PSII-7
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Complete genome analysis of a novel mucolytic bacterium, *Prevotella mucinisolvens* sp. nov., isolated from bovine rumen epithelium

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ABSTRACT

We report the complete genome sequence of a novel mucolytic bacterium, *Prevotella mucinisolvens* sp. nov. Mucolytic bacteria were isolated from rumen epithelium of the dorsal sac of Korean cattle steer using a targeted cultivation on a mucin defined medium as a sole carbon source in anaerobic conditions. The genome of P. mucinisolvens was sequenced by means of both the Illumina HiSeq™ X and PacBio RSII platforms. The genome (2,730,135 bp) was found to contain 2,445 genes, 2,374 coding sequences, 61 transfer RNA, 1 transfer-messenger RNA, and 9 ribosomal RNA. The P. mucinisolvens had a total 51 glycoside hydrolases (GHs), of which 14 GHs, including β-galactosidases (GH2, GH20), α-N-acetylgalactosaminidases (GH101), α-N-acetylglucosaminidase (GH89), sialidase (GH33), and fucosidases (GH29, GH95), were identified as enzymes involved in mucin degradation. Following the KEGG pathways, the putative mucolytic pathway was constructed, including the metabolism of carbon sources such as galactose, N-acetylglucosamine, sialic acid (N-acetylneuraminic acid), and mannose. The presence of putative extracellular polysaccharide biosynthesis pathways, including Wzx/Wzy-dependent pathway (4 putative glycosyltransferases, 3 acetyltransferases, 1 flippase, 1 polymerase, 1 polysaccharide co-polymerase, and 1 outer membrane transport protein) and synthase-dependent pathway (1 putative synthase, 3 precursors of synthesis), was confirmed in P. mucinisolvens. Twelve putative virulence factors associated with adherence (hasB, KpsF, and htpB), stress reactions (clpP and clpC), antiphagocytosis (hasB and bcs1), O-antigen (gmd, fcl, and galE), and metabolic adaption (panD) were identified. This study contributes to a better understanding of epimural bacteria in putative mucin-degrading ability.

METERIALS & METHODS

Purposes
- To analysis the complete genome sequence of a novel mucolytic bacterium, *Prevotella mucinisolvens* sp. nov. isolated from bovine rumen epithelium

Sample collection
- The rumen epithelium tissue samples were collected from the dorsal sac of rumen of Korean cattle, directly after slaughtering at the abattoir

Isolation of mucolytic bacteria
- A targeted cultivation on a mucin defined medium as a sole carbon source in anaerobic **conditions**

Identification of mucolytic bacteria
- DNA extraction and 16S rRNA gene amplicon PCR
- Sequencing of 16S rRNA gene and data analysis

Whole genome sequencing
- Performed using both the Illumina HiSeq™ X and PacBio RSII platforms at Macrogen to obtain a high-quality sequence by error correction
- Annotated using the Prokka Galaxy tool (version 1.13)
- Functional annotation of predicted proteins was evaluated using the BlastKOALA tool of Kyoto Encyclopedia of Genes and Genomes (KEGG)
- To analyze carbon-metabolism related enzymes, the genome sequence of *Prevotella mucinisolvens* was annotated according to the Carbohydrate-Active enZYmes (CAZy) database using a meta server for automated carbohydrate-active enzyme annotation (dbCAN2)
- Prediction of virulence factors and antibiotic-resistant genes was performed using VRprofile

CONCLUSION

- ❖ *P. mucinisolvens* had the mucin-degrading metabolic process using a starch utilization system (Sus)-like system
- ❖ *P. mucinisolvens* was identified the presence of putative extracellular polysaccharide biosynthesis mechanisms, Wzx/Wzy-dependent pathway and synthase-dependent pathway
- ❖ *P. mucinisolvens* had 12 putative virulence factors related to adherence, stress reactions, O-antigen, and metabolic adaption without genes coding bacterial toxins as showing low-pathogenicity for the host

RESULTS

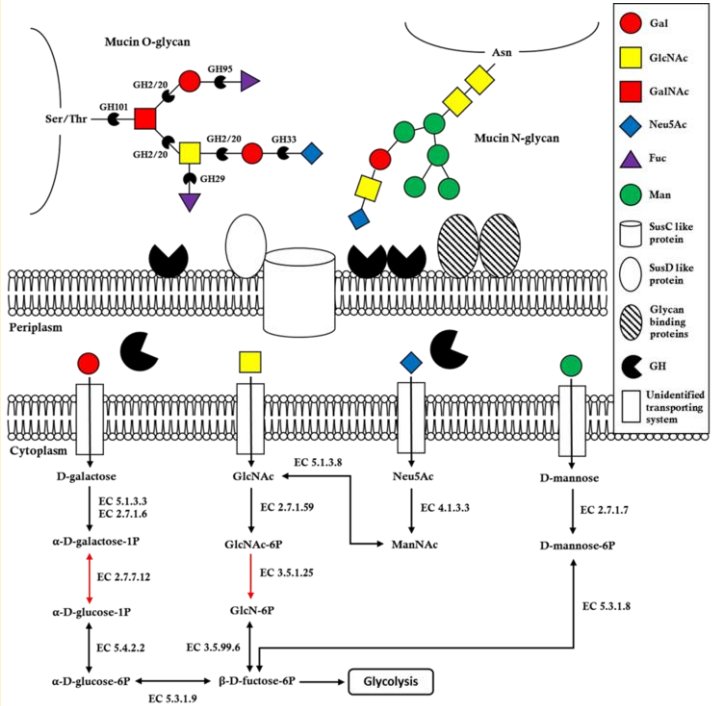
Table 1. Genomic features of the *P. mucinisolvens*

Number of Contigs	1		
Genome Size (bp)	2,730,135		
G+C content (mol%)	47.69		
Number of Genes	2,445	Protein encoding genes	2,374
		transfer RNA	61
		transfer-messenger RNA	1
		ribosomal RNA	9
Proteins with Predicted Functions	1,118		
Hypothetical or Uncharacterized Proteins	1,327		
Proteins with KEGG Annotations	962		
Carbohydrate-Active Enzymes	106	Glycoside Hydrolase	51
		Carbohydrate-Binding Module	2
		Glycosyltransferases	45
		Polysaccharide Lyase	1
		Carbohydrate Esterase	6
		Auxiliary Activities	1
Virulence Factors	12		
Antibiotic-Resistant Genes	4		

Table 2. List of predicted mucolytic enzymes in the complete genome of the *P. mucinisolvens*

Mucolytic enzymes	Glycoside hydrolases (GHs)	Locus ID[a]	KEGG ID	Prokka annotation
β-galactosidases	GH2	DJPCDFCF_01074	K01190 (β-galactosidase)	β-galactosidase
		DJPCDFCF_02139	K01190 (β-galactosidase)	β-galactosidase
	GH20	DJPCDFCF_00755	K12373 (β-hexosaminidase)	Hypothetical protein
		DJPCDFCF_01740	–	Hypothetical protein
		DJPCDFCF_01799	K12373 (β-hexosaminidase)	β-hexosaminidase
		DJPCDFCF_02310	K12373 (β-hexosaminidase)	Hypothetical protein
	GH42	–	–	–
Endo-β1,4-galactosidases	GH98	–	–	–
α-N-acetylgalactosaminidases	GH101	DJPCDFCF_01301	K01604 (methylmalonyl-CoA decarboxylase subunit alpha)	Propionyl-CoA carboxylase beta chain
	GH129	–	–	–
Exo- and endo-β-N-acetylglucosaminidases	GH84	–	–	–
	GH85	–	–	–
α-N-acetylglucosaminidases	GH89	DJPCDFCF_00315	K01205 (α-N-acetylglucosaminidases)	Hypothetical protein
Sialidases	GH33	DJPCDFCF_00633	–	Hypothetical protein
		DJPCDFCF_00914	K01186 (sialidase-1)	Sialidase
		DJPCDFCF_01114	–	Hercynine oxygenase
Fucosidases	GH29	DJPCDFCF_01076	K01206 (α-L-fucosidase)	Hypothetical protein
		DJPCDFCF_02312	K01206 (α-L-fucosidase)	Hypothetical protein
	GH95	DJPCDFCF_02222	K15923 (α-L-fucosidase 2)	Hypothetical protein

❖ Among 51 GHs, *P. mucinisolvens* had 14 GHs related to putative mucolytic enzymes, including β-galactosidases (GH2, GH20), α-N-acetylgalactosaminidases (GH101), α-N-acetylglucosaminidases (GH89), sialidases (GH33), fucosidases (GH29, GH95)



Asn = asparagine, Fuc = fucose, Gal = galactose, GalNAc = N-acetylgalactosamine, GH = glycoside hydrolases, GlcNAc = N-acetylglucosamine, Man = mannose, Neu5Ac = N-acetylneuraminic acid, Ser = serine, Sus = starch utilization system, Thr = threonine.

❖ *P. mucinisolvens* had genes involved in the metabolism of carbon sources such as galactose, GlcNAc, sialic acid (Neu5Ac), and mannose according to the results of KEGG pathways
❖ However, it is also incomplete because of the absence of galactose 1-phosphate uridyltransferase (EC 2.7.7.12) and N-acetylglucosamine-6-phosphate deacetylase (EC 3.5.1.25) associated with galactose and GlcNAc metabolisms, respectively

Figure 1. Putative mucin-degrading pathway of *P. mucinisolvens*. Metabolic pathways that are present in the *P. mucinisolvens* are depicted in black, and metabolic pathways that are absent in the *P. mucinisolvens* are depicted in red.

Table 3. List of predicted starch utilization system (Sus) genes in the complete genome of the *P. mucinisolvens*

Gene	Locus ID	KEGG ID	Prokka annotation
SusC	DJPCDFCF_00002	–	Hypothetical protein
	DJPCDFCF_00066	–	Hypothetical protein
	DJPCDFCF_00068	–	Hypothetical protein
	DJPCDFCF_00072	–	TonB-dependent receptor SusC
	DJPCDFCF_00180	–	Hypothetical protein
	DJPCDFCF_00363	–	TonB-dependent receptor SusC
	DJPCDFCF_00481	–	TonB-dependent receptor SusC
	DJPCDFCF_00483	–	Hypothetical protein
	DJPCDFCF_01522	–	Hypothetical protein
	DJPCDFCF_01753	K01951 (GMP synthase)	TonB-dependent receptor SusC
	DJPCDFCF_01951	–	Hypothetical protein
	DJPCDFCF_01956	–	Hypothetical protein
SusD	DJPCDFCF_00065	K21572 (starch-binding outer membrane protein)	Hypothetical protein
	DJPCDFCF_00362	K21572 (starch-binding outer membrane protein)	SusD-like protein
	DJPCDFCF_00484	K21572 (starch-binding outer membrane protein)	Hypothetical protein

❖ *P. mucinisolvens* was predicted to use a starch utilization system (Sus)-like system for the utilization of mucin glycan
❖ It was identified that *P. mucinisolvens* had putative SusCD through a BlastP analysis against the genome of *P. mucinisolvens*

❖ *P. mucinisolvens* had 12 putative virulence factors
❖ They contain factors associated with adherence (hasB, KpsF, and htpB), stress reactions (clpP and clpC), antiphagocytosis (hasB and bcs1), O-antigen (gmd, fcl, and galE), and metabolic adaption (panD)

Table 5. List of predicted virulence factors in the complete genome of the *P. mucinisolvens*

No.	Locus ID	Length (aa)	VRprofile[a] ID	Ha-value	Product	VFDB[b] category
1	DJPCDFCF_00298	221	VFG0077	0.45	clpP (ATP-dependent Clp protease proteolytic subunit)	Stress protein
2	DJPCDFCF_00549	862	VFG0079	0.46	clpC (Chaperone protein ClpB)	Stress protein
3	DJPCDFCF_00799	434	VFG0963	0.55	hasB (Nucleotide sugar dehydrogenase)	Antiphagocytosis; Adherence; Tissue invasion
4	DJPCDFCF_00887	318	VFG1971	0.48	kpsF (KpsF/GutQ family protein)	Adherence; Phase variation
5	DJPCDFCF_00939	542	VFG1855	0.58	htpB (60 kDa chaperonin)	Adherence
6	DJPCDFCF_01015	845	VFG0079	0.45	clpC (ATP-dependent Clp protease ATP-binding subunit ClpC)	Stress protein
7	DJPCDFCF_01134	361	VFG2365	0.64	gmd (GDP-mannose 4,6-dehydratase)	O-antigen
8	DJPCDFCF_01135	390	VFG2364	0.44	fcl (GDP-L-fucose synthase)	O-antigen
9	DJPCDFCF_01304	115	VFG1416	0.51	panD (Aspartate 1-decarboxylase)	metabolic adaptation
10	DJPCDFCF_01438	345	VFG2361	0.46	galE (UDP-galactose 4-epimerase)	O-antigen
11	DJPCDFCF_02188	255	VFG0700	0.44	bcs1 (bifunctional; ribulose 5-phosphate reductase; CDP-ribitol pyrophosphorylase)	Antiphagocytosis
12	DJPCDFCF_02392	420	VFG1823	<0.64	mbtC (3-oxoacyl-)	Iron uptake; Siderophore

[a]VRprofile: a web-based tool for in silico profiling of virulence and antibiotic resistance traits encoded within genome sequences of bacteria. [b]VFDB: a reference database for bacterial virulence factors.

Table 4. List of predicted genes related to extracellular polymeric substances biosynthesis in the complete genome of the *P. mucinisolvens*

Pathway	Function	Locus ID
Wzx/Wzy-dependent pathway	Glycosyltransferase	DJPCDFCF_00841
		DJPCDFCF_00842
		DJPCDFCF_00844
		DJPCDFCF_00852
	Acetyltransferase	DJPCDFCF_00827
		DJPCDFCF_00835
		DJPCDFCF_00845
	Flippase	DJPCDFCF_00787
	Polymerase	DJPCDFCF_00850
	Polysaccharide co-polymerase	DJPCDFCF_00782
	Outer membrane transport protein	DJPCDFCF_01169
Synthase-dependent pathway	Synthase (HasA)	DJPCDFCF_00894
	Precursors of synthesis (HasBC)	DJPCDFCF_00799
		DJPCDFCF_00834
		DJPCDFCF_00851

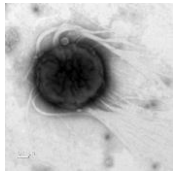


Figure 2. A transmission electron micrograph (TEM) of a negatively stained cell of the *P. mucinisolvens* incubated in mucin agar medium at 39°C for 3 days. Bar, 0.1μm

❖ Characteristic phenotype trait of *P. mucinisolvens* included the formation of branched-shaped extracellular structures
❖ Through Blastp, *P. mucinisolvens* were confirmed the presence of putative extracellular polysaccharide biosynthesis mechanisms, including Wzx/Wzy-dependent pathway and synthase-dependent pathway