

***Herbivorax saccincola* A7, a novel alkaliphilic and thermophilic anaerobe, effectively degrades xylan-rich lignocellulosic biomass**

Highly efficient degradation of lignocellulosic materials (which contain a mixture of cellulose, hemicellulose, and lignin) by biological saccharification is required for developing a cost-effective method of producing fuel and chemicals from biomass. The known thermophilic anaerobes *Clostridium thermocellum* and *C. clariflavum* can degrade and assimilate cellulose. However, these species cannot utilize xylan, which is the main component of hemicellulose contained in lignocellulosic biomass. The amount of cellulose and hemicellulose in lignocellulosic biomass is similar, hence we explored and isolated a novel thermophilic anaerobe for its ability to degrade and assimilate both cellulose and xylan from a cellulose-degrading bacterial community inhabiting bovine manure compost in Ishigaki Island, Japan (Fig. 1A).

This strain was identified as *Herbivorax saccincola* A7 (hereinafter referred to as A7) based on 16S rRNA gene sequence similarity. A7 is closely related to the cellulose-degrading bacteria of *C. thermocellum* and *C. clariflavum* in the family *Ruminococcaceae* (Fig. 1B). The optimal growth pH of A7 was alkaline pH 9.0, but *C. thermocellum*'s and *C. clariflavum*'s are at around neutral pH (Table 1). The genome size of A7 was 3.76 Mb with a G + C content of 34.9%. The genome contained 3346 protein-coding regions, nine rRNA genes, and 54 tRNA genes from a total of 3642 genes (Table 1). The 38 genes encoding glycosyl hydrolase were contained in the A7 genome. The ratio of xylanase to all glycosyl hydrolase was higher in A7 than in *C. thermocellum* and *C. clariflavum* (Table 1). In addition, A7 has genes that encode related protein to the metabolic pathway of xylose and xylooligosaccharide and for their uptake; *C. thermocellum* and *C. clariflavum*, on the other hand, do not possess these genes (Fig. 2). The metabolic pathway and transporters of xylose and xylooligosaccharide would enable A7 to assimilate xylan.

A7 can degrade lignocellulose biomass at alkaline and high temperature conditions, lowering the risk of bacterial contamination. In addition, the alkaliphilic microbe A7 can tolerate pH decrease with organic acid production by fermentation, sustaining the degradation activity longer than neutrophilic cellulose-degrading bacteria. As mentioned earlier, A7 can also assimilate xylan, thus this strain has great potential to effectively degrade xylan-rich lignocellulosic biomass such as empty fruit bunch, oil palm trunk, and corn stover (Patent application number PCT/JP2017/021784). A7 can be obtained from the Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) and the Japan Collection of Microorganisms (JCM), RIKEN BioResource Center (RIKEN BRC).

(S. Aikawa, A. Kosugi)

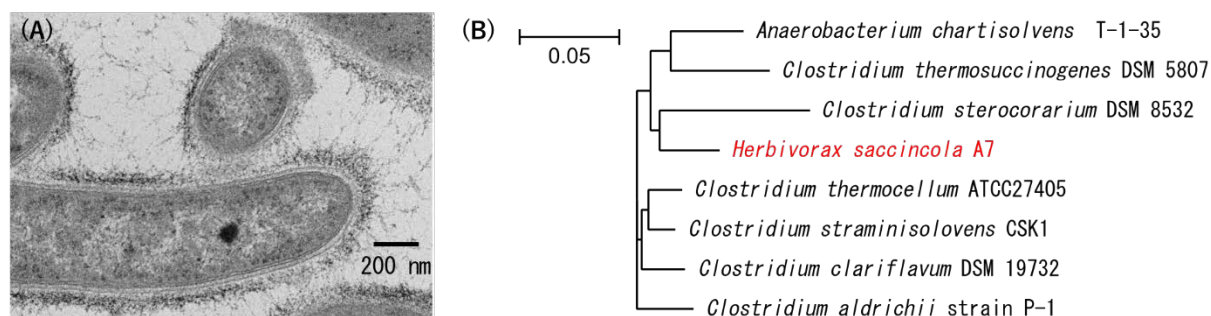


Fig. 1. Electron micrograph (A) and phylogenetic tree (B) of *H. saccincola* A7.

Black-colored bar in photograph is 200 nm long.

Black-colored bar at the upper left of phylogenetic tree shows the ration of different base sequence (0.05).

Table 1. Physiological properties and comparative genome analysis of *H. saccincola* A7 with related species

Characteristic	<i>H. saccincola</i> A7	<i>C. clariflavum</i> DSM 19732	<i>C. thermocellum</i> ATCC27405
Optimal growth pH	9	7.5	7
Xylan assimilation	Yes	No	No
Genome size [Mb]	3.76	4.9	3.84
Total number of genes ^{*1}	3,346	3,906	3,204
Total number of glycolytic enzymes ^{*2}	38	47	42
Number of xylanases	8 (21%) ^{*3}	6 (12%) ^{*3}	1 (2%) ^{*3}

^{*1} Total number of protein coding genes

^{*2} Total number of glycosyl hydrolases containing cellulase, xylanase, and other activities

^{*3} Percentages in parentheses show the ratio of xylanase to total glycosyl hydrolases

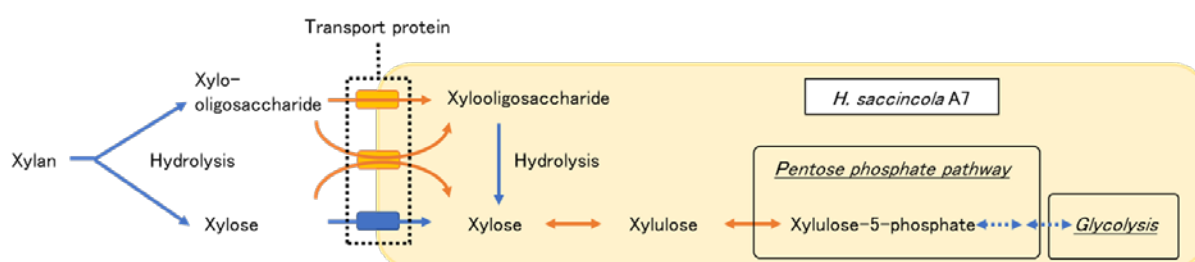


Fig. 2. Metabolic pathway of xylose and xylooligosaccharides in *H. saccincola* A7.

Orange-colored pathways are specific genes in A7 and blue-colored pathways are possessed in common among related species.