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Short Genome Communications

Complete genome sequence of *Maribacter* sp. T28, a polysaccharide-degrading marine flavobacteria

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ABSTRACT

The degradation of plant polysaccharides by enzymes is an industry of increasing importance. Here we present the complete genome sequence of a marine flavobacteria, Maribacter sp. T28 (=CGMCC 1.15788). The genome comprises 4,271,158 bp in a circular chromosome with a G + C content of 34.4% and contains genes encoding xylanolytic, alginolytic and pectinolytic enzymes. Genes encoding alginate lyases and a pectin degradation protein (kdgF) are located on a polysaccharide utilization locus. Maribacter sp. T28 has the ability to utilize xylan, alginate and pectin for growth. The key degradation products xylose and 2-keto-3- deoxy-gluconate were detected from xylan and pectin, respectively. The Maribacter species genomes provide genetic information regarding polysaccharide-degrading enzymes.

Products derived from the degradation of plant polysaccharides have important industrial applications for the paper, food, and feed industries and for fermentation to fuels or chemicals (Harding et al., 2002; Tan et al., 2016). Enzyme technology for conversion of plant polysaccharides advantageously preserve the original carbohydrate structures in the form of sugars, in contrast to traditional chemical conversion, which is accomplished by acid hydrolysis and leads to destruction of the carbohydrates (Horn et al., 2012; Garg et al., 2016). Plant cell wall polysaccharides-degrading enzymes including cellulolytic, xylanolytic and pectinolytic enzymes are used extensively in the industry (Harding et al., 2002; Glöckner and Joint, 2010). Much progress has been made in characterizing the enzymes involved in polysaccharide degradation and the genes of biotechnologically relevant microorganisms encoding these enzymes (Kaur et al., 2011; Singh et al., 2015; Garg et al., 2016).

Members of marine flavobacteria harbor a set of carbohydrate-active enzymes (CAZyme) for polysaccharide decomposition, including glycoside hydrolases and polysaccharides lyases (Tang et al., 2017). We isolated flavobacteria strain T28 from surface seawater off the northeast coast of Taiwan (Supplementary Table S1). Phylogenetic analysis based on 16S rRNA gene sequences showed that strain T28 could be assigned to the genus *Maribacter* belonging to the family *Flavobacteriaceae* within the class *Flavobacteria* (Fig. 1), and that it was most closely related to *Maribacter arcticus* with 97.72% sequence similarity. Here, we report the complete genome sequence of *Maribacter* sp. T28, which possesses enzymes involved in the degradation of xylan, alginate and pectin.

The genomic DNA of T28 was extracted using a TIANamp Bacteria DNA Kit (China) and sequenced using the Pacbio RS II sequencing platform (Pacific Biosciences, Menlo Park, CA, USA) (Supplementary Table S1). The low quality reads were filtered by the SMRT 2.3.0, and the filtered reads were assembled to generate one contig without gaps (Berlin et al., 2015). The final assembled genomes were automatically annotated and analyzed via the RAST platform (http://rast.nmpdr.org). The amino acid sequences were then submitted to the CAZyme Annotation Toolkit (Lombard et al., 2014) (http://mothra.ornl.gov/cgi-bin/cat/cat.cgi) for sequence-based annotation, with an E-value of 1e-40, as well as Pfam-based annotation with an e-value of 0.00001. The results were then further manually checked.

Maribacter sp. T28 has a circular chromosome of 4,271,158 bp with a G + C content of 34.4% (Fig. 2). A total of 3695 genes were predicted in the chromosome, including 42 tRNA, nine rRNA and three sRNA genes (Table 1). Strain T28 genome harbors 38 glycoside hydrolases and six polysaccharides lyases. The genome of strain T28 contains two xylanases (CAZyme superfamily, GH5 and GH43) that can hydrolyze the main chain of xylan to xylose. The T28 genome harbors five genes encoding alginate lyases (CAZyme superfamily, PL6, PL7 and PL17). Four alginate lyase genes in the genome are organized in a predicted polysaccharide utilization locus (PUL) for alginate, along with regulatory elements and transporter systems involved in the binding and uptake of oligosaccharides, including SusD-like oligosaccharide-binding proteins and SusC-like TonB-dependent receptors, as well as other predicted transporters (Fig. 3). The homologous sequences at the gene cluster level in NCBI GenBank were detected using a MultiGeneBlast (v1.1.14) (Medema et al., 2013). More than half of genes in the PUL of

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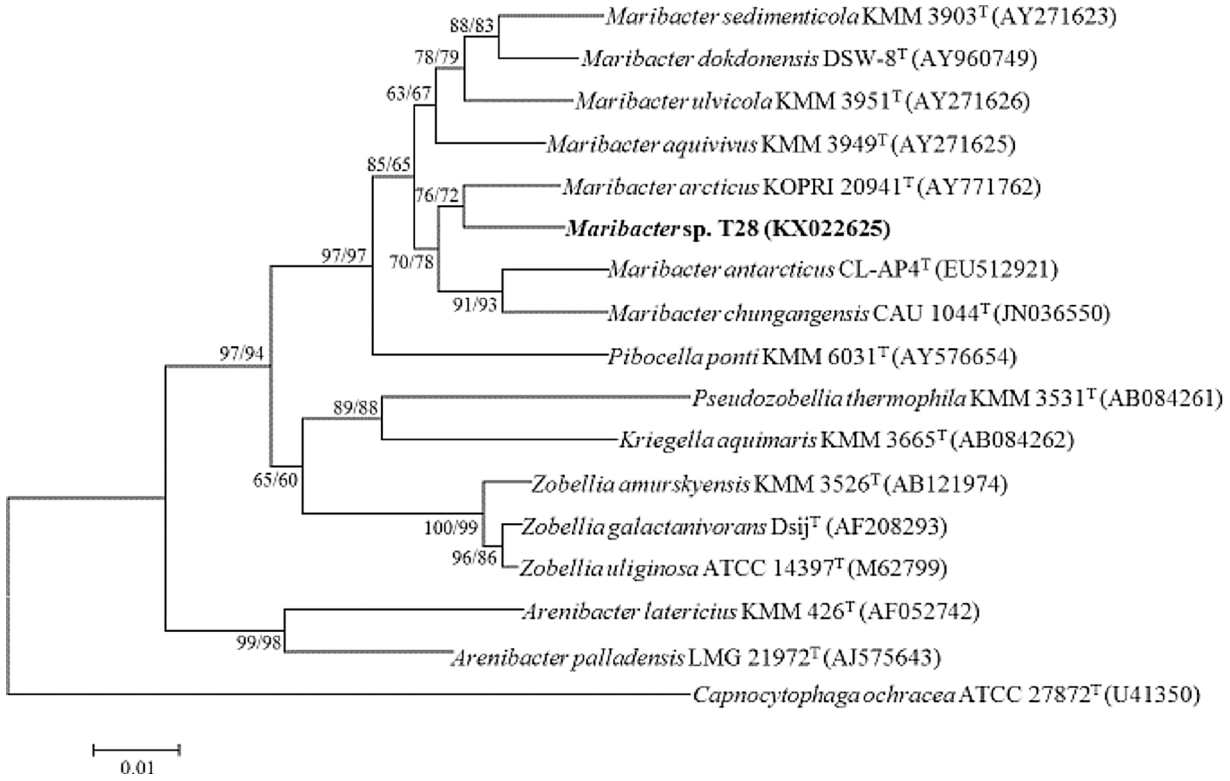

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the position of strain T28 within the genus *Maribacter*. Bootstrap values (using both neighbour-joining and maximum-likelihood algorithms) are shown at nodes as percentages of 1000 replicates. Bar, 0.01 changes per nucleotide position. The Genebank accession numbers are shown in the brackets.

strain T28 were homologous to those in the alginate PUL of a marine flavobacteria *Gramella forsetii* KT0803 (Fig. 3). The alginate PUL in *G. forsetii* KT0803 could function in the degradation of alginate indicated by proteomics analysis (Kabisch et al., 2014). Furthermore, strain T28 contains a gene that encodes a pectin degradation protein, kdgF, as well as other genes (including *kdgK* and *kdgA*) located downstream of the alginate PUL that may have been involved in the degradation of pectin (Fig. 3) (Bauer et al., 2006; Hobbs et al., 2016). Enzyme kdgF contributes to efficient production of the key metabolite 2-keto-3-deoxygluconate (KDG) from lyase-catalyzed depolymerization of pectin and alginate (Hobbs et al., 2016). The gene encoding a pectate lyase (PL9) was found in the genome, which might cleave pectin into small oligogalacturonates during pectin hydrolysis.

Growth experiments showed that strain T28 could utilize xylan, alginate and pectin (Fig. 4). Metabolites were extracted from the quenched strain T28 cells at the exponential growth phase as previously described (Tang et al., 2017), then examined by gas chromatography-mass spectrometry (GC–MS) using an Agilent Technologies 7890 gas chromatograph system coupled to a Pegasus HT time-of-flight mass spectrometer (LECO Corporation, USA). GC–MS analysis of bacterial metabolites showed that the highest concentration of xylose and KDG, which are the degradation products of xylan and pectin, were observed in xylan-grown cells and pectin-grown cells, respectively (Table 2).

Among the 18 available *Maribacter* species genomes, four lack any gene encoding xylanase, and five lack any gene encoding alginate lyases (Table 3). Additionally, seven genomes contain a gene encoding pectate lyase, whereas only strains T28 and HTCC2170 harbored genes encoding both kdgF and pectate lyases (Table 3) and strain DSM21422 genome lacks genes encoding all of these enzymes (Table 3). In addition, the alginate PUL was only found in strain T28 genome. The functional profiles of the CAZyme show variation in *Maribacter* species genomes (Table 3), suggesting that they have different potentials for hydrolyzing polysaccharides. Further growth experiments showed that the four other selected strains possess the ability to degrade alginate, but that they lacked the ability to degrade xylan (two strains) or pectin (four strains) since they lacked genes associated with these polysaccharides degradation (Table 3, Supplementary Figs. S1 and S2).

In summary, this study provides insight into the degradation of xylan, alginate and pectin in *Maribacter* sp. T28. The *Maribacter* species genomes provide genetic information regarding enzymes that are specific for the degradation of polysaccharides.

Strain and nucleotide sequence accession numbers

The strain has been deposited in China General Microbiological Culture Collection Center (=CGMCC 1.15788) and Japan Collection of Microorganisms (=JCM 31510). The complete genome sequence has been deposited in GenBank under the accession CP018760.

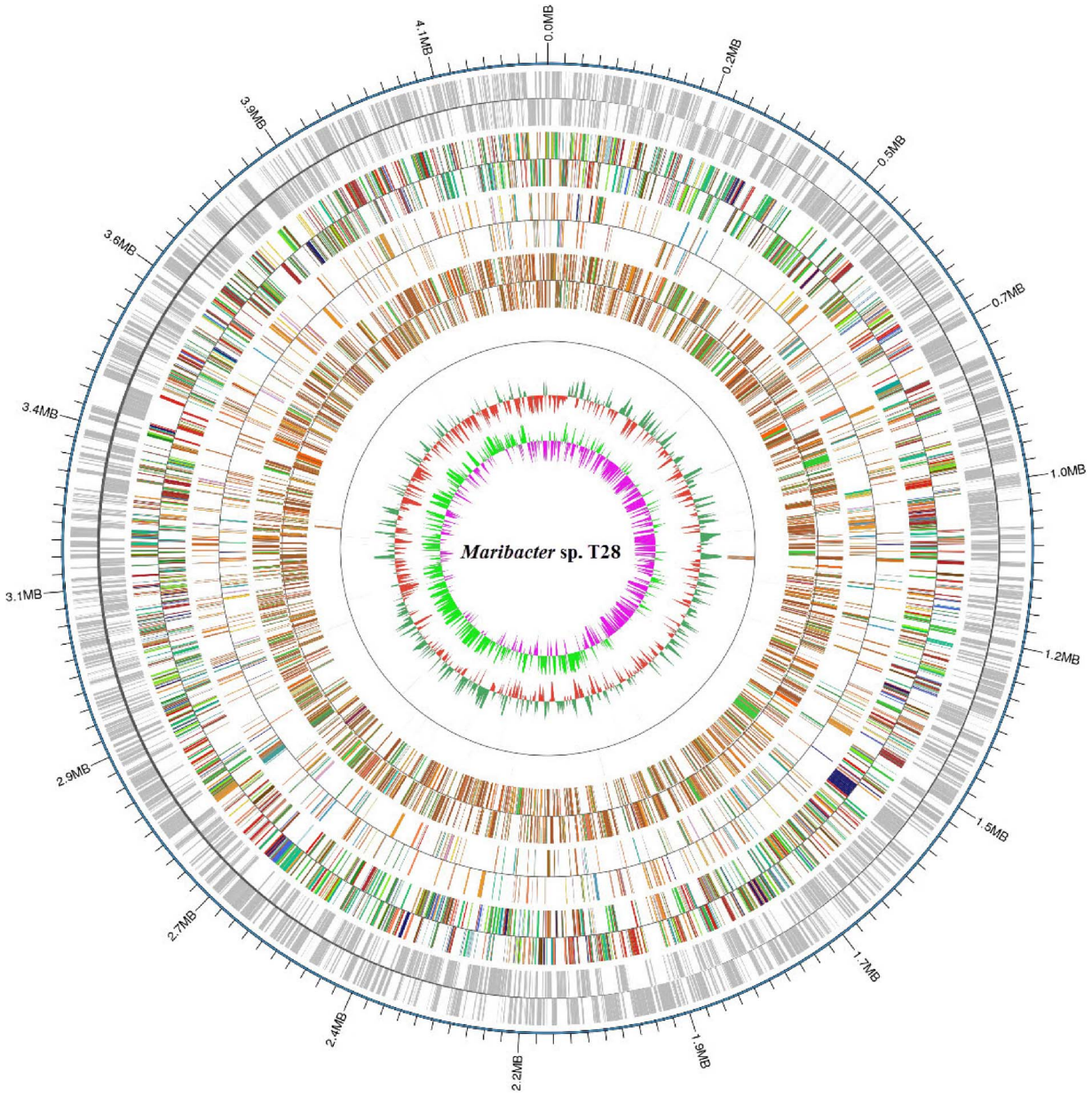

Fig. 2. Complete genome map of *Maribacter* sp. T28. From the outermost circle to the innermost circle, circle represents the coding genes (circles 1,2), COG (3,4), KEGG (5,6) and GO (7,8) function categories, tRNA/rRNA/sRNA genes (9), GC content (10) and GC skew (11), respectively.

Table 1
Genomic features of the strain T28.

Features	Value
Genome size (bp)	4,271,158
Contig numbers	1
G + C content (%)	34.4
Total number of genes	3695
rRNA genes	9
tRNA genes	42

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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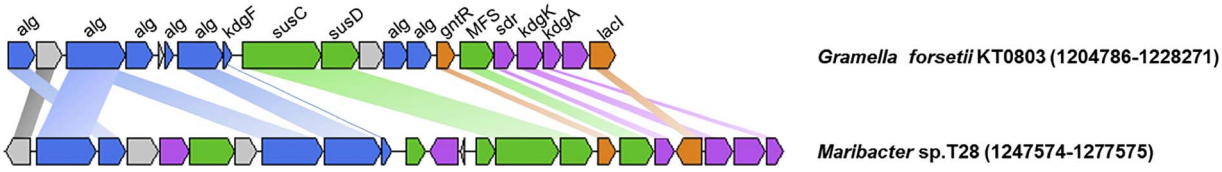

Fig. 3. Genetic organization of the alginate PULs in two bacteria. The range of sequences of the gene cluster in the genome is shown in a bracket. The functions of the proteins are color-coded: blue, hydrolase; green, transporters; purple, other enzymes; orange, regulation factor; grey, unknown function. Homologous genes are connected by colored bars between two PULs. *alg*, Alginate lyase; *kdgF*, Pectin degradation protein; *sdr*, Short-chain dehydrogenase/reductase; *kdgK*, 2-dehydro-3-deoxygluconokinase; *kdgA*, Keto-hydroxyglutarate-aldolase; *susC*, TonB-dependent receptor; *susD*, Carbohydrate-binding protein; *MFS*, Hexuronate transporter; *gntR*, GntR family transcriptional regulator; *lacI*, LacI family transcriptional regulator. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

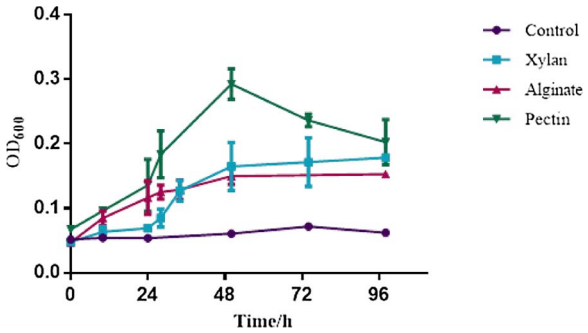

Fig. 4. Growth curves of strain T28. Bacteria were cultured in marine minimal media (2.3% (w/v) sea salts, 0.05% (w/v) yeast extract, 0.05% (w/v) NH_4Cl and 50 mM Tris-HCl, pH 7.8), with a final concentration of 0.2% of one of the following carbon sources: xylan, alginate and pectin. Cultures using only minimal media were treated as controls.

Table 2
Relative fold-change in selected metabolites in strain T28 grown in a marine minimal medium with xylan and pectin as a carbon source (28 °C), respectively (*t*-test, $p < 0.05$)*.

Metabolite	Similarity[a]	R.T.[b]	Unique Mass	log_2 fold change (xylan/control)[c]	log_2 fold change (pectin/control)[c]
Xylose	864	15.32	217	7.40	4.70
2-keto-3-deoxy-gluconate	675	17.99	93	4.31	7.54

*. Metabolites extraction and identification as Tang et al., 2017.
[a] Similarity, the LECO/Fiehn Metabolomics Library was used to identify the compounds.
[b] R.T., retention time of gas chromatography.
[c] Fold-change values using the ratios of the mean of standardized peak intensities between two groups of samples. Cultures using only marine minimal media were treated as controls.

Table 3
Distributions of genes encoding polysaccharide-degrading enzymes among the *Maribacter* species genomes.

Genome Name/Gene Name	xylanase	alginate lyase	pectate lyase	kdgF
M. sp. T28*	3	5	1	1
M. sedimenticola DSM19840*	1	5	1	–
M. dokdonensis DSW-8*	1	9	–	–
M. forsetii DSM18668*	–	8	–	–
M. ulvicola DSM15366*	–	7	1	–
M. sp. HTCC2170	3	–	1	1
M. thermophilus HT7-2	1	3	–	–
M. sp. 1_2014MBL_MicDiv	–	8	–	–
M. arcticus DSM23546	1	–	1	–
M. sp. Hel_I _7	1	7	1	–
M. aquivivus DSM16478	1	8	–	–
M. orientalis DSM16471	2	–	–	–
M. stanieri DSM19891	1	7	–	–
M. antarcticus DSM21422	–	–	–	–
M. sp. MAR_2009_60	1	8	–	–
M. polysiphoniae DSM23514	4	5	–	–
M. dokdonensis MAR_2009_71	1	8	–	–
M. sp. MAR_2009_72	1	–	1	–

–, absent. *, five strains selected for growth experiment and the positive results: alginate, all strains; xylan, strains T28, DSM19840 and DSW-8; pectin, strain T28.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jbiotec.2017.08.009.

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