

**Characterization of β -glucosidase activity of a *Lactiplantibacillus plantarum* 6-phospho-
 β -glucosidase**

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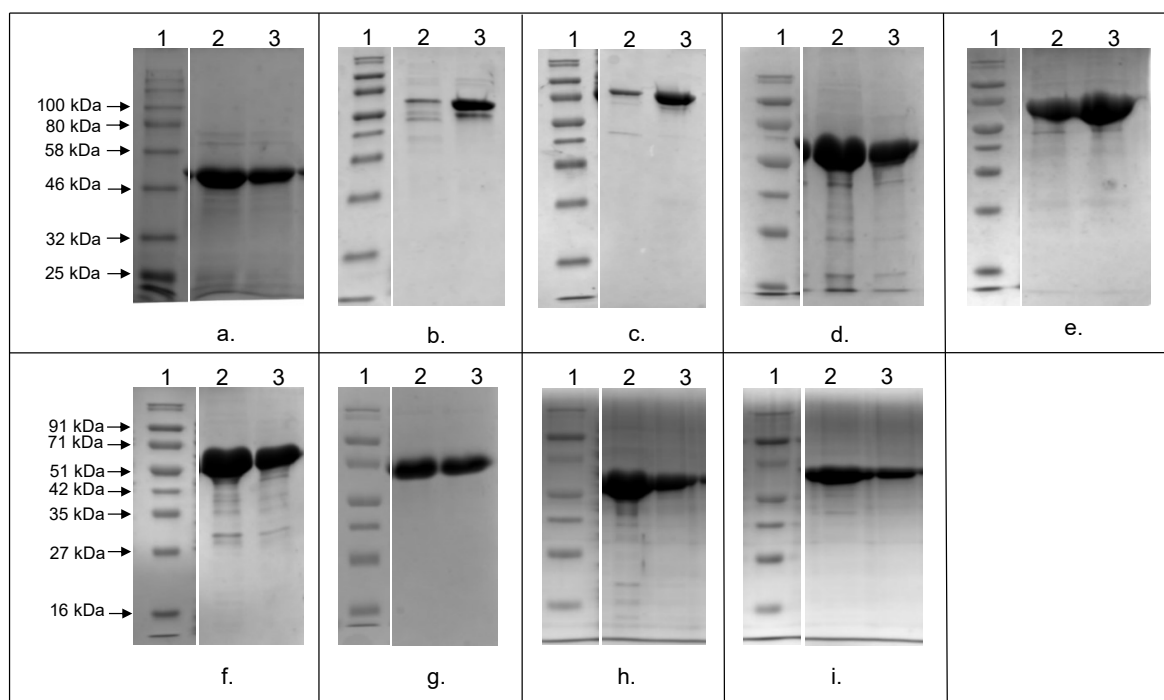


Fig. S1 Purification of the recombinant glycosyl hydrolases from *L. plantarum* NCIM 2903 expressed in the soluble fraction of *E. coli* by immobilized affinity chromatography as analyzed by SDS-PAGE. (a) LpBgl1; (b) LpBgl3; (c) LpBgl4; (d) LpBgl5; (e) LpBgl6; (f) LpBgl7; (g) LpBgl8; (h) LpBgl9; and (i) LpBgl10. Lane 1: Protein ladder; lane 2 and 3: elution with 150 mM and 250 mM imidazole, respectively.

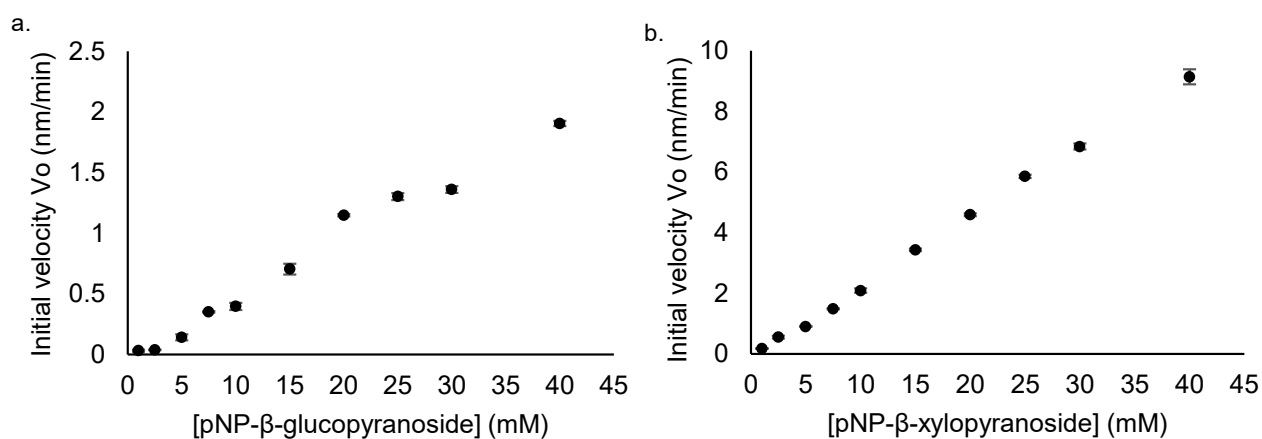


Fig. S2 Effect of various concentrations of pNP-β-glucopyranoside (a) and pNP-β-xylopyranoside (b) on the initial velocities of the reactions catalyzed by LpBgl5.

Table S1: Primers used for amplifying and vectors for cloning putative glycosyl hydrolases from *L. plantarum* NCIM 2903.

Gene	Orientation	RE site	Sequence (5' to 3') ^a	Cloning vector
<i>lpbgl1</i>	Forward	Sac I	TAGCGAGCTCCATGAGTAAATTACCAAATGGATTC	pEXP5-CT/TOPO-TA
	Reverse	Sac I	TAGCGAGCTCTTAAGAGAGGTCAGCACCG	
<i>lpbgl2</i>	Forward	EcoR I	AAAAGAATTTCGATGCAATTTCCGGCTG	pEXP5-CT/TOPO-TA
	Reverse	EcoR I	AAAAGAATTCTTAATTTGAGGCCGAGGG	
<i>lpbgl3</i>	Forward	Sac I	TAGCGAGCTCCATGGATAAGGAGCGACAG	pEXP5-CT/TOPO-TA
	Reverse	Sac I	TAGCGAGCTCTTACAATTCATCTCCATTTTTTG	
<i>lpbgl4</i>	Forward	Xho I	TAGCCTCGAGATGGATGTTTACAAAACGAG	pET-30b
	Reverse	Xho I	TAGCCTCGAGTCATTGAACGACTATAC	
<i>lpbgl5</i>	Forward	Not I	AAAAGCGGCCGCAATGTCAGAGTTCCCAG	pET-30b
	Reverse	Not I	AAAAGCGGCCGCCTATTTCTTTGTCAGCCC	
<i>lpbgl6</i>	Forward	Nco I	TAGCCCATGGCGATGTATTCAAAAAC	pET-30b
	Reverse	Nco I	TAGCCCATGGCTATCCCAAATCTTCC	
<i>lpbgl7</i>	Forward	EcoR I	AAAAGAATTCAATGACAAAAGGATAC	pET-30b
	Reverse	EcoR I	AAAAGAATTCCTATAAATCTTCACCATC	
<i>lpbgl8</i>	Forward	EcoR I	AAAAGAATTCTATGGTAGAGTTTCCGGAAGGCTTTG	pET-32b
	Reverse	EcoR I	AAAAGAATTCTCAAAACCCATTCCGTTCCCAAGC	
<i>lpbgl9</i>	Forward	BamH I	AAAAGGATCCGATGTATAAACCAACTTATCCTAAAAC	pET-30b
	Reverse	BamH I	AAAAGGATCCTTAAGCTAGATCTTACC	
<i>lpbgl10</i>	Forward	EcoR I	AAAAGAATTTCGATGACGATTAAAGGACGAGCGTTTC	pET-30b
	Reverse	EcoR I	AAAAGAATTCCTACTCAATTCGGCACCATTGTC	

^a sequence in bold and italics represents the restriction site and gene-specific region, respectively.

Table S2: Details of the selected putative glycosyl hydrolase genes and their predicted proteins from *L. plantarum* NCIM 2903.

Glycosyl hydrolase	Nucleotide length (bp)	Amino acid length	Molecular weight (kDa)	pI	GH family prediction using dbCAN3	GenBank accession number	<i>L. plantarum</i> WCFS1 homolog ^a
LpBgl1	1434	490	54.36	5.21	GH1	PQ876087	-
LpBgl2	1473	460	55.9	5.24	GH1	PQ876088	Lp_1401
LpBgl3	1383	477	53.4	5.04	GH1	PQ876089	Lp_3011
LpBgl4	1503	500	57.8	5.3	GH1	PQ876090	Lp_0906
LpBgl5	1443	480	54.5	4.96	GH1	PQ462659	Lp_3525
LpBgl6	1464	487	55.2	5.05	GH1	PQ876091	Lp_3526
LpBgl7	1440	479	54.9	4.87	GH1	PQ876092	Lp_2778
LpBgl8	1386	461	52.7	5.16	GH1	PQ876093	Lp_3629
LpBgl9	1461	486	55.82	5.64	GH1	PQ876094	Lp_3512
LpBgl10	1437	478	54.83	5.18	GH1	PQ876095	Lp_0440

^a > 98% identity as assessed by BLASTP, with the proteins reported in *L. plantarum* WCFS1 (Plaza-Vinuesa et al. 2022).

Table S3: The percent identity matrix of the amino acid sequences of the selected putative glycosyl hydrolases from *L. plantarum* NCIM 2903.

	LpBgl 1	LpBgl 2	LpBgl 3	LpBgl 4	LpBgl 5	LpBgl 6	LpBgl 7	LpBgl 8	LpBgl 9	LpBgl 10
LpBgl1	100									
LpBgl2	48.4	100								
LpBgl3	36.0	33.8	100							
LpBgl4	41.0	40.3	32.7	100						
LpBgl5	51.4	50.9	34.1	44.0	100					
LpBgl6	51.4	49.1	34.2	46.2	53.0	100				
LpBgl7	64.4	50.1	36.9	40.7	49.4	49.5	100			
LpBgl8	29.3	27.2	29.1	23.2	29.5	26.4	28.9	100		
LpBgl9	47.9	47.1	36.1	45.7	49.9	72.2	47.9	28.7	100	
LpBgl10	65.8	51.6	33.9	40.3	53.6	50.6	67.2	29.1	47.3	100