predicted using server SignalP 4.1 (www.cbs.dtu.dk/services/signalP/). This helps to predict the cleavage site and signal peptide based on combination of several artificial neural network. pI/Mw (Isoelectric point/ Molecular weight) was estimated by tool compute pI/Mw (www.expasy.org/compute_pi/) which is a tool that allows...
the computation of theoretical pI and Mw for user entered sequences.

**Homology Modeling:** The homology modeling was done by MODELLER9.21 that was downloaded and installed from https://salib.org/modeller/download__installation.html. The modeled protein was validated by Protein Structure Validation Software Suite (PSVS) (http://psvs-1_5-dev.nesg.org/) which integrates and analyses from a number of widely-used structure quality evaluation tools including PROCHECK\(^2\), ERRAT\(^3\), PROVE\(^8\) and Verify3D\(^4\).

**Results and Discussion**

Based on sequence similarity search, total 9 PGH encoding genes were identified in *Pediococcus acidilactici* D3 genome that encodes for different PGH activity i.e. 2 genes (flgJ and flgJ2) encoded Muramidase activity, csxA and amiD2 genes encoded for glucosaminidase activity, cwID, PAD3_0540 and PAD3_0577 encoded for N-acetyl muramoyl-L-alanine amidase, PAD3_1720 and PAD3_0463 genes encoded for endopeptidase.

Among identified proteins, 5 genes encoded for extracellular proteins (flgJ, FlgJ2, csxA, amiD2, PAD3_0540), 3 for cytoplasmic (PAD3_0463, PAD3_1720, PAD3_0577) and one cwID for cell wall. The identified proteins may be secretory proteins as they all had high positive high scores in signal peptides because for non-secretory proteins score should ideally be very low (close to -0.1). Some of them had high isoelectric point (pI) above 7.0 which suggests these proteins to be of basic nature. One is acidic (pI 4.84) and one near neutral (pI 6.78). Computed parameters for all the proteins are summarized in table 1.

**Homology modeling:** Determination of protein structure using NMR spectroscopy or X-ray crystallography is of course more reliable but it is time consuming and not successful for all proteins\(^5\). Homology is a computational tool for structure determination. Though, homology modelling is less accurate than experimentally determined structures, still it is useful in suggesting hypothesis in molecular biology. It can also serve as starting model for solving structures from electron microscopy, NMR and X-ray crystallography\(^1\).

Therefore, three dimensional structures of all predicted proteins were elucidated by modeller (Figure 1) by selecting best templates (Table 1). All elucidated protein structures were validated by PSVS that computes Z score (overall global quality score) based on calibration with a set of high-resolution X-ray crystal structures. The Z score for modeled proteins was greater than recommended value of -0.5. The verified 3-D results also showed positive scores which indicated reliability of the models. These studies indicated the validation of the model.

**Conclusion**

PGHs exhibit autolytic activity, release cytoplasmic enzymes that produce free amino acids, peptides and organoleptic substances which are important in fermentation and cheese ripening. These enzymes can be explored for industrial applications but their role in bacterial physiology needs further studies. PGH genes in *P. acidilactici* D3 were analyzed using computational tools and elucidated protein structures will help in identification of appropriate ligand-binding site and preferred substrates or ligands. *In silico* studies also form the basis of further studies of role of PGHs in targeting deadly pathogens which is the major thrust area.

<table>
<thead>
<tr>
<th>Enzyme Name</th>
<th>Gene Name</th>
<th>Similarity</th>
<th>Position</th>
<th>Signal peptide</th>
<th>pI/MW</th>
<th>PDB ID of template</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muramidase</td>
<td>flgJ</td>
<td>83%</td>
<td>Extracellular</td>
<td>1-45</td>
<td>9.69/24.5</td>
<td>3wo</td>
</tr>
<tr>
<td>flgJ2</td>
<td></td>
<td>84%</td>
<td>Extracellular</td>
<td>1-25</td>
<td>9.47/40.4</td>
<td>2zyc</td>
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<tr>
<td>Glucosaminidase</td>
<td>csxA</td>
<td>69%</td>
<td>Extracellular</td>
<td>1-32</td>
<td>6.78/97.1</td>
<td>2vzsA</td>
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<tr>
<td>amiD2</td>
<td></td>
<td>83%</td>
<td>Extracellular</td>
<td>1-44</td>
<td>9.69/24.5</td>
<td>3fi7A</td>
</tr>
<tr>
<td>N-acetyl muramoyl-</td>
<td>cwID</td>
<td>100%</td>
<td>Cell wall</td>
<td>1-36</td>
<td>9.62/32.2</td>
<td>4bin</td>
</tr>
<tr>
<td>L-alanine amidase</td>
<td>PAD3_0540</td>
<td>89%</td>
<td>Extracellular</td>
<td>1-29</td>
<td>9.00/40.8</td>
<td>3LAT</td>
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<tr>
<td>PAD3_0577</td>
<td>80%</td>
<td>Cytoplasmic membrane</td>
<td>1-10</td>
<td>8.98/14.1</td>
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<td>Endopeptidase</td>
<td>PAD3_1720</td>
<td>100%</td>
<td>Cytoplasmic membrane</td>
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<td>4.84/72.02</td>
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<tr>
<td>PAD3_0463</td>
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<td>1-40</td>
<td>9.64/51.4</td>
<td>4c2eA</td>
<td></td>
</tr>
</tbody>
</table>

Table 1

Predicted parameters of PGHs analyzed by computational tools.
Fig. 1: Predicted structure of PGHs
References

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