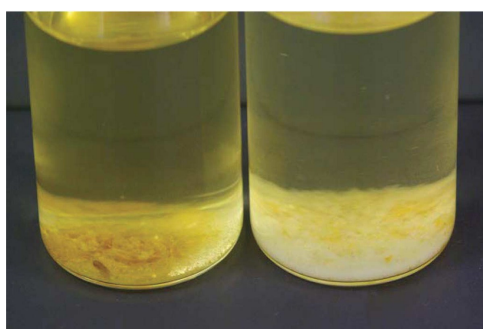




Identification of the *Clostridium cellulovorans* cellulosomal subunits on soft biomass degradation

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Mie University

+ Degradation of 3% rice straw by *Clostridium cellulovorans*



Control

Inoculation with
C. cellulovorans

***C. cellulovorans* can directly degrade soft biomass!!**

GENOME ANNOUNCEMENT

Genome Sequence of the Cellulosome-Producing Mesophilic Organism *Clostridium cellulovorans* 743B[∇]

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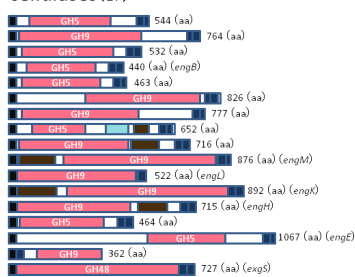
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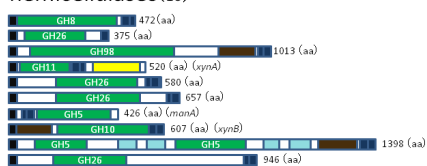
Clostridium cellulovorans 743B was isolated from a wood chip pile and is an anaerobic and mesophilic spore-forming bacterium. This organism degrades native substrates in soft biomass such as corn fiber and rice straw efficiently by producing an extracellular enzyme complex called the cellulosome. Here we report the genome sequence of *C. cellulovorans* 743B.

+ Structure of cellulosome-related proteins from *C. cellulovorans*

cellulases(17)



hemicellulases(10)



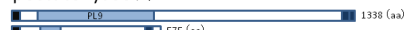
scaffolding protein(4)



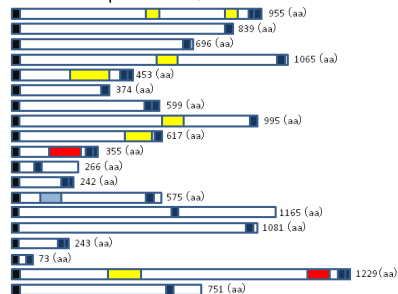
peptidase or protease inhibitor(5)



pectate lyase(2)



the other protein(19)



+ Polysaccharolytic enzyme genes encoding GHs and PLs in the cellulosome-producing clostridia

Organism	Total GHs + PLs	Cellulosomal GHs and PLs		Non-cellulosomal GHs and PLs	
		GHs	PLs	GHs	PLs
<i>C. cellulovorans</i> 743B	92 (100%)	27 (29%)	2 (2%)	53 (58%)	10 (11%)
<i>C. cellulolyticum</i> H10	89 (100%)	43 (48%)	4 (5%)	42 (47%)	0 (0%)
<i>C. thermocellum</i> ATCC 27405	67 (100%)	49 (73%)	4 (6%)	14 (21%)	0 (0%)

GHs, glycosyl hydrolases; PLs, polysaccharolytic lyases.

(Y. Tamaru et al.: Microbial Biotechnol. 2010)

***C. cellulovorans* has a total of 27 genes encoding cellulosomal GHs and PLs which of number was lowest among other cellulosome-producing clostridia.**

+ Cellulosomal large gene clusters are conserved among mesophilic clostridia

Clostridium cellulovorans



Clostridium cellulolyticum



Clostridium josui



Clostridium acetobutylicum



+ Identified cellulosomal proteins by proteome analysis with several carbon sources

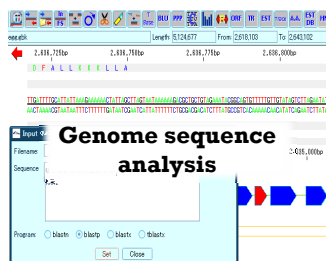
Question:
What kinds of cellulosomal enzymes are most important to degrade soft biomass ?

Table 1 Identified cellulosomal proteins by proteome analysis

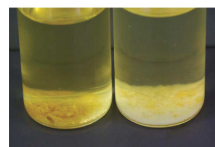
Functions	Gene name	CAZy ^a	Carbon sources			Accession No ^b
			Cellobiose	Avicel	Xylan	
Cellulases						
endoglucanase		GH5		○		ZP_04806172
endoglucanase		GH9	●	●	●	ZP_04806149
endoglucanase		GH5		○		ZP_04806690
endoglucanase		GH5		○		ZP_04804560
endoglucanase		GH5	●	●	●	ZP_04804999
endoglucanase	<i>EngF</i>	GH5	●	●	●	AA039739
endoglucanase	<i>EngH</i>	GH9		○		ZP_04807564
endoglucanase	<i>EngK</i>	GH9		○		ZP_04807563
endoglucanase	<i>EngL</i>	GH9	●	●	●	ZP_04807561
endoglucanase	<i>EngY</i>	GH9	●	●	●	ZP_04804221
Hemicellulases						
mannanase	<i>ManA</i>	GH5	●	●	●	ZP_04807560
mannanase		GH26	●	●	●	YP_003845544
mannanase		GH26	●	●	●	ZP_04805612
mannanase		GH26	●	●	●	ZP_04806148
xylanase	<i>XynA</i>	GH11		○		ZP_04805534
xylanase	<i>XynB</i>	GH10		○		ZP_04807887
exocellulase	<i>ExgS</i>	GH48	●	●	●	AAC38571
Pectate lyases						
pectate lyase		PL1		○		YP_003842527
pectate lyase	<i>PelA</i>	PL9		○		AAG59609
Other proteins						
peptidase inhibitor				○		ZP_04807292
peptidase inhibitor				○		ZP_04807290
peptidase				○		ZP_04804668
salicylic acid-specific 9-O-acetyltransferase				○		ZP_04805106
hypothetical protein				○		ZP_04804379
hypothetical protein			●	●	●	YP_003843744
Scalfoid proteins						
cellulose binding protein	<i>CbpA</i>		●	●	●	AAA23218
hydrophobic protein	<i>HbpA</i>			○		AAF06108

^a Refer to <http://www.cazy.org/>
^b Refer to <http://www.ncbi.nlm.nih.gov/>
 ● (closed circles): Common proteins identified in each substrate
 ○ (open circles): Not common proteins identified in each substrate
(Morisaka H., et al.: AMB express, 2012)

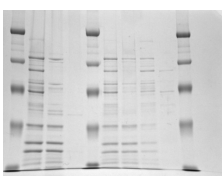
+ Proteome analysis of cellulosomal subunits



Cultivation on a variety of carbon sources



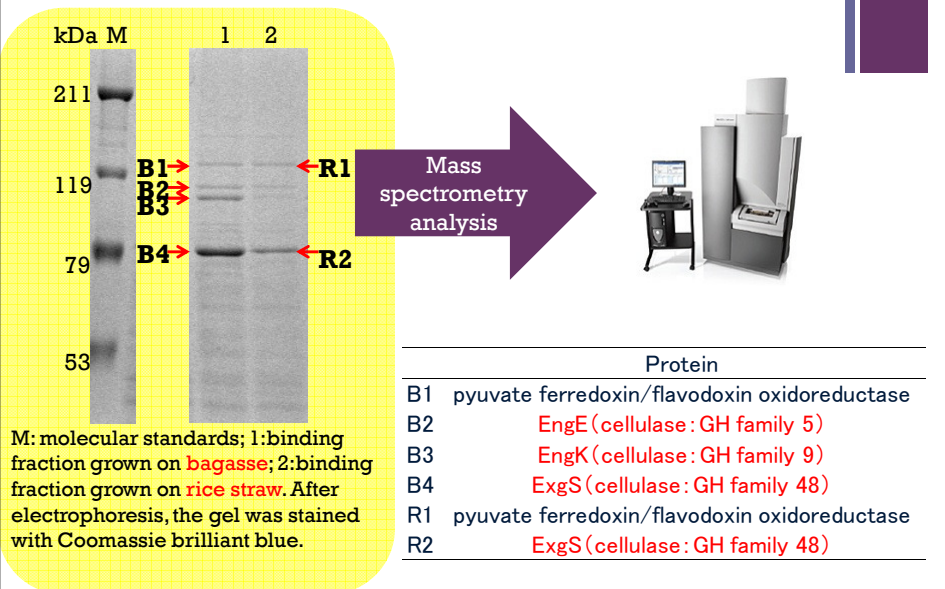
Purify the cellulosomes



Mass spectrometry analysis

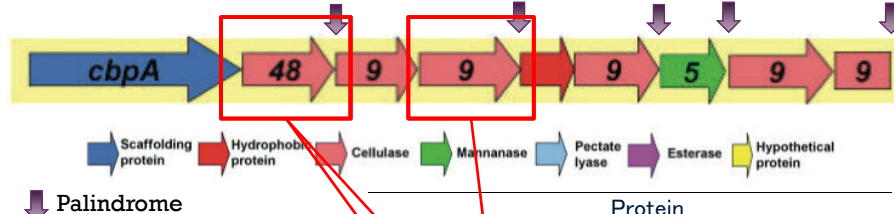


+ Comparison of the cellulosomal subunits between bagasse and rice straw



+ ExgS and EngK are located in the cellulosomal gene cluster

The largest cellulosomal gene cluster in *C. cellulovorans*

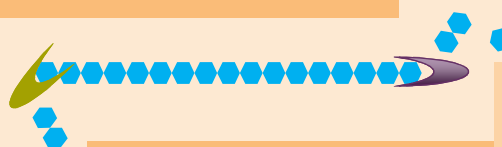


Protein	
B1	pyruvate ferredoxin/ flavodoxin oxidoreductase
B2	EngE (cellulase : GH family 5)
B3	EngK (cellulase : GH family 9)
B4	ExgS (cellulase : GH family 48)
R1	pyruvate ferredoxin/ flavodoxin oxidoreductase
R2	ExgS (cellulase : GH family 48)

+ Enzymatic properties between EngK and ExgS

- Classified into **GH family 48**
- Acts from the reducing end
- Endo-processive cellulase
- Produces main products as cellobiose

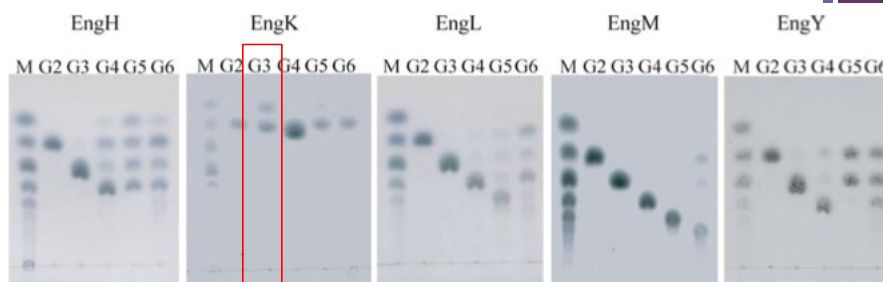
ExgS



EngK

- Classified into **GH family 9**
- Acts from the non-reducing end
- Exo-processive cellulase
- Produces main products as cellobiose
- If cellotriose is cleaved by EngK, glucose and cellobiose are produced.

+ How special property of EngK is?



(T. Arai et al.: Microbial Biotechnol. 2006)

- EngK has enzymatic activity for cellotriose among other cellulosomal GH9.
- EngK is estimated that the enzyme is exo-processive glucanase.

+ Conclusions



- When *C. cellulovorans* was inoculated on rice straw or bagasse, the cellulosomes from each culture supernatants revealed different patterns that depended on each biomass.
- EngK (family 9) was identified in the cellulosome purified from only the culture supernatant of bagasse in comparison that ExgS (family 48) was identified in the cellulosome from the culture supernatants of both rice straw and bagasse.
- Since EngK was detected when *C. cellulovorans* was inoculated by xylan, bagasse was more hemicelulosic substrate than rice straw.

Explanation in detail will be shown in my poster.

+

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