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Genomic analysis of a lignocellulose degrading strain from the underexplored genus *Meridianimaribacter*

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Abstract

The genus *Meridianimaribacter* is one of the least-studied genera within *Cytophaga-Flavobacteria*. To date, no genomic analysis of *Meridianimaribacter* has been reported. In this study, *Meridianimaribacter* sp. strain CL38, a lignocellulose degrading halophile was isolated from mangrove soil. The genome of strain CL38 was sequenced and analyzed. The assembled genome contains 17 contigs with 3.33 Mbp, a GC content of 33.13% and a total of 2982 genes predicted. Lignocellulose degrading enzymes such as cellulases (GH3, 5, 9, 16, 74 and 144), xylanases (GH43 and CE4) and mannanases (GH5, 26, 27 and 130) are encoded in the genome. Furthermore, strain CL38 demonstrated its ability to decompose empty fruit bunch, a lignocellulosic waste residue arising from palm oil industry. The genome information coupled with experimental studies confirmed the ability of strain CL38 to degrade lignocellulosic biomass. Therefore, *Meridianimaribacter* sp. strain CL38, with its halotolerance, could be useful for seawater based lignocellulosic biorefining.

Keywords: *Meridianimaribacter*; genomics; lignocellulolytic enzymes; empty fruit bunch; halophile

1.0 Introduction

Lignocellulosic biomass which comprises of cellulose, hemicellulose, lignin and pectin, is the most abundant form of fixed carbon on Earth (10^9 tons/annum) and its breakdown is a critical component of the global carbon cycle [1]. It is of major interest due to its renewable and sustainable nature as a feedstock to replace fossil fuels for the production of biofuels and chemicals [2]. The use of agricultural residues including rice straw, nut shell, maize stover and oil palm empty fruit bunch (EFB) as feedstocks are favourable as they are not used for food and are not compromising food security [3-5]. Palm oil is the most widely used edible oil and the waste generated at palm oil mills is currently creating a major disposal problem particularly EFB, shells, and fruit palm kernel, in addition to waste produced in the plantations, such as palm trunks and fronds. The generation of large amounts of lignocellulosic waste are mainly in the form of EFB [5]. The valorization of this abundant waste is highly encouraged in countries like Malaysia and Indonesia.

Many bacteria are able to decompose plant biomass by secreting lignocellulolytic enzymes, including cellulases, hemicellulases, ligninases and pectinases [6-9]. These enzymes have been further classified, based on structure in the Carbohydrate-Active Enzyme database (CAZy), into glycosyl hydrolases (GHs), carbohydrate esterases (CEs), polysaccharide lyases (PLs) and auxiliary activities (AAs) [10]. Common well-known bacteria such as *Bacillus* spp., *Brevibacillus* spp., *Cellulomonas* spp., *Streptomyces* spp. and *Pseudomonas* spp. have been widely studied for their biomass degrading abilities for biorefining applications [6, 11, 12]. Lignocellulolytic enzymes from thermophiles, for example, *Anoxybacillus* spp. and *Geobacillus* spp. have also received considerable attention due to their enzyme stability [13, 14].

Bacteria affiliated to *Cytophaga-Flavobacteria* have also been found to be wood degrading prokaryotes based on 16S rRNA amplicon analyses [15, 16]. To date, limited studies have been reported on wood degradation of this group of bacteria and culturing these bacteria remains challenging [15]. Among these studies, some of the members of *Cytophaga-Flavobacteria* such as *Flavobacterium* sp. and *Chryseobacterium* sp. isolated from decaying wood were recently found to be able to produce lignocellulolytic enzymes to degrade wheat straw [17, 18]. Other members of *Cytophaga-Flavobacteria* for instance *Zhouia*, *Aquibacter*, *Flavimarina*,

Seonamhaeicola, *Hanstruepera* and *Meridianimaribacter* are so far underexplored, and these genera are represented by less than three species each [19]. Furthermore, degradation of lignocellulose by these *Cytophaga-Flavobacteria* has not been studied.

To date, only one species from the genus *Meridianimaribacter* has been reported [20]. Here we describe the genomic analysis of a halophilic bacterium which is able to produce extracellular cellulases and hemicellulases and show that the strain is phylogenetically related to *Meridianimaribacter*.

2.0 Materials and methods

2.1 Bacterial isolation and identification

Mangrove soil was collected from Tanjung Piai, Johor, Malaysia (1°16'06.0"N 103°30'31.2"E) with the permit number of CJB F No. 734342. A 0.1 ml of serially diluted sample was transferred onto marine agar (MA) 2216 (BD Difco) and incubated at 35°C for 1 to 7 days. The isolates were then inoculated on MA plates supplemented with 10.0 g/L carboxymethyl cellulose (CMC) (Merck) and 5.0 g/L xylan from beechwood (Sigma) respectively for qualitative screening of cellulolytic and xylanolytic abilities. These plates were then stained with Congo red for MA-CMC and Lugol's iodine for MA-xylan. The screening was regarded as positive if clear zone formed surrounding the colonies.

The bacterial genomic DNA (strain CL38) was extracted using DNeasy Blood and Tissue kit (Qiagen) and was purified using DNA Clean and Concentrator™-25 (Zymo Research) following the manufacturer's protocol. The purity and concentration of harvested genomic DNA were checked using a Nanodrop™ 1000 spectrophotometer and Qubit® 3.0 fluorometer (Thermoscientific) respectively, which were 2.03 (A_{260/280}) and 820 ng/μl. The 16S rRNA gene of strain CL38 was amplified using primers: 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1525R (5'-AAGGAGGTGWTCCARCC-3') [21] with conditions as stated [22]. Sequencing of 16S rRNA gene was performed at Apical Scientific Pte. Ltd., Seri Kembangan, Malaysia. The nearly full length of 16S rRNA gene was trimmed with Bioedit software and searched against sequences in EzBioCloud [23] and National Center for Biotechnology Information (NCBI) databases for bacterial identification.

2.2 Genome sequencing, assembly and annotation

The whole-genome shotgun sequencing of strain CL38 was performed using pair-end sequencing (2×150 bp) in an Illumina HiSeq 2500 sequencing platform (Illumina, California, USA). After sequencing, the reads with low-quality nucleotides, contained N nucleotides and overlapped with adapter were eliminated. The quality-filtered data (1,356 Mbp data size) was *de novo* assembled using SOAPdenovo version 2.04 [24] into contigs and then scaffolds. Gaps between contigs were closed and optimized by krskgf (version 1.2) and gapclose (version 1.12). The resulting assembled genome was annotated and analyzed using NCBI Prokaryotic Genome Annotation Pipeline (PGAP) version 4.5 [25]. Protein coding genes were predicted by GeneMarkS+ using “best-placed reference protein set” method [26]. Non-coding RNA sequences were anticipated by tRNAscan-SE version 1.21 [27], rRNAmmer version 1.2 [28] and Rfam version 12.0 [29] for tRNA, rRNA and small nuclear RNA respectively. The functional prediction of protein coding genes was accomplished by searching against Clusters of Orthologous Groups (COG) via WebMGA server [30] utilizing RPS-BLAST version 2.2.15 [31]. Metabolic pathways were predicted using KEGG Automatic Annotation Server (KAAS) [32].

2.3 Phylogenetics and average nucleotide identity (ANI)

Multilocus sequence typing (MLST) analysis was performed on three housekeeping genes: 16S rRNA, *rpoB* and *dnaK*. The sequences of housekeeping genes were acquired from genome data, aligned separately and concatenated in the following order: *16S rRNA-rpoB-dnaK*. The phylogenetic trees of 16S rRNA gene and concatenated housekeeping genes were constructed by neighbor joining method [33] using Molecular Evolutionary Genetics Analysis (MEGA) version 7.0 [34] with following parameters: bootstrap value based on 1000 replications [35] and Kimura 2-parameter model (K2P). The phylogenetic tree based on whole genome sequence was built using REALPHY 1.12 [36] and edited using MEGA 7.0. The ANI values based on BLAST (ANiB) and MUMmer (ANIm) between strain CL38 and its closely related taxa were determined by JSpeciesWS server [37].

2.4 Comparative genomic analyses

The genome of the only type species: *Meridianimaribacter flavus* NH57N^T (Ga0079837) is available at DOE-JGI Integrated Microbial Genomes/Expert Review (IMG/ER) system and was downloaded for the comparative genomic analyses. The analyses of COGs using RSP-BLAST through WebMGA server, metabolic pathways using KAAS and genes of CAZymes using dbCAN 2 meta server (refer to section below) were performed and the results were compared for both strains.

2.5 Analysis of CAZymes and mining of lignocellulose degrading genes

Putative genes for carbohydrate active enzymes (CAZymes) encoded in the genome of strain CL38 were classified by dbCAN (using HMMER), CAZy (using DIAMOND) and PPR (using Hotpep) databases respectively, in the integrated dbCAN2 meta server using default settings [38]. The resulting data was imported into R studio and organized. The further screening of CAZyme genes was performed manually and confirmed based on the rule that the sequence shall at least be recognized by two of the aforementioned databases. The lignocellulose degrading genes were further revealed by cross-checking with the annotations available in CAZy database [10]. Other features of the lignocellulose degrading genes were assessed using InterProScan 5 [39].

2.6 Degradation of oil palm empty fruit bunch by monitoring lignocellulolytic enzymes activities

For production of lignocellulolytic enzymes by strain CL38, empty fruit bunch (EFB) biomass obtained from a palm oil mill at Johor, Malaysia was used as an induction biomass. Prior to use, the collected EFB was washed, dried and grinded into 2 mm fine fibre form. A 5% (v/v) inoculum of strain CL38 was cultured in a medium (pH 7.5) containing yeast extract (1.0 g/L), peptone (5.0 g/L), MgCl₂ (5.0 g/L), MgSO₄·7H₂O (2.0 g/L), CaCl₂ (0.5 g/L), KCl (1.0 g/L), NaCl (20.0 g/L) and EFB (10.0 g/L), incubated at 35°C and 180 rpm for 24 to 96 h.

The flask containing EFB and cells was centrifuged at 4°C and 4500 rpm for 20 min and the cell free supernatant was used as a crude enzyme extract for assays. Endoglucanase, exoglucanase, 1,4-β-xylanase and β-mannanase activities were determined by 3,5'-dinitrosalicylic acid (DNS) method [40] using 1% (w/v) CMC,

Avicel[®], xylan from beechwood and locust bean gum as substrate, accordingly. While β -glucosidase and β -xylosidase assays were conducted by using 5mM of *p*-nitrophenyl- β -D-glucopyranoside (*p*NPG) and *p*-nitrophenyl- β -D-xylopyranoside (*p*NPX) as substrate, respectively. All enzyme assays (biological triplicates) were carried by incubating the crude enzymes and substrate (1:1 ratio) in a reaction tube containing 50 mM sodium phosphate buffer (pH 7.5), at 35°C for 30 min. The optical density at 540 nm (for reducing sugar assays using DNS) and at 430 nm (for detecting *p*-nitrophenol (*p*NP) released from *p*NPG/ *p*NPX) were subsequently measured. For all the assays, one unit of enzyme activity (U/ml) is defined as the amount of enzyme that releases 1 μ mol of respective product per minute under assay conditions.

The remaining EFB obtained was washed three times using 1 \times PBS added with 0.5% Tween 20, dried at 60°C and weighed on an electronic balance until constant weight was obtained. The weight loss of EFB was calculated by comparing to control (EFB without inoculation). The structural changes of EFB before and after bacterial inoculation were observed under Phenom Pro G5 scanning electron microscope (SEM) (Phenom-World BV) with accelerating voltage of 10 kV and 1000 \times magnification.

2.7 Data access

The genome sequence of *Meridianimaribacter* sp. strain CL38 has been deposited at DDBJ/ENA/GenBank and DOE-JGI Genome Online Database (GOLD) under accession **QKWS00000000** and GOLD analysis project ID: **Ga0311192**, respectively. The 16S rRNA gene of strain CL38 is available at DDBJ/ENA/GenBank under accession **MH819767**.

3.0 Results and discussion

3.1 Taxonomic affiliation of strain CL38

A halophilic bacterium with cellulolytic and xylanolytic capabilities, designated as strain CL38, was isolated from soil collected from a mangrove forest at Tanjung Piai, Johor, Malaysia. The bacterium was found to be Gram negative, yellow pigmented and grew well in range of 0.5 – 9% (w/v) NaCl. To identify this strain taxonomically, the 16S rRNA gene was amplified and compared with genomic data to obtain the

full-length sequence (1514 bp). Based on sequence searches against EzBioCloud and NCBI databases, the 16S rRNA gene of strain CL38 is 100% identical (97% query coverage and E value of 0.0) to *Meridianimaribacter flavus* strain NH57N^T (FJ360684.1), which was isolated from Mischief Reef of the South China Sea [20].

Besides the 16S rRNA gene, the alignment of housekeeping genes: *rpoB* and *dnaK* of strain CL38 with strain NH57N^T demonstrated 99% similarity. The 16S rRNA, concatenated housekeeping genes and whole genome sequences phylogenetic analyses of strain CL38 demonstrated that it clustered with strain NH57N^T with 100% of support (Fig. 1), and with distinct lineage to other closely related genera including *Mangrovimonas*, *Psychroserpens*, *Hanstruepera* and *Seonamhaeicola*. The pairwise ANIb and ANIm values between strain CL38 and strain NH57N^T were 97.80% and 98.06% respectively (S. Table 1), indicating that strain CL38 could be a new strain of *M. flavus*.

3.2 Genome metrics of strain CL38

The genome of strain CL38 was sequenced and its genome features are summarized in Table 1. The assembly generated 17 contigs in 17 scaffolds, with a mapped coverage of 395-fold of the genome. The largest contig is 1,143,547 bp with N₅₀ and N₉₀ values 595,671 bp and 230,604 bp respectively. These contigs were joined and the genome size of strain CL38 determined to be 3,332,696 bp with a GC content of 33.13% (Table 1). The genome size is slightly greater than strain NH57N^T (3.22 Mbp) and both genomes of *Meridianimaribacter* spp. are notably larger than its close relative in the family: *Mangrovimonas yunxiaonensis* LY01^T (2.67 Mbp) [41]. The GC content of strain CL38 is similar to related members: *Winogradskyella psychrotolerans* RS-3^T, *Psychroserpens mesophilus* JCM 13413^T and *Seonamhaeicola aphaedonensis* CECT 8487^T (33.4 – 33.7%). In contrast, *Mangrovimonas yunxiaonensis* LY01^T has higher GC content (39.3%) as compared to strain CL38.

The composition of the genome of strain CL38: protein coding genes, non-coding RNA genes, pseudogenes and putative horizontal transferred genes is displayed in Table 1, with total of 2982 genes predicted using NCBI PGAP annotator. A closer analysis of the protein coding genes demonstrated that a total of 801 genes (27.33%) are assigned as hypothetical proteins, with 77 genes sharing no similarity

to any protein sequences in NCBI nr-protein database. These statistics suggested that more than 1/4 fraction of protein coding genes of strain CL38 could potentially serve as novel targets for future experimental studies [42]. Apart from that, a total of 17 horizontal transferred genes was encoded in the genome of strain CL38 (S. Table 2). These genes which originated from other genera such as *Mangrovibacter*, *Thioalkalivibrio* and *Reichenbachiella* could lead to phenotypic variation and new adaptability of strain CL38 [43].

3.3 Strain CL38 versus the only species of *Meridianimaribacter*

Genomes of strain CL38 and strain NH57N^T were compared in aspects of COGs, KEGG pathway and CAZymes. Based on Table 2, strain CL38 and strain NH57N^T seem to have a slightly different COG assignment profile. Both strains have the highest counted genes under the classification of general function prediction (12.95 – 13.36%) followed by amino acid transport and metabolism (8.97 – 9.30%). The quantity of genes related to carbohydrate metabolism according to COG classification in strain CL38 represents 5.34% in the genome and higher than strain NH57N^T (4.72%), as described in Table 2. This group of genes shows the highest difference (0.62%) between both strains.

Following the closer inspection in KEGG metabolic pathway analysis, both strains encode parallel set of genes in the metabolism of starch and cellulose to produce glucose (S. Fig. 1A). Strain CL38 encompasses an extra fructokinase (EC: 2.7.1.4) to phosphorylate D-fructose to D-fructose-6-phosphate in sucrose metabolism (S. Fig. 1A). Additionally, there are seven genes presented in strain CL38 that are connected to pentose and glucuronate interconversions, which are absent in its counterpart (S. Fig. 1B).

The genomes of both strains were annotated in dbCAN 2 meta server for CAZymes analyses, as shown in Fig. 2. Considering one annotated protein may contain more than one CAZyme domains, the total number of CAZyme domains may be higher than total annotated proteins. The genome of strain CL38 encompasses a total of 106 annotated proteins with 112 CAZyme domains, while only 90 annotated proteins with 94 CAZyme domains were detected in the genome of strain NH57N^T.

Taken individually, the annotated domains of CAZymes in strain CL38, including GHs, CEs, PLs and glycosyl transferases (GTs) are consequentially more

abundant than strain NH57N^T (Fig. 2). In total, 44 GHs, 10 CEs, 9 PLs and 44 GTs were found in strain CL38 while strain NH57N^T has 39 GHs, 7 CEs, 1 PL and 42 GTs. Both strains do not possess encoded proteins assigned to auxiliary activities (AA) and they incorporate the same number of annotated carbohydrate binding modules (CBMs) (2 CBM48, 1 CBM32, 1 CBM50 and 1 CBM57). These CBMs whilst non-catalytic promote the proximity of associated catalytic domains with the polysaccharide [44]. Usually the CBM is attached with catalytic domains of CAZymes, however, CBM50 in both strains are not associated to any known catalytic domains.

In depth analysis of CAZyme differences between both strains (Fig. 3) revealed that several CAZyme domains, CE8, CE10, CE12, GH28, GH100, GH105, GT11, PL6, PL7, PL10 and PL17 are unique to strain CL38. Some of these CAZyme domains are responsible for pectin degradation such as CE8, CE12 and PL10 [45]. While PL6, PL7 and PL17 are categorized as alginate lyase, a group of enzymes that deconstruct alginate, a key component of brown algae consisting of 40 % its dry weight [46]. In another study, it was found that purified halotolerant PL6, PL7 and PL17 could degrade algae material which is known to contain large amounts of salt. [47-50]. This suggests the potential application of PL6, PL7 and PL17 in marine algae degradation to release simple sugars as feedstock for bioethanol production. Apart from that, strain CL38 has an additional GH5 (WP_019386503.1) and GH43 (WP_007845049.1) which are linked to cellulose and hemicellulose deconstruction respectively (Fig. 3).

The divergence of these two strains, in particular in the composition of their CAZymes, most likely are a result of the different environmental niches they occupy. While strain NH57N^T was isolated from Mischief Reef of the South China Sea, the strain CL38 was isolated from a mangrove saline environment, an area located along ocean coastlines with numerous trees tolerant to salt and is associated with muddy sediments influenced by tidal waves [51]. These environments, situated in equatorial regions are some of the most productive in the world [52], and as such microorganisms, including strain CL38, have adapted to utilize biomass from this environment as carbon sources for growth. Therefore, the expansion of CAZymes in CL38 is not surprising.

3.4 Lignocellulose degrading enzymes of strain CL38

Depolymerization of complex plant biomass into simple sugar requires the synergistical action of wide spectrum of lignocellulolytic enzymes such as GHs, CEs, PLs and associated domains of CBMs. Based on Table 3, a total of 30 encoded proteins in the genome of strain CL38 were found to be related to lignocellulose degradation. Among these proteins, 11 are categorized as cellulases, including members of the GH3, GH5, GH9, GH16, GH74 and GH144 families, which are key enzymes that contribute to the decomposition of cellulose into glucose [6]. Only one GH9 was found in the genome of strain CL38 and members from this family have previously been described as cellulases exhibiting either endoglucanase or cellobiohydrolase activities [53]. Sequence analysis through InterProScan detected a Type 9 Secretion System (T9SS) present at the C-terminal of the GH9 in strain CL38 (S. Fig. 2A), with 69% similarity to GH9 with a T9SS from *Winogradskyella pacifica* (WP_115807879.1) based on BLASTp search. T9SS was recently reported as a novel secretion system involved the degradation of complex biopolymers, and has been reported in the phylum *Bacteroidetes* [54] under genera *Porphyromonas* [55], *Flavobacterium* [56], *Rhodothermus* and *Cytophaga*. Specifically, the T9SS was detected in a xylanase from *Rhodothermus marinus* [57] and cellulolytic enzymes sequences from *Cytophaga hutchinsonii* [58].

Six β -glucosidases (3 GH3, 2 GH144 and 1 GH16) that release glucose from the hydrolysis of beta-D-glucosides and oligosaccharides [10], were also revealed in the genome of strain CL38 (Table 3). Interestingly, all the GH3s are fused with fibronectin type 3 domains (S. Fig. 2B). This domain could promote cellulose hydrolysis, as demonstrated with the cellobiohydrolase from *Clostridium thermocellum* [59].

A variety of annotated GHs and CEs of strain CL38 are predicted to participate in hemicellulose degradation. A total of 15 hemicellulases, including xylanases and mannanases were found in the genome of strain CL38, as depicted in Table 3. For instance, 1,4- β -xylanase (GH43 sub-family 28 associated with CBM32) and β -xylosidase (GH43 sub-family 10) work cooperatively on xylan to release xylose [7]. In addition, a series of mannanases: β -mannanase (GH5 sub-family 7), β -mannosidase (GH26) and α -galactosidase (GH27) were annotated in strain CL38. These enzymes were reported to be essential for mannan degradation (the second

major part of hemicellulose beside xylan) to liberate mannose as simple sugars [8]. Notably, two hemicellulases were found coupled with CBMs: GH43 sub-family 28 and GH2, attached with CBM32 and CBM57 respectively (Table 3). A GH associated with a CBM was proved to have a better efficiency in hydrolyzing cellulose and hemicellulose as compared to GHs without their CBM domains [60].

3.5 *Empty fruit bunch decomposition by strain CL38: reveal its polysaccharide degrading abilities*

The ability of strain CL38 to degrade lignocellulose was investigated by inoculating strain CL38 in medium with EFB. Structural and weight changes of EFB were monitored over a period of time. Around 22.4% of EFB was lost after a 96 h incubation period (Fig. 4A), compared to less than 0.05% of EFB reduction was observed in control (EFB without cells). In terms of structural changes (Fig. 4B), EFB before inoculation was smooth and intact, the fibrils were rigid and in a fairly ordered manner [Fig. 4B(i)], as described elsewhere [61]. The structure of EFB was significantly altered after 96 h of incubation with strain CL38 [Fig. 4B(ii)] with rough and broken surfaces being observed.

In terms of lignocellulolytic enzyme activities including endoglucanase, exoglucanase, β -glucosidase, β -1,4-xylanase, β -xylosidase and β -mannanase, changes in activities were monitored across different incubation periods (24 h, 48 h, 72 h and 96 h) (Fig. 5). Five lignocellulolytic enzymes activities were detected (except for β -1,4-xylanase) during the first 24 hours of incubation, with highest activities seen for exoglucanase and β -mannanase (Fig. 5). Subsequently, all tested lignocellulolytic enzymes showed variation in activities across 48 h and 72 h of incubation. Diminished activities were seen for four of the lignocellulolytic enzymes at 96 h (endoglucanase, exoglucanase, β -1,4-xylanase and β -mannanase). Both β -glucosidase and β -xylosidase activities remained steady throughout the incubation period.

Exoglucanase activity reached its peak after 24th h of incubation, while no activity was detected by 96 h. Unlike exoglucanase, the peak activities for endoglucanase, β -1,4-xylanase and β -mannanase were observed after 48 h of incubation (Fig. 5). In other study, the enzyme activity of β -1,4-xylanase and endoglucanase from *Exiguobacterium* sp. AS2B were monitored throughout 6 days

of incubation using wheat bran as carbon source. Maximum activities of β -1,4-xylanase and endoglucanase were found at 24 h and 72 h of incubation respectively [62]. While for *Klebsiella* sp. MD21, by utilizing saw dust as substrate, the maximum activities for endoglucanase, exoglucanase and β -1,4-xylanase were observed at 96 h of incubation [63]. Collectively, these indicated that the maximum activity of lignocellulose degrading enzymes could occur at different time point depends on different bacteria and/or lignocellulosic substrates.

Based on analysis of the genome (Table 3), GH9 and GH74 could potentially contribute to exoglucanase activity. Two GH5 (sub-family 26 and 42) present in genome of strain CL38 (Table 3) are annotated as endoglucanases, these enzymes are likely to responsible for the activity disclosed. The presence of β -1,4-xylanase and β -mannanase activities in this study could be contributed by GH43 sub-family 28 associated with CBM32 and GH5 sub-family 7 respectively (Table 3).

To the best of our knowledge, this is the first genome sequence and analysis describing the biomass degradation (particularly EFB) by *Meridianimaribacter* sp. strain CL38. Such genomic and experimental data could provide new lignocellulose degrading enzymes for biomass processing.

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Conflict of interest statement

All authors declared that they have no conflicts of interest.

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Fig. 1 Phylogenetic trees of strain CL38 constructed using neighbor-joining method with respect to related members based on 16S rRNA (A), concatenated housekeeping genes *16S rRNA-rpoB-dnaK* (B) and whole genome sequences (C). The sequences of *Capnocytophaga canis* were used as an outgroup. Bootstrap values (>50%) based on 1000 replications are expressed as percentages at nodes. The scale bar represents the substitutions per nucleotide position.

Fig. 2 Number of CAZymes domains (GHs, CEs, PLs, GTs and CBMs) encoded in genome of strain CL38 and strain NH57N^T, annotated using dbCAN 2 meta server.

Fig. 3 Comparative analysis in terms of the CAZymes abundance between strain CL38 (1) and strain NH57N^T (2).

Fig. 4 Empty fruit bunch (EFB) degradation by strain CL38 with respect to total biomass weight loss (A) and structural changes (B). EFB structure before inoculation (i) and after 96 h of incubation (ii), viewed under scanning electron microscope.

Fig. 5 Lignocellulolytic enzymes activities of strain CL38 across different incubation period. Mean values (n=3) are expressed and standard deviations are indicated as error bars.

S. Fig. 1 KEGG metabolic pathway annotated in genome of strain CL38 and strain NH57N^T: starch and sucrose metabolism (A) and pentose & glucuronate interconversions (B). Green coloured box, genes annotated in both strain CL38 and strain NH57N^T; yellow coloured box, genes annotated exclusive for strain CL38 only.

S. Fig. 2 Domain organization of GH9 (A) and GH3s (B) encoded in genome of strain CL38. SP, signal peptide; E set, Immunoglobulin E-set domain; GH, glycosyl hydrolase; T9SS, Type 9 secretion system; FN3, fibronectin type 3 domain.

Table 1 General genome statistics of strain CL38 and strain NH57N^T.

| Category | Strain CL38 | | Strain NH57N ^T | |
|---------------------------|-------------|------------|---------------------------|------------|
| | Number | % of total | Number | % of total |
| Number of contigs | 17 | - | 16 | - |
| Number of scaffolds | 17 | - | 14 | - |
| Genome size (bp) | 3,332,696 | 100.00 | 3,223,525 | 100.00 |
| G + C content | 1,104,122 | 33.13 | 1,069,019 | 33.16 |
| Total genes predicted | 2982 | 100.00 | 2894 | 100.00 |
| Protein coding genes | 2930 | 98.26 | 2827 | 97.68 |
| with COGs | 2230 | 74.78 | 2139 | 73.91 |
| connected to KEGG pathway | 1313 | 44.03 | 1300 | 44.92 |
| Noncoding RNA genes | 43 | 1.44 | 52 | 1.80 |

| | | | | |
|---------------------------------------|----|------|----|------|
| rRNA genes | | | | |
| 5S rRNA | 1 | 0.03 | 3 | 0.10 |
| 16S rRNA | 1 | 0.03 | 3 | 0.10 |
| 23S rRNA | 1 | 0.03 | 3 | 0.10 |
| tRNA | 36 | 1.21 | 39 | 1.35 |
| ncRNA | 4 | 0.13 | 4 | 0.14 |
| Pseudogenes | 9 | 0.30 | 15 | 0.52 |
| Putative horizontal transferred genes | 17 | 0.57 | 34 | 1.17 |

Table 2 Clusters of Orthologous Groups (COGs) assignment of protein coding genes of strain CL38 and NH57N^T.

| COG class | COG functional categories | Strain CL38 | | Strain NH57N ^T | |
|-----------|---|-------------|----------------|---------------------------|----------------|
| | | Count | Percentage (%) | Count | Percentage (%) |
| R | General function prediction only | 298 | 13.36 | 277 | 12.95 |
| E | Amino acid transport and metabolism | 200 | 8.97 | 199 | 9.30 |
| M | Cell wall/membrane/envelope biogenesis | 199 | 8.92 | 195 | 9.12 |
| S | Function unknown | 170 | 7.62 | 164 | 7.67 |
| J | Translation, ribosomal structure and biogenesis | 154 | 6.91 | 157 | 7.34 |
| K | Transcription | 141 | 6.32 | 132 | 6.17 |
| P | Inorganic ion transport and metabolism | 119 | 5.34 | 113 | 5.28 |
| G | Carbohydrate transport and metabolism | 119 | 5.34 | 101 | 4.72 |
| T | Signal transduction mechanisms | 107 | 4.80 | 102 | 4.77 |
| C | Energy production and conversion | 106 | 4.75 | 107 | 5.00 |
| H | Coenzyme transport and metabolism | 102 | 4.57 | 101 | 4.72 |

| | | | | | |
|---|---|----|------|-----|------|
| L | Replication, recombination and repair | 98 | 4.39 | 102 | 4.77 |
| O | Posttranslational modification, protein turnover, chaperones | 98 | 4.39 | 97 | 4.53 |
| I | Lipid transport and metabolism | 94 | 4.22 | 81 | 3.79 |
| F | Nucleotide transport and metabolism | 64 | 2.87 | 64 | 2.99 |
| V | Defense mechanisms | 53 | 2.38 | 49 | 2.29 |
| U | Intracellular trafficking, secretion, and vesicular transport | 41 | 1.84 | 40 | 1.87 |
| Q | Secondary metabolites biosynthesis, transport and catabolism | 39 | 1.75 | 29 | 1.36 |
| D | Cell cycle control, cell division, chromosome partitioning | 20 | 0.90 | 20 | 0.94 |
| N | Cell motility | 7 | 0.31 | 7 | 0.33 |
| Z | Cytoskeleton | 1 | 0.04 | 0 | 0.00 |

Table 3 List of potential lignocellulose degrading enzymes found in the genome of strain CL38.

| Category | CAZy family | Activities in the family | Locus tag (scaffold: gene position) |
|-----------|----------------------|--------------------------|--|
| Cellulase | GH3 | β -glucosidase | Scaffold1: 415157 – 417442 |
| | | | Scaffold3: 138356 – 140503 |
| | | | Scaffold3: 469288 – 471630 |
| | GH5 sub-family 26 | Endoglucanase | Scaffold1: 392649 – 393644 |
| | GH5 sub-family 42 | Endoglucanase | Scaffold1: 618054 – 619568 |

| | | | |
|-----------|-------------------------------|---|--|
| | GH9 | Endoglucanase/Exoglucanase | Scaffold1: 412891 – 415044 |
| | GH16 | Glucan endo-1,3- β -D-glucosidase | Scaffold3: 137595 – 138359 Scaffold3: 140663 – 142294 |
| | GH74 | Endoglucanase/Exoglucanase | Scaffold1: 862541 – 865630 |
| | GH144 | β -glucosidase | Scaffold3: 472429 – 473796 Scaffold3: 473797 – 475179 |
| Xylanase | GH43 sub-family 28 with CBM32 | 1,4- β -xylanase | Scaffold3: 462208 – 464016 |
| | GH43 sub-family 10 | β -xylosidase | Scaffold1: 189046 – 190662 |
| | CE4 | Polysaccharide deacetylase | Scaffold5: 183683 – 184324 |
| Mannanase | GH5 sub-family 7 | β -mannanase | Scaffold1: 411532 – 412884 |
| | GH26 | β -mannosidase | Scaffold1: 404605 – 405762 Scaffold1: 409991 – 411529 Scaffold1: 1118683 – 1119762 |
| | GH27 | α -galactosidase | Scaffold1: 393641 – 394852 |
| | GH130 | β -1,4-mannooligosaccharide phosphorylase | Scaffold1: 407607 – 408794 |
| Other | GH2 with | β -galactosidase | Scaffold3: 466675 |

| | | | |
|---------------|----------------|--|-------------------|
| hemicellulase | CBM57 | | – 469278 |
| | GH2 | β -galactosidase | Scaffold1: 389791 |
| | | | – 392574 |
| | | | Scaffold1: 582786 |
| | | | – 585224 |
| | GH28 | Glycosyl hydrolase | Scaffold1: 171089 |
| | | | – 172441 |
| | | | Scaffold1: 185327 |
| | | | – 186763 |
| | GH53 | Arabinogalactan endo-1,4- β -galactosidase | Scaffold1: 581626 |
| | | | – 582783 |
| Pectinase | GH105 | Glycosyl hydrolase | Scaffold1: 187796 |
| | | | – 189040 |
| | CE12 | Rhamnogalacturonan acetyl esterase | Scaffold1: 172445 |
| | | | – 173221 |
| | PL1 sub-family | Pectate lyase | Scaffold1: 173208 |
| 2 | | – 175208 | |
| | PL10 + CE8 | Pectate lyase | Scaffold1: 184000 |
| | | | – 185316 |

Highlights

- This is the first genome analysis of genus *Meridianimaribacter*.
- Genome data mining of *Meridianimaribacter* sp. strain CL38 revealed that a series of lignocellulose degrading enzymes such as cellulases (GH 3, 5, 9, 16, 74 and 144), xylanases (GH43 and CE4) and mannanases (GH5, 26, 27 and 130) are encoded in the genome.
- The genome information coupled with experimental studies confirmed the abilities of strain CL38 in degrading lignocellulosic biomass, therefore could be useful for lignocellulosic biorefining.