

# 读书报告

吉伟利

2016-11-26

# Genomic analysis of six new *Geobacillus* strains reveals highly conserved carbohydrate degradation architectures and strategies

*Phillip J. Brumm*<sup>1,2\*</sup>, *Pieter De Maayer*<sup>3,4</sup>, *David A. Mead*<sup>1,2,5</sup> and *Don A. Cowan*<sup>3</sup>

<sup>1</sup> C5•6 Technologies, Middleton, WI, USA, <sup>2</sup> Great Lakes Bioenergy Research Center, University of Wisconsin, Madison, WI, USA, <sup>3</sup> Centre for Microbial Ecology and Genomics, Genomics Research Institute, University of Pretoria, Pretoria, South Africa, <sup>4</sup> Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, South Africa, <sup>5</sup> Lucigen Corporation, Middleton, WI, USA

Front Microbiol

Published: 12 May 2015

IF:4.165



# Introduction

嗜热木聚糖酶



食品、饲料、制浆造纸、生物脱胶.....

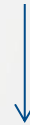
木聚糖是半纤维素的最丰富的形式

纤维素



$\beta$ -1, 4 糖苷键连接的葡萄糖均聚物

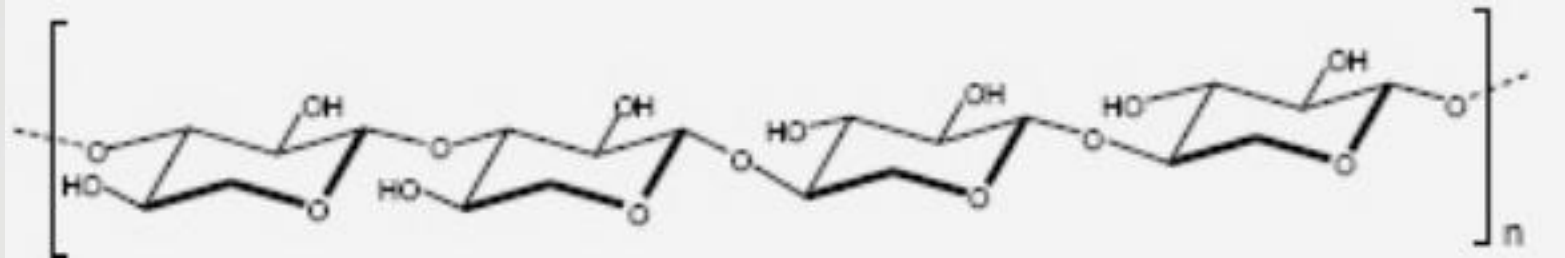
半纤维素



由几种不同类型的单糖构成的异质多聚体  
(主要成分为木糖)



# Introduction



木糖： $\beta$ -1, 4 糖苷键连接的木糖残基作为主链

1

阿拉伯糖

2

半乳糖

3

葡萄糖

4

葡萄糖醛酸

5

乙酰化



未取代的木聚糖、阿拉伯木聚糖、  
葡糖醛酸木聚糖、阿拉伯葡糖醛酸木聚糖.....



# 木聚糖



# Introduction

各种修饰的结果 → 木聚糖的化学组成和结构的多样性



需要宽范围的酶和酶活性来降解

*Geobacillus*  
*Stearothermophilus*  
strain T-6  
(嗜热脂肪土芽孢杆菌)

两种木聚糖酶

$\alpha$ -葡糖醛酸糖苷酶

三种木糖苷酶

阿拉伯呋喃糖苷酶

阿拉伯吡喃糖苷酶



# Introduction

*Geobacillus*  
*stearothermophilus*  
strain **T-6**  
(嗜热脂肪土芽孢杆菌)

23.55kb基因组DNA片段含有细胞外和细胞内木聚糖酶， $\beta$ -木糖苷酶和参与葡萄糖醛酸转运和代谢的12个基因



完整的基因组序列尚未公开 ——> 木聚糖和阿拉伯糖苷代谢的有限理解

## 全基因组测序

嗜热毁丝杆菌 ( *G.thermodenitrificans* )  
嗜热毁丝霉 ( *G. kaustophilus* )  
地芽孢杆菌 菌株GHH01  
地芽孢杆菌 菌株JF8  
热葡萄糖苷酶TNO-09.020  
*G. thermoleovorans* CCB\_US3\_UF5



## Introduction

six novel xylanolytic *Geobacillus* strains

GenBank

JGI

该分析的结果显示，碳水化合物代谢的组织 and 个体基因在整个属中是高度保守的。

此外，许多这些碳水化合物降解簇位于单一的200kb保守基因组区域。



# Materials and Methods

**TABLE 1 | *Geobacillus* genomes sequenced in this work.**

	<b>MC52, MC61, YS93</b>	<b>1MC16</b>	<b>56T2</b>	<b>56T3</b>
Source	Obsidian hot spring WY, USA	Grass compost WI, USA	Double hot springs NV, USA	Sandy's spring west NV, USA
Latitude	44.376262	43.111566	41.051289	40.651893
Longitude	-110.690383	-89.518892	-119.028790	-119.376659
Temperature	79°C	60°C	79.6°C	80°C
pH	6.7	unknown	8.0	7.4

Geobacillus strains were isolated from environmental samples





# Materials and Methods

## YTP-2 (每升)

2.0g 酵母提取物,  
2.0g 胰蛋白胨,  
2.0g 丙酮酸钠,  
1.0g KCl,  
2.0g KNO<sub>3</sub>,  
2.0g Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O  
0.1g MgSO<sub>4</sub>,  
0.03g CaCl<sub>2</sub>,  
8.0g 琼脂  
2.0ml 澄清的番茄汁  
在70°C下培养。



# Materials and Methods

## 制备基因组DNA

1L分离菌株的培养物

在70°C下在以200rpm搅拌的烧瓶中生长18小时



离心收集



SDS和蛋白酶K组合裂解细胞浓缩物



苯酚/氯仿提取分离基因组DNA  
并用RNA酶处理除去残留的RNA



# Materials and Methods

## 样品的内向活性

1.0 ml YT2培养基

70°C 1000rpm 72小时

离心回收细胞

0.1 ml Cellytic IIB试剂处理裂解细胞

0.5 ml含有0.2%AZCL不溶性底物和  
50 µl上清或者

10 µl裂解澄清物的

50mM乙酸盐缓冲液 (Ph5.8)

测定上清或者裂解物样品的内活性

## 样品的外向活性

5.0 µl澄清裂解物

含有10mM 4-甲基伞形酮基的  
琼脂平板上点样

70°C 2小时

手持UV灯检查 阴性阳性对照

测定样品的外向活性



# Materials and Methods

Joint Genome Institute (JGI)

对6种分离的土芽孢杆菌进行基因组测序

6个测序菌株的16s rRNA基因序列

以发表的所有的地芽孢杆菌属的菌株的16s rRNA基因序列

确定新型土芽孢杆菌菌株的系统发育

**TABLE 2 | Enzymatic activities of *Geobacillus* strains grown on various carbohydrate substrates.**

Strain	Pyruvate 丙酮酸盐	Xylose 木糖	Glucose 葡萄糖	Arabinose 阿拉伯糖	XO <sup>a</sup> 木寡糖	AG <sup>b</sup> 阿拉伯半乳聚糖
<b>EXTRACELLULAR ENZYMIC ACTIVITY</b>						
YS93	xylanase	xylanase	n.d.	xylanase	xylanase	xylanase
1MC16	xylanase	xylanase	n.d.	xylanase	xylanase	n.d.
MC52	xylanase	xylanase	n.d.	n.d.	xylanase	xylanase arabinase
MC61	xylanase	xylanase	n.d.	n.d.	xylanase	xylanase arabinase
56T2	xylanase	xylanase	n.d.	xylanase	n.d.	xylanase
56T3	xylanase	xylanase	n.d.	n.d.	n.d.	n.d.
<b>INTRACELLULAR ENZYMIC ACTIVITY</b>						
YS93	n.d.	xylosidase	n.d.	xylosidase	xylosidase	xylosidase
1MC16	n.d.	xylosidase	n.d.	n.d.	n.d.	n.d.
MC61	arabinosidase	xylosidase	n.d.	n.d.	xylosidase	arabinosidase
MC52	arabinosidase	xylosidase	n.d.	n.d.	xylosidase	arabinosidase
56T2	n.d.	xylosidase	n.d.	n.d.	n.d.	n.d.
56T3	n.d.	xylosidase	n.d.	n.d.	n.d.	n.d.
YS93	n.d.	xylosidase	n.d.	xylosidase	xylosidase	xylosidase

*Geobacillus* strains grown and assayed as described in Methods.

<sup>a</sup>XO, xylo-oligosaccharides.

<sup>b</sup>AG, arabinogalactan.

n.d. – none detected.

## Enzymatic activities of *Geobacillus* strains grown on various carbohydrate substrates.

**TABLE 3 | *Geobacillus* sequencing results.**

<i>Geobacillus</i> species	Genome size	Genome contigs	Plasmids	GC content	GenBank ID
YS93	3,993,793	1	2	43.9	NC_015660
1MC16	3,545,187	31	n.d.	48.8	ABVH01000001-ABVH01000031
MC52	3,673,940	1	1	52.3	NC_014915
MC61	3,667,901	1	1	52.3	NC_013411
56T2	3,545,944	1	2	52.4	NA*
56T3	3,650,813	1	0	52.5	NC_014206

*Geobacillus* strains isolated and sequenced as described in Methods. \*Genome available at <http://gp-next.jgi-psf.org:1090/GeospC56T2/GeospC56T2.info.html>.

## Geobacillus sequencing results

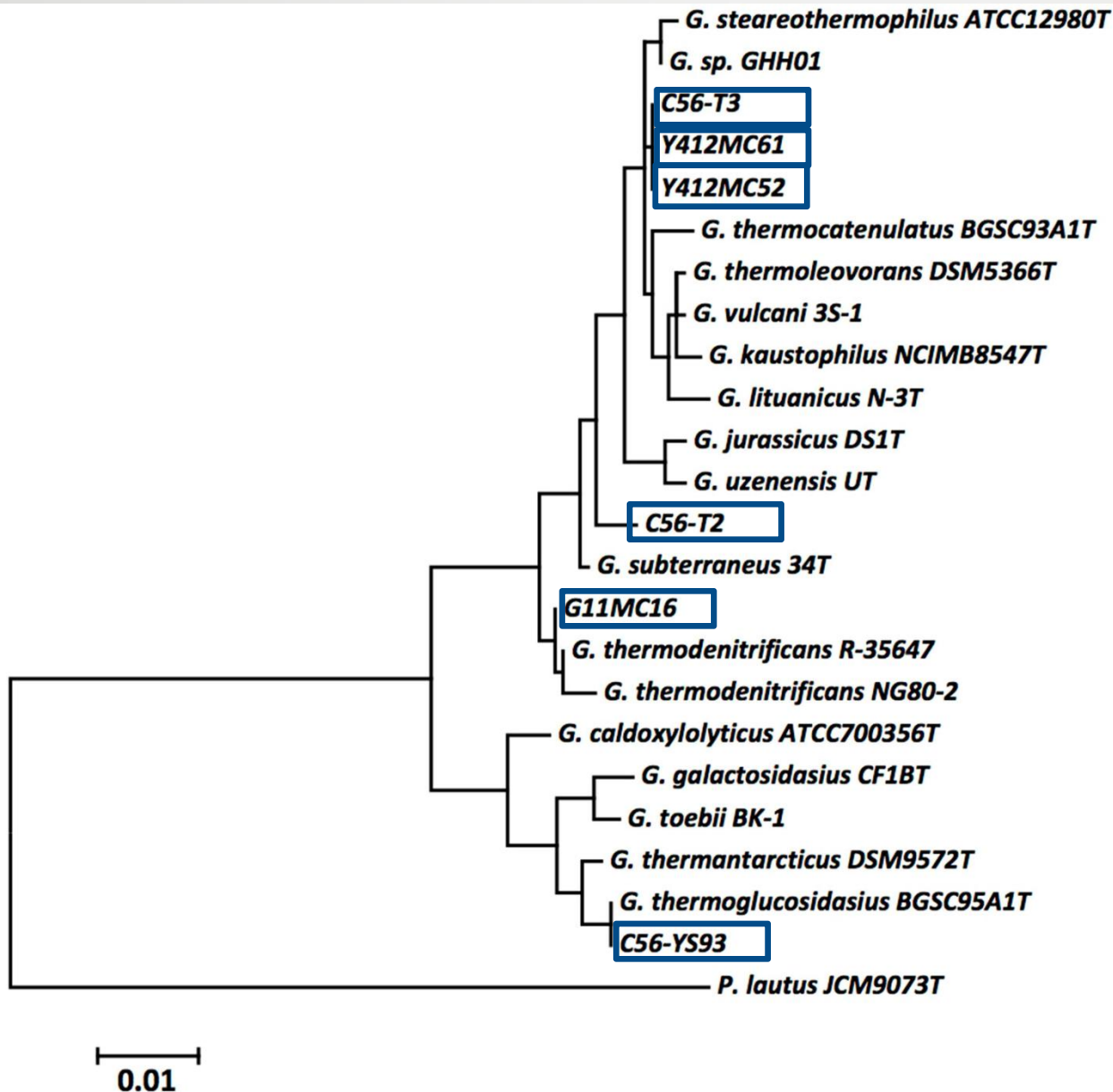


FIGURE 1 | The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). The tree with the highest log likelihood (-3118.4467) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 24 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1260 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura et al., 2011). The type strains of all validly described species are included (NCBI accession numbers): *G. caldoxylolyticus* ATCC700356T (AF067651), *G. galactosidasius* CF1BT (AM408559), *G. jurassicus* DS1T (FN428697), *G. kaustophilus* NCIMB8547T (X60618), *G. lituanicus* N-3T (AY044055), *G. stearothermophilus* R-35646T (FN428694), *G. subterraneus* 34T (AF276306), *G. thermantarcticus* DSM9572T (FR749957), *G. thermocatenulatus* BGSC93A1T (AY608935), *G. thermodenitrificans* R-35647T (FN538993), *G. thermoglucosidasius* BGSC95A1T (FN428685), *G. thermoleovorans* DSM5366T (Z26923), *G. toebii* BK-1T (FN428690), *G. uzenensis* U T (AF276304), and *G. vulcani* 3S-1T (AJ293805). The 16S rRNA sequence of *Paenibacillus lautus* JCM9073T (AB073188) was used to root the tree.

**TABLE 4 | Geobacillus average nucleotide identity (ANI) results.**

Genome 2	YS93	1MC16	MC52	MC61	56T2	56T3
<i>G. caldioxylsilyticus</i> C1C9	83.68	75.96	76.13	76.13	75.82	75.97
<i>G. caldioxylsilyticus</i> NBRC 107762	83.68	76.46	76.01	76.00	76.15	75.89
<i>G. kaustophilus</i> GBlyS	75.92	84.20	96.60	96.61	85.87	96.65
<i>G. kaustophilus</i> HTA426	75.88	83.96	96.73	96.73	85.66	96.84
<i>G. kaustophilus</i> NBRC 102445	75.91	84.21	96.44	96.44	85.68	96.57
<i>G. sp.</i> A8	75.83	84.05	96.72	96.72	85.62	96.65
<i>G. sp.</i> C56-T2	75.40	84.00	85.32	85.39	100.00	85.35
<i>G. sp.</i> C56-T3	75.66	83.79	98.33	98.33	85.34	100.00
<i>G. sp.</i> Y412MC52	75.75	83.77	100.00	100.00	85.30	98.32
<i>G. sp.</i> Y412MC61	75.71	83.83	100.00	100.00	85.36	98.32
<i>G. sp.</i> CAMR12739	75.57	83.70	97.63	97.63	85.12	97.29
<i>G. sp.</i> CAMR5420	75.97	83.91	96.14	96.13	85.39	96.30
<i>G. sp.</i> FW23	75.79	84.05	96.69	96.70	85.60	96.71
<i>G. sp.</i> GH101	76.00	83.85	96.46	96.48	85.44	96.55
<i>G. sp.</i> JF8	75.68	85.60	85.56	85.55	83.69	85.42
<i>G. sp.</i> MAS1	75.79	84.03	96.90	96.90	85.59	96.87
<i>G. sp.</i> WCH70	86.75	75.48	75.62	75.81	75.93	75.42
<i>G. sp.</i> WSUCF1	76.33	83.78	96.20	96.20	85.29	96.31
<i>G. stearothermophilus</i> 22	76.04	84.25	89.57	89.57	84.92	89.53
<i>G. stearothermophilus</i> NUB3621	83.85	76.22	76.25	76.16	75.93	76.21
<i>G. stearothermophilus</i> X1	71.13	70.23	69.81	69.80	69.89	70.03
<i>G. subterraneus</i> PSS2	75.61	83.55	85.37	85.36	97.58	85.37
<i>G. thermocatenulatus</i> GS-1	75.47	83.36	94.66	94.66	84.84	94.88
<i>G. sp.</i> G11MC16	75.71	100.00	83.81	83.88	84.01	83.82
<i>G. thermodenitrificans</i> DSM 465	75.54	99.41	83.66	83.65	83.78	83.65
<i>G. thermodenitrificans</i> NG80-2	75.88	99.46	83.70	83.77	83.98	83.74
<i>G. thermoglucosidasius</i> C56-YS93	100.00	75.63	75.76	75.77	75.41	75.65
<i>G. thermoglucosidans</i> YU	99.35	75.53	75.50	75.72	75.52	75.53
<i>G. sp.</i> Y4.1MC1	99.34	75.72	75.59	75.73	75.44	75.50
<i>G. thermoglucosidasius</i> GT20	99.35	75.56	75.65	75.66	75.54	75.73
<i>G. thermoglucosidasius</i> M10EXG	99.36	75.44	75.53	75.54	75.20	75.48
<i>G. thermoglucosidasius</i> NBRC 107763	99.36	75.80	75.72	75.72	75.81	75.66
<i>G. thermoleovorans</i> B23	75.84	84.09	96.71	96.71	85.53	96.72
<i>G. thermoleovorans</i> CCB_US3_UF5	75.74	84.17	96.72	96.74	85.68	96.65
<i>G. vulcani</i> PSS1	75.47	83.98	91.27	91.26	88.71	91.23

Strains forming a clade with YS93 are shown in bold and highlighted in pink, strains forming a clade with 1MC16 are shown in bold and highlighted in blue, the clade formed by 12MC52, 12MC61, and 56T3 is shown in bold and highlighted in yellow, and 56T2 is highlighted in gray.



Cob:钴胺素生物合成簇	Ara:阿拉伯糖和阿拉伯聚糖利用簇和核糖转运蛋白簇
NO <sub>3</sub> :硝酸还原酶簇	Pep:肽利用簇
Fruc:果糖利用簇	Urea:尿素酶和尿素利用簇
Cell:纤维二糖利用簇	Inos:肌醇磷酸利用簇
NO <sub>2</sub> :亚硝酸还原酶簇	αMan:α-甘露糖苷利用簇
Xyn:木糖和木聚糖利用簇	Glen:葡萄糖酸盐利用簇
	Mtl:甘露醇利用簇

	Cob	NO <sub>3</sub>	Fruc	Cell	NO <sub>2</sub>	Xyn	Ara	Pep	Urea	Inos	αMan	Glen	Mtl
MC52	←	⇒	←	←	←	←	←	←	←			←	←
MC61	←	⇒	←	←	←	←	←	←	←			←	←
56T2	←		←	←	←	←	←		←			←	←
YS93	←	⇒	←	←	←	←		←	←			←	←
56T3	←	⇒	←	←	←	←	←		←			←	←
1MC16	←	⇒	←	←	←	←	←			←	←	←	←

**FIGURE 2 | Diagram of major functional clusters found in the conserved regions; carbohydrate utilization clusters are shown in red, non-carbohydrate clusters in blue.** Cob, cobalamin biosynthetic cluster; NO<sub>3</sub>, nitrate reductase cluster; Fruc, fructose utilization cluster; Cell, cellobiose utilization cluster; NO<sub>2</sub>, nitrite reductase cluster; Xyn, xylose and xylan utilization cluster; Ara, arabinose and arabinan utilization cluster, and ribose transporter cluster; Pep, peptide utilization cluster;

Urea, urease and urea utilization cluster; Inos, inositol-phosphate utilization cluster; αMan, α-mannoside utilization cluster; GLcn gluconate utilization cluster; Mtl, mannitol utilization cluster. The gene sequence values for the corresponding genomes regions are (start-end): MC61, 2635441-2855821; MC52, 1775380-1995757; 56T2, 1737107-1912324; 56T3, 1646809-1858633; YS93, 2080803-2255158; 1MC16, contig ABVH01000004 28446-229812.

# Carbohydrate Clusters Found in the ~ 200 Kb Region of the Sequenced Geobacillus Strains



- 甘露醇代谢
- $\alpha$ -甘露糖苷和肌醇磷酸代谢
- 阿拉伯糖和阿拉伯糖代谢
- 木糖和木聚糖代谢
- 纤维二糖和果糖代谢

## 甘露醇代谢

三组分磷酸转移酶系统 (PTS)

使用磷酸烯醇式丙酮酸将糖运输至细胞中，并将其磷酸化

甘露醇-1-磷酸

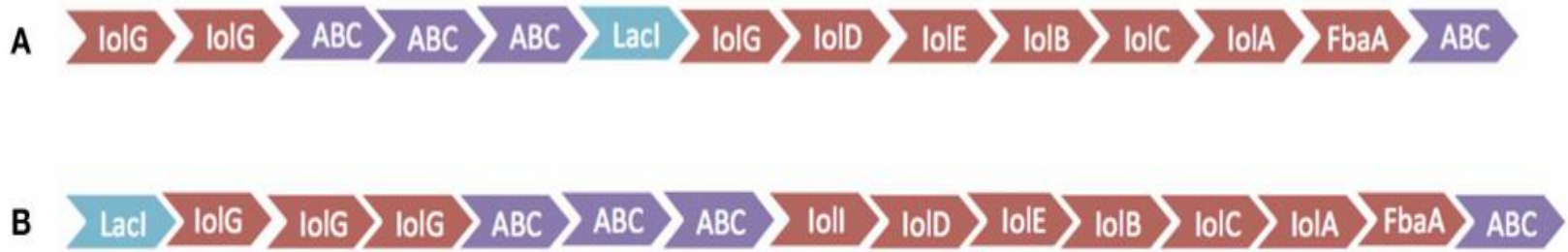
甘露醇-1-磷酸-5-脱氢酶

果糖-1-磷酸

## 1MC16肌醇磷酸代谢簇

**TABLE 5 | 1MC16 Inositol-phosphate metabolic cluster.**

Annotation	Gene
Inositol 2-dehydrogenase, lolG	1528
Oxidoreductase domain protein, lolG	1530
ABC transporter-related protein	1531
ABC-type transport systems, permease	1532
ABC-type sugar transport system, periplasmic component	1533
Transcriptional regulator, LacI family	1534
<i>myo</i> -Inositol 2-dehydrogenase, lolG	1535
Trihydroxycyclohexane-1,2-dione hydrolase, lolD	1536
Inosose dehydratase, lolE	1537
5-Deoxy-glucuronate isomerase, lolB	1538
5-Dehydro-2-deoxygluconokinase, lolC	1539
methylmalonate-semialdehyde dehydrogenase, lolA	1540
Fructose 1,6-bisphosphate aldolase, lolJ	1541



**FIGURE 3 | Diagram of inositol utilization clusters. (A)** Cluster found in 1MC16, *G. thermodenitrificans* DSM 465 and *G. thermodenitrificans* NG80-2. **(B)** Cluster found in *G. kaustophilus* HTA426, *Geobacillus subterraneus* PSS2, *G. thermoglucosidasius* M10EXG, and *G. thermocatenuatus* GS-1.

肌醇利用集群图。

(A) 在1MC16, *G.thermodenitrificans* DSM 465和*G.thermodenitrificans* NG80-2中发现的簇。

(B) 嗜热毁灭霉 HTA426, 地球芽孢杆菌PSS2, 热葡萄球菌M10EXG和热疣菌GS-1中发现的簇。

# 阿拉伯糖和核糖代谢簇

TABLE 6 | Arabinose and ribose metabolic cluster.

	Annotation	YS93	1MC16	MC52	MC61	56T2	56T3
1	Sugar ABC transporter sugar-binding protein	-	1543	-	-	-	1614
2	Multi-sensor signal transduction histidine kinase	-	1544	-	-	-	1615
3	AraC family transcriptional regulator	-	1546	-	-	-	1616
4	ABC transporter substrate-binding protein	-	1547	-	-	-	1617
5	ABC transporter	-	1548	-	-	-	1618
6	Inner-membrane translocator	-	1549	-	-	-	1619
7	GntR family transcriptional regulator	-	1550	1867	2737	1890	1620
8	L-ribulose-5-phosphate 4-epimerase	-	1551	1866	2736	1889	1621
9	L-ribulokinase	-	1552	1865	2735	1888	1622
10	L-arabinose isomerase	-	1553	1864	2734	1887	1623
11	Arabinopyranosidase	-	-	1863	2733	-	-
12	Intracellular endo- $\alpha$ -(1-5)-L-arabinanase	-	-	1862	2732	-	-
13	Family 1 extracellular solute-binding protein	-	-	1861	2731	-	-
14	Binding-protein-dependent transporters inner membrane protein	-	-	1860	2730	-	-
15	Sugar ABC transporter permease	-	-	1859	2729	-	-
16	Extracellular arabinanase	-	-	1858	2728	-	-
17	$\alpha$ -L-arabinofuranosidase	-	-	1857	2727	-	-
18	Unknown 88 a.a. protein	-	-	1856	2726	-	-
19	Family 1 extracellular solute-binding protein	-	1554	-	-	-	1624
20	Binding-protein-dependent transporters inner membrane protein	-	1555	-	-	-	1625
21	Sugar ABC transporter permease	-	1556	-	-	-	1626
22	$\alpha$ -N-arabinofuranosidase	-	-	-	-	1885	-
23	$\alpha$ -L-arabinofuranosidase	-	1557	1855	2725	1884	1627
24	Oxidoreductase domain-containing protein	-	1558	1854	2724	1883	1628
25	Aldose 1-epimerase	-	1569	1853	2723	1878	1629
26	HAD-superfamily hydrolase	-	1559	1852	2722	1881	1630
27	Glycerol-1-phosphate dehydrogenase	-	1560	1851	2721	1880	1631
28	$\beta$ -L-arabinofuranosidase	-	-	1850	2720	-	-



## Results

**TABLE 7 | 56T3 Arabinose and arabinan metabolic cluster.**

<b>Annotation</b>	<b>Gene</b>
Transcriptional regulator, ArsR family	1352
Extracellular solute-binding protein family 1	1353
Binding-protein-dependent transport systems inner membrane component	1354
Binding-protein-dependent transport systems inner membrane component	1355
GH43 Intracellular endo- $\alpha$ -(1-5)-L-arabinanase	1356
GH2 $\alpha$ -L-arabinofuranosidase	1357
Oxidoreductase	1358
Galactokinase, GalK	1361
UDP-glucose 4-epimerase, GalE	1362
Gal-1-phosphate uridylyltransferase, GalT	1363
Transcriptional regulator, LacI family	1364

**TABLE 8 | Xylose and xylan metabolic cluster.**

	<b>Annotation</b>	<b>YS93</b>	<b>1MC16</b>	<b>MC52</b>	<b>MC61</b>	<b>56T2</b>	<b>56T3</b>
1	Integral membrane sensor signal transduction histidine kinase	2272	1564	1849	2719	1877	1634
2	AraC family transcriptional regulator	2271	1565	1848	2718	1876	1635
3	Family 1 extracellular solute-binding protein	2270	1566	1846	2716	1875	1636
4	Binding-protein-dependent transporters inner membrane component	2269	1567	1845	2715	1874	1637
5	Binding-protein-dependent transporters inner membrane component	2268	1568	1844	2714	1873	1638
6	Aldose 1-epimerase	2267	1569	1843	2713	-	1639
7	Polysaccharide deacetylase	2266	1570	1842	2712	-	1640
8	Xylan 1,4-beta-xylosidase	2265	1571	1841	2711	1872	1641
9	Endo-1,4-beta-xylanase	2264	1572	1840	2710	1871	1642
10	Family 1 extracellular solute-binding protein	2262	1574	1839	2709	1873	1643
11	Binding-protein-dependent transporters inner membrane component	2261	1575	1838	2708	1874	1644
12	Binding-protein-dependent transporters inner membrane component	2260	1577	1837	2707	1875	1645
13	$\alpha$ -glucuronidase	2259	1578	1836	2706	1870	1646
14	Xylan 1,4-beta-xylosidase	2258	1579	1835	2705	1869	1647
15	PfkB domain-containing protein	2257	1580	1834	2704	-	1648
16	2-dehydro-3-deoxyphosphogluconate aldolase	2256	1581	1833	2703	1867	1649
17	GntR family transcriptional regulator	2255	1582	1832	2702	1866	1650
18	Uronate isomerase	2254	1583	-	-	-	1651
19	Mannonate dehydratase	2253	1584	1828	2699	1864	1652
20	Short-chain dehydrogenase	2252	1585	1829	2698	1863	1653
21	Hypothetical protein	2251	1586	1827	2697	-	1654
22	Endo-1,4-beta-xylanase	2250	1587	1825	2695	1860	1655
23	Hypothetical protein	2247	1588	1823	2693	1858	1656
24	G-D-S-L family lipolytic protein	-	1589	1822	2692	1857	1657
25	AraC family transcriptional regulator	-	-	-	-	1856	1658
26	Integral membrane sensor signal transduction histidine kinase	-	-	-	-	1855	1659
27	Family 1 extracellular solute-binding protein	-	-	-	-	1854	1660
28	Binding-protein-dependent transporters inner membrane component	-	-	-	-	1853	1661
29	ABC transporter permease	-	-	-	-	1852	1662
30	Arabinofuranosidase/xylosidase	-	1564	-	-	1851	-
31	Xylose isomerase	2243	1565	1818	2688	1850	1664
32	Xylulokinase	2242	1566	1817	2687	1849	1665



# Carbohydrate Clusters Found Outside the ~ 200 Kb Region



- 淀粉代谢
- 半乳糖和半乳糖苷代谢
- 蔗糖代谢

## $\alpha$ -1,4-连接的葡萄糖寡糖代谢簇

**TABLE 9 |  $\alpha$ -1,4-linked Glucooligosaccharide metabolic cluster.**

<b>Annotation</b>	<b>YS93</b>	<b>1MC16</b>	<b>MC52</b>	<b>MC61</b>	<b>56T2</b>	<b>56T3</b>
$\alpha$ -amylase (cyclomaltodextrinase)	-	0573	0632	1510	0721	2858
Extracellular solute-binding protein family 1	-	0572	0633	1511	0722	2857
Binding-protein-dependent transport systems inner membrane component	-	0571	0634	1512	0723	2856
Binding-protein-dependent transport systems inner membrane component	-	0570	0635	1513	0724	2855
Secreted $\alpha$ -amylase	-	0569	0636	1514	0725	2854
Transcriptional regulator, Lacl family	-	0568	0637	1515	0726	2853
Secreted amylopullulanase	-	-	3302	3272	2870	3189
Secreted $\alpha$ -amylase (AmyS)	-	-	3303	3273	2871	3190

## 半乳糖和半乳糖苷代谢簇

**TABLE 10 | Galactose and galactoside metabolic cluster.**

Annotation/corresponding gene	YS93	1MC16	MC52	MC61	56T2	56T3
$\alpha$ -galactosidase	1518	-	2132	0528	-	-
Uncharacterized protein	1519	-	2131	0529	-	-
$\beta$ -galactosidase, GH42	-	-	2130	0530	2119	-
Extracellular solute-binding protein family 1	-	-	2129	0531	2118	-
Binding-protein-dependent transport systems inner membrane component	-	-	2128	0532	2117	-
Binding-protein-dependent transport systems inner membrane component	-	-	2127	0533	2116	-
$\beta$ -galactosidase, GH2	-	1068	2126	0534	2115	-
Galactokinase, GalK	1520	1066	2124	0536	2113	1361
UDP-glucose 4-epimerase, GalE	1521	1065	2123	0537	2112	1362
Gal-1-phosphate uridylyltransferase, GalT	1522	1064	2122	0538	2111	1363
Transcriptional regulator, LacI family	1523	1063	2121	0539	2110	1364

**TABLE 11 | Summary of carbohydrate utilization capabilities.**

	<b>YS93</b>	<b>1MC16</b>	<b>MC52</b>	<b>MC61</b>	<b>56T2</b>	<b>56T3</b>
Fructose	PTS	PTS	PTS	PTS	PTS	PTS
Arabinose	ABC	ABC	ABC	ABC	ABC	ABC
Xylose	ABC	ABC	ABC	ABC	ABC	ABC
Galactose	ABC	ABC	ABC	ABC	ABC	-
Gluconate	PER	PER	PER	PER	PER	PER
Inositol	-	PTS	-	-	-	-
Mannitol	PTS	PTS	PTS	PTS	PTS	PTS
Cellobiose	PTS	PTS	PTS	PTS	PTS	PTS
Sucrose	PTS	-	PTS	PTS	-	-
Lactose	-	-	ABC	ABC	-	-
Starch	-	ABC	ABC	ABC	ABC	ABC
$\alpha$ -Mannosides	-	ABC	-	-	-	-
Arabinan	-	-	ABC	ABC	-	-
Xylan	ABC	ABC	ABC	ABC	ABC	ABC
Panose/pullulan	ABC	ABC	ABC	ABC	ABC	ABC

*Gene clusters for utilization of listed substrates as described in text. ABC, three component ABC transporter system; PTS, three component phosphotransferase system; PER, permease system.*

**恳请各位老师同学  
批评指正**