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Research Article

Characterization of β-glucosidases from *Meridianimaribacter* sp. CL38

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ABSTRACT

The production of second-generation biofuel requires a huge amount of freshwater. It is estimated that at least three gal of freshwater is used to produce one gal of biofuel. The replacement of freshwater with seawater serves as a potential alternative in biofuel generation. Therefore, salt-tolerant enzymes play an important role in saccharification and fermentation process. Halophilic β-glucosidase is one of the key enzymes for the process. In this study, the β-glucosidase of halophile *Meridianimaribacter* sp. CL38 isolated from mangrove soil was characterized. Strain CL38 achieved maximum production of β-glucosidase at 12th hour of growth. The β-glucosidase showed highest activity at 2% (*w/v*) NaCl while highly stable at salt concentration ranging from 1-2% (*w/v*) (more than 96% of relative activity). Its β-glucosidase activity remained active in the presence of 5mM Mn^{2+}, Mg^{2+}, Ca^{2+} ions, and 1% (*v/v*) Tween-20 and Tween-80. The draft genome sequence of strain CL38 was retrieved from GenBank database and submitted to dbCAN meta server for CAZymes annotation. Strain CL38 harbors 44 GHs and GH3 are annotated as β-glucosidases. The β-glucosidases of *Meridianimaribacter flavus* (99.61%) and *Mesoflavibacter sabulilitoris* (97.44%) showed the closest identity with Bgl3a and Bgl3b protein sequences from strain CL38, respectively. Glycoside hydrolase family 3 domain was identified in both enzymes via InterPro scan server. The presence of signal peptides indicated that both enzymes were secreted extracellularly. Five motifs were identified in Bgl3a and Bgl3b, with the active site (nucleophile) found at Asp296 and Asp297, respectively. Collectively, these β-glucosidases could be potentially used in the biofuel production, in particular the lignocellulosic biomass pretreatment process. This is the first attempt to characterize the β-glucosidase in genus *Meridianimaribacter* as so far none of the lignocellulolytic enzymes from this genus were characterized.

Keywords: β-glucosidase, GH3, Halophiles, Lignocellulosic biomass, Meridianimaribacter

Introduction

Fossil fuel is the main source of energy that has been widely used worldwide [1, 2]. Due to the extensive use of fossil fuels, it is currently facing a depletion crisis [3]. The combustion of fossil fuels is also known to cause air pollution and greenhouse effect [1]. Collectively, this encouraged scientists to seek new energy sources. Second-generation biofuel seems to be a good replacement for fossil fuels [4]. The production of the second-generation biofuel from lignocellulosic biomass is a sustainable process. It is not only able to ensure a continuous supply of energy source but also help to reduce the environmental pollution arising from agricultural waste processing[5].

The production of biofuel requires a vast amount of freshwater. Around three to four gal of freshwater is needed to produce one gal of biofuel [6]. Hence, seawater has been proposed to replace freshwater in the process [7]. Seawater has a general salinity of 3.2% (*w/v*) [8]. As the saccharifica-

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tion and fermentation of the lignocellulosic biomass involve microbial degradation and enzymatic reaction, seawater uses biofuel production requires halophiles or enzymes with halotolerant characteristics [9–11]. A wide range of places promotes halophiles growth, for instance, oceans, salt lakes, coastal lagoons, and estuaries [12]. Besides, the mangrove environment is also a place with a high possibility of isolating salt-tolerant microorganisms that secrete halotolerant enzymes [13, 14]. As the mangrove environment consists of a high amount of organic matter (i.e., weathered log and leaf litter), it is also a good place for discovering novel lignocellulolytic enzymes [15].

Lignocellulose biomass consists mainly of cellulose, hemicellulose, and lignin [16]. In among, cellulose occupies nearly 40-60% of the lignocellulose biomass [17]. Due to this reason, cellulase is the key enzyme for the conversion of lignocellulosic waste into biofuel [18]. Cellulase is a group of enzymes, including endoglucanase, exoglucanase, and β-glucosidase. These three enzymes work synergistically to achieve complete saccharification of the cellulose into glucose [19]. β-glucosidase plays the most significant role as it governs the rate-limiting enzyme reaction during cellulose complete degradation [19]. Without β-glucosidase, the intermediate products (i.e., cellobiose and cellotriose) will accumulate and this will cause feedback inhibition on endoglucanase and exoglucanase activities [20].

Meridianimaribacter sp. CL38 was isolated from mangrove soil collected from Tanjung Piai, Johor, Malaysia (1°16′06.0″N 103°30′31.2″E) [11]. It is a bacterium identified as Gram-stained negative, yellow-pigmented, rod-shaped, and considered moderate halophile that can thrive in salt concentrations up to 9% (*w/v*) [11]. According to a previous genomic study, strain CL38 is closely related to *Meridianimaribacter flavus* NH57N, suggesting that both strains belong to the same genus and species [21]. Nonetheless, the genome of strain CL38 was found to encode different lignocellulolytic genes [11]. Based on the preliminary screening, strain CL38 showed positive results in some lignocellulose degradation tests, including β-glucosidase, endoglucanase, and exoglucanase [11]. When writing, none of the lignocellulolytic enzymes in *Meridianimaribacter* genus had been biochemically characterized and reported. Therefore, in this project, the β-glucosidase of strain CL38 was characterized and bioinformatics analysis of these β-glucosidases was also carried out.

Material and Methods

Bacterial culture of Meridianimaribacter sp. CL38

Meridianimaribacter sp. CL38 was isolated from mangrove soil by Lam et al. [11] and preserved in 20% (*v/v*) glycerol stock. In this project, the bacterium was resuscitated from the stock and was inoculated onto marine agar (pH 7.6). The agar plate culture was incubated at 35°C for 3–7 days before the bacterial colonies were formed.

Bacterial growth and β-glucosidase activity

A loopful of *Meridianimaribacter* sp. CL38 culture was inoculated into production medium (pH7.5), which consists of 5.0g/L peptone, 1.0g/L yeast extract, 5.0g/L $MgCl_2$, 2.0g/L $MgSO_4 \cdot 7H_2O$, 0.5g/L $CaCl_2$, 1.0g/L KCl, and 20.0g/L NaCl [11]. After overnight incubation at 35˚C with 180 rpm shaking, 5% (*v/v*) of the overnight culture was transferred into a fresh production medium supplemented with 1% (w/v) carboxymethyl cellulose (CMC). The culture was incubated at 35˚C, 180 rpm. Samples were collected from the culture at 3-hour intervals until the 24th hour and bacterial growth was measured by OD_{600nm}. Nevertheless, crude enzyme of each sampling point was harvested by centrifugation (4˚C, 4500rpm) was subjected to β-glucosidase assay protocol.

β-glucosidase assay

The β-glucosidase activity was determined by using *p*-nitrophenyl-β-D-glucopyranoside (*p*NPG) as substrate. 5 mM *p*NPG was prepared by dissolving the substrate in 50 mM Tris-HCl buffer (pH 7.0). Then, 0.5 mL crude enzyme was mixed with 0.5 mL substrate. The enzyme reaction was started by incubating the mixture at 35˚C. After 30 mins of incubation, the enzyme reaction was stopped by adding 1 mL of 1M NaOH. The β-glucosidase activity was determined by releasing the *p*-nitrophenol (*p*NP) compound after the reaction and the absorbance changes were measured at OD_{405nm} using a spectrophotometer. One unit of enzyme activity was defined as the amount of β-glucosidase that can release one µmol of *p*NP per minute per mL under the assay condition. All enzyme reactions were conducted in at least triplicate.

Effects of salt concentration on β-glucosidase activity and stability

The β-glucosidase activity of CL38 was conducted by incubating the mixture of enzyme-substrate at different concentrations of salt (1-10% NaCl, *w/v*). The stability of β-glucosidase was carried out by preincubating the crude enzyme of CL38 with different concentrations of NaCl as mentioned, for 1 hour at 35°C. Then, the activity and stability of the enzyme were measured by standard enzyme assay. Crude enzyme without any salt added (1% NaCl, *w/v*) was treated as the assay control with its relative activity expressed as 100%. The experiment was conducted in at least triplicate.

Effects of metal ions, detergents and organic solvents on β-glucosidase stability

The crude enzyme of CL38 was preincubated for 1 hour at 35°C with 5mM of metal ion (Ca^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Mn^{2+}), 1% detergent (sodium dodecyl sulfate in *w/v*, Triton X-100, Tween-20, Tween-40, Tween-60, Tween-80 in *v/v*) and 25% organic solvent (methanol, acetone, isopropanol, chloroform, ethanol in *v/v*). After preincubation, an enzyme assay was carried out. Crude enzyme without any additives added was treated as the assay control with its relative activity expressed as 100%. The experiment was conducted in at least triplicate.

Protein sequence analysis

Draft genome sequence of *Meridianimaribacter* sp. CL38 was retrieved from NCBI GenBank database with the accession number of QKWS00000000.1. Then, the genome sequence of strain CL38 was submitted to the dbCAN meta server to identify the Carbohydrate-active enzymes (CAZymes) [22, 23]. Sequences listed as glycoside hydrolase (GH) family that encode for β-glucosidase were selected for further analysis. The protein sequence was submitted to the InterPro v86.0 server to determine the functional domain [24]. β-glucosidase sequences from other species were selected from the NCBI database and used in multiple sequence alignment via Cluster Omega [25]. A phylogenetic tree was constructed using MEGA-X software with a neighbor-joining method, and was bootstrapped 1000 times [26, 27].

Results and Discussions

Bacterial growth and β-glucosidase activity

The bacterial growth of *Meridianimaribacter* sp. CL38 and β-glucosidase activity in production medium supplemented with carboxymethyl cellulose (CMC) were monitored (Figure 1). According to the growth curve, the lag phase was observed from 0 to the 3rd hour, and then the bacterium growth achieved an exponential phase from the 3rd to 12th hour and entered the stationary phase thereafter. As for enzyme activity, it was low during the lag and early of the exponential phase. The β-glucosidase activity reached its peak at 12 hours of bacterial growth (Figure 1).

Effects of salt concentration on β-glucosidase activity and stability.

The β-glucosidase activity and stability in the presence of salt were determined (Figure 2). The β-glucosidase demonstrated optimal activity at 2% NaCl (*w/v*). When the salt concentration was increased (from 2% to 10%), the enzyme activity decreased. The enzyme activity remains more than 50% at 10% NaCl (*w/v*) (64.19 ± 1.18%). Whereas the enzyme is most stable at 1-2% NaCl (*w/v*) and the stability decreased with the increase of salinity. The halotolerant ability of β-glucosidase produced by strain CL38 could be due to the adaptation of strain CL38 to its habitat. The mangrove which connected the sea and the land create a salty environment that require the bacteria to secrete halotolerant hydrolytic enzyme to degrade the carbon source into simple sugar [28].

Effects of metal ions, detergents, and solvents on β-glucosidase stability

Table 1 summarizes the results of the β-glucosidase stability when the crude enzymes were preincubated with different metal ions, detergents, and solvents. The β-glucosidase activity of strain CL38 was relatively stable in the presence of Ca^{2+}, Mg^{2+}, and Mn^{2+} ions as compared to other metal ions, which retains more than 80% of relative activities. Ca^{2+} and Mg^{2+} were commonly found in inorganic salts that use in the chemical pretreatment of lignocellulosic biomass [29, 30]. These metal salts have lower corrosivity as compared to acid solution and this makes it a more environmental friendly pretreatment method [31]. However, trace amount of metal ions could be found in

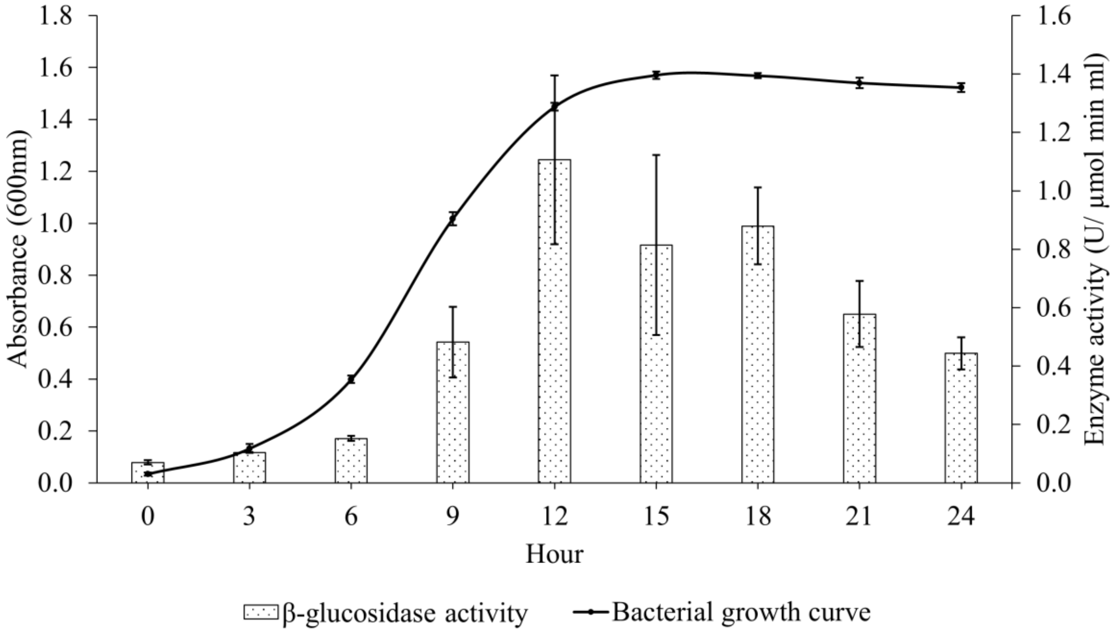

Figure 1. β-glucosidase activity and growth curve of *Meridianimaribacter* sp. CL38 cultured in production medium supplemented with 1% (*w/v*) CMC.

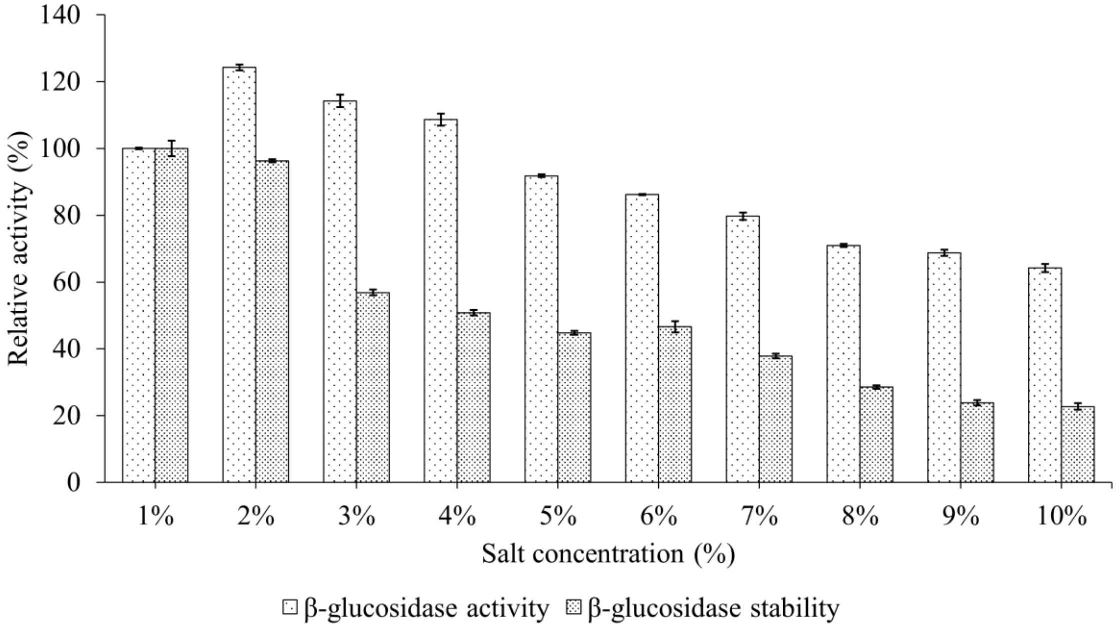

Figure 2. The effect of different salt concentration to the β-glucosidase activity and stability

the biomass after chemical pretreatment [32]. After the chemical pretreatment, the lignocellulosic biomass will go for enzymatic pretreatment. The β-glucosidase of strain CL38 that can withstand these trace metal ions will outperform other intolerance enzymes during the saccharification process of the biomass. Based on Table 1, the Cu^{2+} showed strong inhibition on the enzyme activity (24.47 ± 0.49%), this may be due to the autooxidation catalyzed by the Cu^{2+} on the cysteine residues of the enzyme, and in turn causing disulfide interchange and dysfunctional protein structure [33]. The inhibition caused by Co^{2+} (54.01 ± 0.85%) might be due to the formation of cobaltous-amino acid complexes, which are irreversible oxidation compounds that formed between Co^{2+} ions and amino acid residues [34].

The β-glucosidase of strain CL38 showed the

Table 1. Effect of metal ions, detergents, and organic solvents on β-glucosidase activity.

Chemicals	Concentration of chemicals	Relative activity (%)
Control	-	100.00 ± 0.49
Ca^{2+}	5mM	81.43 ± 0.36
Mg^{2+}	5mM	92.97 ± 0.28
Co^{2+}	5mM	54.01 ± 0.85
Ni^{2+}	5mM	73.28 ± 0.52
Cu^{2+}	5mM	24.47 ± 0.49
Mn^{2+}	5mM	99.86 ± 0.81
Sodium dodecyl sulfate (SDS)	1% (*w/v*)	13.20 ± 0.44
Triton X-100	1% (*v/v*)	27.45 ± 0.31
Tween-20	1% (*v/v*)	94.21 ± 0.88
Tween-40	1% (*v/v*)	36.20 ± 1.15
Tween-60	1% (*v/v*)	56.23 ± 0.67
Tween-80	1% (*v/v*)	97.48 ± 0.28
Methanol	25% (*v/v*)	3.19 ± 0.49
Acetone	25% (*v/v*)	0
Isopropanol	25% (*v/v*)	0
Chloroform	25% (*v/v*)	35.71 ± 0.16
Ethanol	25% (*v/v*)	0

Table 2. Selected β-glucosidase sequence from closely related species compared to Bgl3a

Species	**Origin**	**Protein Length (aa)**	**Percent identity (%)**	**Accession**	**Ref.**
Meridianimaribacter sp. CL38 (Bgl3a)	Mangrove soil	761	100	WP_131505993.1	[11]
Meridianimaribacter flavus	Marine sediment	761	99.61	WP_134198609.1	[21]
Flavobacteriaceae bacterium 144Ye	Near-bottom water and surface sediments of Benham Bank	761	98.55	RYH75728.1	[40]
Winogradskyella vidalii	Seawater surface	759	74.54	WP_179353224.1	[41, 42]
Kordia zhangzhouensis	Freshwater surface	753	74.34	WP_046745035.1	[43]
Flavobacterium sediminis	Tidal flat sediment	760	69.80	WP_109570126.1	[44]
Olleya aquimaris	Seawater	766	68.69	WP_111659610.1	[45]
Mangrovimonas xylaniphaga	Estuarine mangrove sediment	776	48.85	WP_053978191.1	[46]
Winogradskyella wichelsiae	Seawater surface	780	47.07	WP_179374618.1	[41, 42]
Winogradskyella ludwigii	Seawater surface	780	47.01	WP_179337019.1	[41, 42]
Meridianimaribacter sp. CL38 (Bgl3b)	Mangrove soil	780	46.76	WP_131507911.1	[11]
Mesoflavibacter sabulilitoris	Seashore sand	780	46.25	WP_106680313.1	[47]
Winogradskyella litoriviva	Coastal seawater	780	46.06	WP_173299877.1	[48]
Winogradskyella echinorum	Sea urchin	780	45.66	WP_186844354.1	[49]

highest stability against Tween-80 followed by Tween-20, which retained more than 94% enzyme activity after an hour of preincubation with the detergents. Detergents like Tween-40, Tween-60, Triton X-100, and sodium dodecyl sulfate (SDS) inhibited the enzyme activity after preincubation, which the relative activities are less than 60% as compared to the control. SDS is an ionic surfactant that is more likely to disrupt and denature enzyme structure due to the interaction of SDS with positively charged amino acids [35]. In contrast, the non-ionic detergents (i.e. Triton and Tween family) are less likely to affect the enzyme activity because they will only bind to the enzyme with weak hydrophobic interactions and hydrogen bonds [36].

According to Table 1, all organic solvents tested were inhibitors to the β-glucosidase of strain CL38. The organic solvents act as chaotropic agents which denatured the enzyme by disrupting the hydrogen bonds between protein subunits [37]. Nonetheless, the β-glucosidase of *Olleya aquimaris* was also found to be inhibited by organic solvents [38]. More research on physical or chemical modification on the enzyme is needed if the non-tolerance β-glucosidase needs to be involved in a process with high concentration of organic solvents.

Analysis of protein sequence related to β-glucosidase activity

Draft genome of strain CL38 was retrieved from GenBank database with accession number of QKWS0000000.1 and the genome sequence were uploaded to dbCAN meta server for carbohydrate-active enzymes (CAZymes) identification. Based on the result, there were 157 CAZymes identified in CL38. Among the glycoside hydrolases (GHs), GH2, 3, 5, 9, 16, and 144 were the possible GH families that were detected in the dbCAN meta server which encoded for β-glucosidases. Since GH3 is the most common GH family encoded for β-glucosidases [39], two of the protein sequences identified as GH3 in the strain CL38 genome were selected for further study. The two sequences with the code of Bgl3a (TBV27841.1) and Bgl3b (TBV26231.1) were used thereafter. Closely related sequences with Bgl3a and Bgl3b were selected by NCBI BlastP and tabulated in Table 2.

Based on Table 2, most of the closely related species were halophile isolated from saline environments such as seawater, marine sediment, sea-

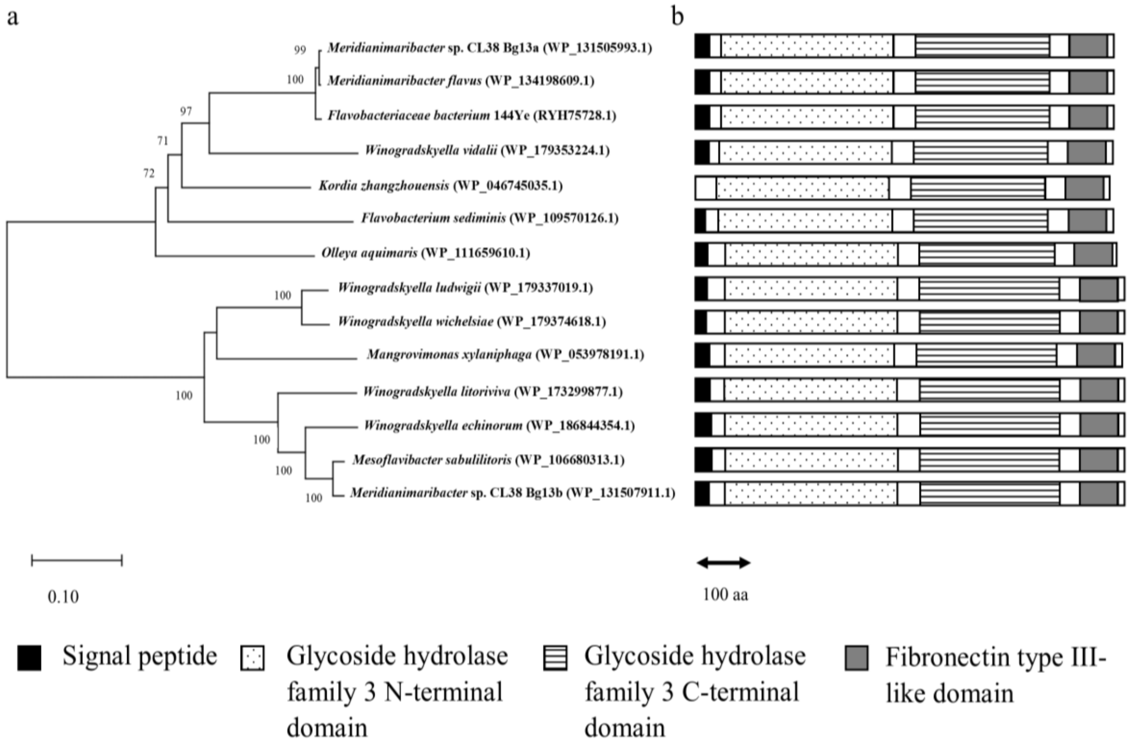

Figure 3. Phylogenetic tree of Bgl3a, Bgl3b, and other closely related β-glucosidases constructed using neighbor-joining method; the phylogenetic tree had been bootstrapped for 1000 times (a) and Domains of Bgl3a, Bgl3b and others closely related sequences determined using InterPro (b).

shore sand, etc. Hence the β-glucosidase isolated from these halophiles have high possibilities to be halotolerant [50]. Although Bgl3a and Bgl3b belong to the same species, there is only 46.76% identity between them (Table 2). β-glucosidase of *Meridianimaribacter flavus* (99.61%) showed the highest percent identity with Bgl3a followed by Flavobacteriaceae bacterium 144Ye (98.55%). Whereas for Bgl3b, the closest sequences are from *Mesoflavibacter sabulilitoris* and *Winogradskyella echinorum* with 97.44% and 90.26% identity respectively. To further analyze the relationship among Bgl3a, Bgl3b, and β-glucosidases of other species, a phylogenetic tree was constructed (Figure 3).

The phylogenetic tree (Figure 3a) showed that Bgl3a amino acid sequence has the closest relationship with the β-glucosidase of *Meridianimaribacter flavus* [21]. Moreover, the Bgl3a protein sequence also formed a big cluster with the β-glucosidases from *Flavobacteriaceae* bacterium 144Ye, *Olleya aquimaris*, *Kordia zhangzhouensis*, *Winogradskyella* vidalii, and *Flavobacteriaceae sediminis* [21, 45, 51, 52]. Whereas for Bgl3b amino acid sequence, it has the closest relationship with β-glucosidase produced by *Mesoflavibacter sabulilitoris* and was clustered with another group of GH3 β-glucosidase from bacteria such as *Winogradskyella* spp. and *Mangrovimonas xylaniphaga* [46, 47]. Interestingly, all the aforementioned bacteria belong to the same family taxon *Flavobacteriaceae*, indicating that the protein sequence of either Bgl3a or Bgl3b is most probably encoded by similar genes that found in the members of the family; these two GH3 proteins might be part of the core genome shared among the family members.

Nonetheless, regardless of Bgl3a or Bgl3b groups of β-glucosidases, these β-glucosidases have the same domain arrangements as shown in Figure 3b. All sequences consist of a glycoside hydrolase family 3 domain which involves in the enzyme hydrolysis function of the glycosidic bond formed between the two D-glucose monomers [53]. Besides, most of these β-glucosidases consist of a signal peptide sequence at the N-terminal, indicating that these enzymes are most probably expressed extracellularly [54]. A fibronectin type-III like domain was found in all sequences at the C-terminal. This domain is involved in ligand-binding function, protein-protein interaction, and could be a novel carbohydrate binding module-like domain [55–57].

As shown in the multiple sequence alignment (Supplementary 1), there are 5 motifs present among the sequences. These motifs could connect to form glycoside hydrolase family 3 domain and are used as the signature for GH3 family identification [58]. By referring to the well-studied β-glucosidase of *Chaetomium thermophilum* (GenBank Accession: ABR57325.2) [39], the active sites of Bgl3a and Bgl3b are found at Asp296 and Asp297, respectively. This active site is functioning as the nucleophile during the hydrolysis reaction and is highly conserved throughout different species. The segregating site indicated that mutation might occur among species to adapt to different habitats or conditions [59].

Conclusion

To the extent of our knowledge, this is the first β-glucosidase characterization of genus *Meridianimaribacter*. The β-glucosidases of *Meridianimaribacter* sp. CL38 remained stable in the presence of 1-2% NaCl (*w/v*) (96.28-100%), 5mM Mn^{2+} (99.86%), Mg^{2+} (92.97%), Ca^{2+} ions (81.43%), and 1% (*v/v*) Tween-20 (94.21%) and Tween-80 (97.48%). Two protein sequences (Bgl3a and Bgl3b) related to the β-glucosidase activity from *Meridianimaribacter* sp. CL38 were identified with closest identity to *Meridianimaribacter flavus* (99.61%) and *Mesoflavibacter sabulilitoris* (97.44%), respectively. A total of five motifs were found in Bgl3a and Bgl3b with the active site (nucleophile) found at Asp296 and Asp297, respectively. Taken together, the β-glucosidases of strain CL38 could serve as good candidates in the production of biofuel that involves Mg^{2+} and Ca^{2+} ions as inorganic metal salt in the lignocellulosic biomass seawater-based pretreatment process.

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Supplementary 1

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Mangrovimonas_xylaniphaga              MKT-LSTQLKTIAVGVFVVSLLGCNQQESKT--EESF---------SFS-----------   37
Winogradskyella_litoriviva             MKKLQFAKIKYFTLIIGVLTVLSCNTNDKKT--T------------ASK-----------   35
Mesoflavibacter_sabulilitoris          MTTLKLTNIKSLAIGFGLLTAISCNTNSKKT--T------------STK-----------   35
Meridianimaribacter_sp._CL38_GH3b      MTTLKLTNIKSLAIGFGLLTAVSCNTNSNKI--K------------SKN-----------   35
Flavobacterium_sediminis               --------MKRTI--ILTLLIMGSYAHA-----QKKK---------KMK-----------   25
Olleya_aquimaris                       -----MNIIKYYILVLAGLLIISCKTNNENDLALNDI---------KTS-----------   35
Kordia_zhangzhouensis                  ----------------MVLCFLGCVTNKKS---GSQM---------EVM-----------   21
Winogradskyella_vidalii                ------MFLKNKL-FALLIISVLGHINQNV---YAQS---------KP------------   29
Flavobacteriaceae_bacterium_144Ye      -----MKTIKITR-VCAVAVILLCLVNTKT---HAQM---------EE------------   30
Meridianimaribacter_sp._CL38_GH3a      -----MKTIKITR-VCAVAVILLCLVNTKT---HAQM---------EE------------   30
Meridianimaribacter_flavus             -----MKTIKITR-VCAVAVILLCLVNTKT---HAQM---------EE------------   30
Chaetomium_thermophilum                -----------MKLAVGLACLVGSHLAATGSLKSKAKLEKRAWATSEPSYPSPWMNPNAA   49
Cochliobolus_heterostrophus            --------------MASLLFLSLSGLAAGAAIQQRDPV--PAGYVAPPYYPAPH-GGWVS   43


Mangrovimonas_xylaniphaga              QENPFQSKVDSVLGLMTLEEKLGQLNLPS-----SGDITTGQAQSSNIAQKIEQGQVGGL   92
Winogradskyella_litoriviva             SDNPFEEKVDSIISLMTLDEKLGQLNLPA-----TGPITTGSSKSTDVVSKIEEGKIGGL   90
Mesoflavibacter_sabulilitoris          NDNPYQTKVDSIIGLMTLDEKIGQLNLPA-----TGPITTGASKSTDVVKKIEDGKIGGL   90
Meridianimaribacter_sp._CL38_GH3b      TDNPYQAKVDSIIGLMTLDEKIGQLNLPA-----TGPITTGSSKSTDVVKKIEDGKIGGL   90
Flavobacterium_sediminis               QQEPIETRIEQLLQKMTLEEKVGQMNQYNGFWDVTGPAPEGGSAA-LKYEHLKKGWVGSM   84
Olleya_aquimaris                       KTLSIEDKVDALLAKMTLEEKIGQMNQYNGFWDVTGPVPKDGDQA-KKYDHLRKGYVGSM   94
Kordia_zhangzhouensis                  KEISVDQRVDSLLAKMTLEEKVGQMNLYNGFWDVTGPTPQGGSAK-LKYEHLKKGWVGAM   80
Winogradskyella_vidalii                SELNTEQKIDALLALMTVEEKVGQLNLYNGFWNVTGPAPEHGGAA-TKYKHLKNGWVGAM   88
Flavobacteriaceae_bacterium_144Ye      TKVDINQKIESLLSQMTIEEKVGQMNLYNGFWDVTGPAPEEGNAA-LKYDHLKKGWVGAM   89
Meridianimaribacter_sp._CL38_GH3a      TKVDINQKVESLLSQMTIEEKVGQMNLYNGFWDVTGPAPEEGNAA-LKYDHLKKGWVGAM   89
Meridianimaribacter_flavus             TKVDINQKVESLLSQMTIEEKVGQMNLYNGFWDVTGPAPEEGNAA-LKYDHLKKGWVGAM   89
Chaetomium_thermophilum                GWEQAYQWAKDFVSQMTLLEKVNLT---TG-----------------------VGWEGEQ   83
Cochliobolus_heterostrophus            DWAESYRKASLLVSNMTLAEKTNLT---AG-----------------------TGIFMGR   77
                                                . .:  **: ** .                               *
                                                                Motif 1                  Motif 2
                                                           ‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾       ‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾
Mangrovimonas_xylaniphaga              FNIKSVEKIREVQRFAVEKSRLKIPLIFGMDVI-----HGYETTFPIPLGLSASWDMDMI   147
Winogradskyella_litoriviva             FNIKDIKNIYEVQKIAVEKSRLGIPLIFGMDVI-----HGFKTTFPIPLGLSASWDMEMI   145
Mesoflavibacter_sabulilitoris          FNIKDPKNIYDVQKIAVEKSRLGIPLIFGMDVI-----HGFKTTFPIPLGLSASWDMDMI   145
Meridianimaribacter_sp._CL38_GH3b      FNIKDPKNIYEVQKIAVEKSRLGIPLIFGMDVI-----HGFKTTFPIPLGLSASWDMEMI   145
Flavobacterium_sediminis               LTVRGVKEVKAVQKIAVEETRLGIPLIIGFDVI-----HGYKTLSPIPLAEAASWDMEAI   139
Olleya_aquimaris                       LNVRGVKDVKAVQKIAVEETRLGIPLIIGFDVI-----HGYETLTPIPLAESASWDLEAI   149
Kordia_zhangzhouensis                  LNIRGVENVKEVQRIAVEETRLGIPLLIGFDVI-----HGYKTLSPIPLAESASWDLEAI   135
Winogradskyella_vidalii                LNVRGTEEVNKVQRIAVEESRLGIPLLIGFDVI-----HGYKTLSPIPLAESASWDLKAI   143
Flavobacteriaceae_bacterium_144Ye      LTVRGAEEVRNVQRIAVEESRLGIPLLIGFDVI-----HGYKTLSPIPLAESASWDLQAI   144
Meridianimaribacter_sp._CL38_GH3a      LTVRGAEEVRNVQRIAVEESRLGIPLLIGFDVI-----HGYKTLSPIPLAESASWDLQAI   144
Meridianimaribacter_flavus             LTVRGAEEVRKVQRIAVEESRLGIPLLIGFDVI-----HGYKTLSPIPLAESASWDLQAI   144
Chaetomium_thermophilum                ----CVGQ-------TGAIPRLGLRSLCLHDSPLGIRGTDYNSAFPSGQTTAATWDRTLF   132
Cochliobolus_heterostrophus            ----CVGN-------TGSALRVGIPQLCLQDGPLGVRNTDHNTAFPAGITAGATWDKDLI   126
                                              .       :    *: :  :   *        ..::  *     .*:**   :
                                                                           Motif 3
                                       ‾‾‾‾                           ‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾
Mangrovimonas_xylaniphaga              KKTAQMAAQEATADGINWTFSPMVD-ISRDPRWGRVSEGNGEDPYLASEIAKAMVAGYQG   206
Winogradskyella_litoriviva             EKTAQIAASEASANGINWTFSPMVD-ISRDPRWGRVSEGNGEDPFLGSEIAKAMVNGYQQ   204
Mesoflavibacter_sabulilitoris          EKTAQIAANEASANGINWTFSPMVD-ISRDPRWGRVSEGNGEDPFLGSAIARAMVRGYQQ   204
Meridianimaribacter_sp._CL38_GH3b      EKTAQIAANEASANGINWTFSPMVD-ISRDPRWGRVSEGNGEDPFLGSAIARAMVRGYQQ   204
Flavobacterium_sediminis               KKSAQVAAGEASASGINWTFGPNVD-VSRDARWGRVMEGAGEDAFLGSKVAEARVKGFQG   198
Olleya_aquimaris                       KNSAEMAAKESSAAGINWTFAPMVD-ISRDARWGRVMEGAGEDTYLGSKIAVARVNGFQG   208
Kordia_zhangzhouensis                  RNSARVAATEASAAGINWTFAPMVD-ISRDPRWGRVMEGGGEDPYLGSKIAKARVLGFQG   194
Winogradskyella_vidalii                EASARIAAIEASSAGINWTFAPMVD-VSRDARWGRVMEGAGEDPFLGSLIAIARVNGFQG   202
Flavobacteriaceae_bacterium_144Ye      EKSARVAATEASAAGINWTFAPMVD-ISRDARWGRVMEGAGEDPYLGSLIAKARVNGFQG   203
Meridianimaribacter_sp._CL38_GH3a      EKSARVAATEASASGINWTFAPMVD-ISRDARWGRVMEGAGEDPYLGSLIAKARVNGFQG   203
Meridianimaribacter_flavus             EKSARVAATEASASGINWTFAPMVD-ISRDARWGRVMEGAGEDPYLGSLIAKARVNGFQG   203
Chaetomium_thermophilum                YKRGYAMGKEARGKGINVLLGPVAGPLGRMPAGGRNWEGFSPDPVLTGIAMAETVKGIQD   192
Cochliobolus_heterostrophus            YRRAVAIGEEFRGKGVNIHLGPSVGPLGRKPRGGRNWEGFGSDPVLQAFGGSLSVEGIQS   186
                                          .   . *  . *:*  :.* .. :.*    **  ** . *  * .      * * *
                                                      Motif 4
                                                  ‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾
Mangrovimonas_xylaniphaga              D---DLTADNTMMACVKHFALY--------GAPEAGRDYNTVDMSHIRMYNEYFPPYKAA   255
Winogradskyella_litoriviva             N---DMSANNTMMACVKHFALY--------GAVEAGRDYNTVDMSRVRMFNEYLPPYKAA   253
Mesoflavibacter_sabulilitoris          D---DMAANNTLMSCVKHFALY--------GAPEAGRDYNTVDMSRIRMYNEYLAPYKAA   253
Meridianimaribacter_sp._CL38_GH3b      D---DMAANNTLMSCVKHFALY--------GAPEAGRDYNTVDMSRIRMYNEYLAPYKAA   253
Flavobacterium_sediminis               ETKEDLGLPNTIAACAKHFAAY--------GFVEAGKEYNLVDIGMPTLYNLVLPPFEAA   250
Olleya_aquimaris                       E---DLSAHHSIAACAKHFAAY--------GFAESGRDYNTADVGTSTLNNIILPPFKAA   257
Kordia_zhangzhouensis                  N---DLTKNNTIAACAKHFAAY--------GFSEAGKEYNTVDIGTSTLYNTVLPPFKAA   243
Winogradskyella_vidalii                D---DLTDANTIAACAKHFAGY--------GFAEAGKEYNTVDVGLSTLYNTILPPFKAA   251
Flavobacteriaceae_bacterium_144Ye      D---DLSAHNTIAACAKHFAAY--------GFAEAGRDYNVADIGNGTLYNTVLPPFKAA   252
Meridianimaribacter_sp._CL38_GH3a      D---DLSAHNTIAACAKHFAAY--------GFAEAGRDYNVADIGNGTLYNTVLPPFKAA   252
Meridianimaribacter_flavus             D---DLSAHNTIAACAKHFAAY--------GFAEAGRDYNVADIGNGTLYNTVLPPFKAA   252
Chaetomium_thermophilum                A---------GVIACAKHLIGDEQEHFRQVGDGFDIDESLSSNIDDRTMHELYLWPFADA   243
Cochliobolus_heterostrophus            T---------GVIATIKHLIANEQEMYRMWN---IVKPGYSSNVDDRTLHELYLWPFAEG   234
                                                  : :  **:           .           ::.   : :  : *:  .
                                                                           Motif 5
                                                                    ‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾
Mangrovimonas_xylaniphaga              VDAGVGSLMASFNEVDGIPATGNKWLMTDLLRHDWGFNGFVVTDYTGIPEMIDHGMGD-L   314
Winogradskyella_litoriviva             VDAGVGSVMASFNEIDGVPATGNKWLLTDLLRDDWAFNGFVVSDYTGIYEMMAHGMGD-E   312
Mesoflavibacter_sabulilitoris          VDEGVGSVMASFNEIDGVPATGNKWLLTDLLRDDWGFDGFVVSDYTGIYEMRAHGMGD-E   312
Meridianimaribacter_sp._CL38_GH3b      VDEGVGSVMASFNEIDGVPATGNKWLLTDLLRDDWGFDGFVVSDYTGIYEMRAHGMGD-E   312
Flavobacterium_sediminis               KNAGVRTFMNSFNTLNGVPATGSKFLQRDILKGKWNFDGFVITDWASIREMINWGFAEDG   310
Olleya_aquimaris                       KEANVRTFMNSFNELNGIPATGDKYLQRDILKGQWGFDGFVVSDWGSITEMIAHGHAKDG   317
Kordia_zhangzhouensis                  ADSGVKTFMNSFNELNGVPVTGSEFLQRDILKGAWDFDGFVVSDWASINEIIFWGHAKDK   303
Winogradskyella_vidalii                QEAGVKTFMNSFNDLNGIPVTASEFLQRDILKNGWDFNGFVVSDWASMNELIAWGVAKDK   311
Flavobacteriaceae_bacterium_144Ye      SEAGVKTFMNSFNTLNGVPVTGSSFLQRDILKRDWNFDGFVVSDWASINEMIPWGHAKDK   312
Meridianimaribacter_sp._CL38_GH3a      SEAGVKTFMNSFNTLNGVPVTGSSFLQRDILKRDWNFDGFVVSDWASINEMIPWGHAKDK   312
Meridianimaribacter_flavus             SEAGVKTFMNSFNTLNGVPVTGSSFLQRDILKRDWNFDGFVVSDWASINEMIPWGHAKDK   312
Chaetomium_thermophilum                VRAGVGSVMCAYNQVNNSYSCHNSKLLNGLLKNELGFQGFVMSDWQAQH-----------   292
Cochliobolus_heterostrophus            VRSGVGSVMIAYNAVNGSACAQNSYMINGLLKDELGFQGFVMSDWLAQI-----------   283
                                          .* :.* ::* ::.     .. :  .:*:    *:***::*: .
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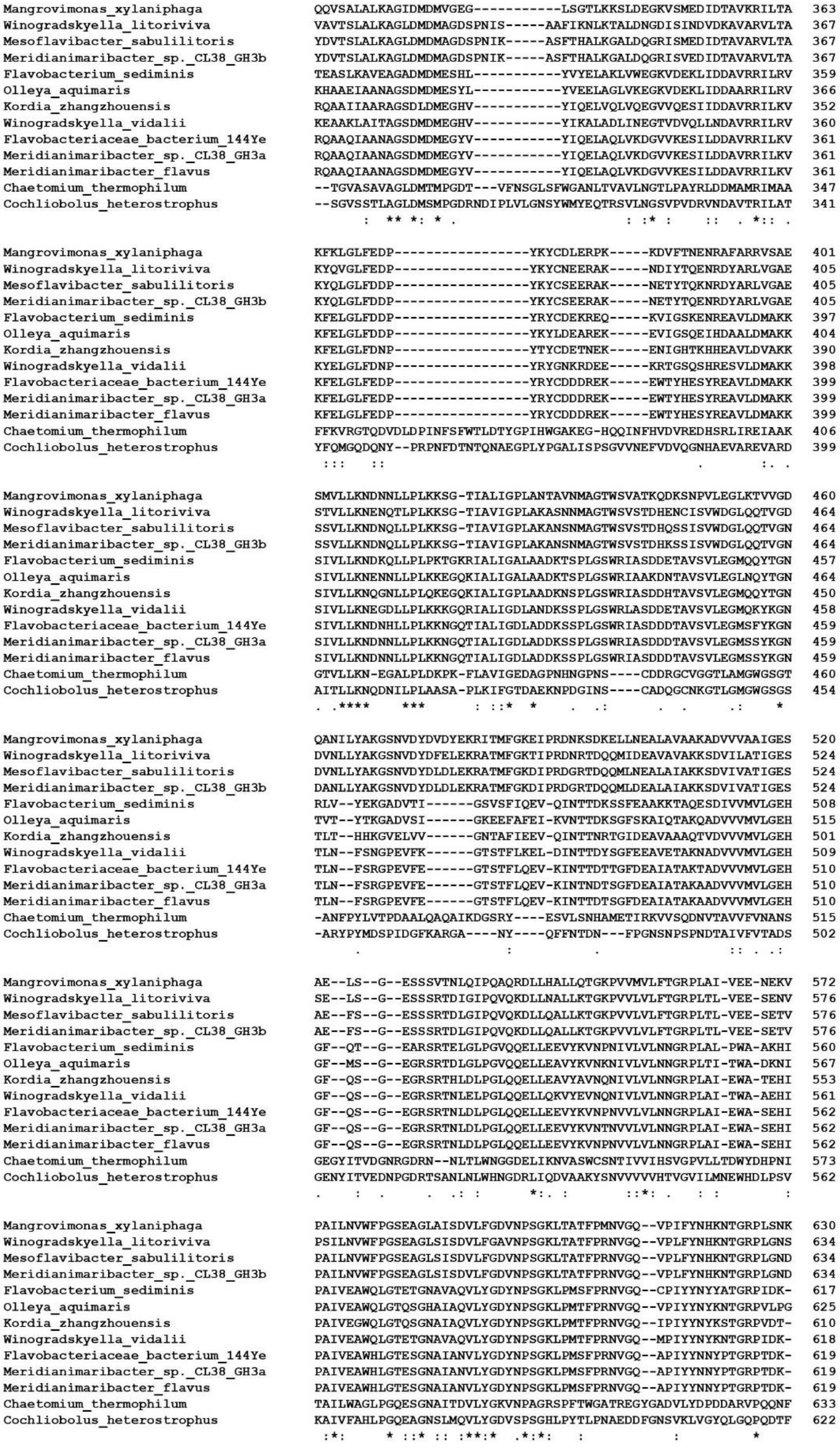
continued



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Mangrovimonas_xylaniphaga            QQVSALALKAGIDMDMVGEG-----------LSGTLKKSLDEGKVSMEDIDTAVKRILTA   363
Winogradskyella_litoriviva           VAVTSLALKAGLDMDMAGDSPNIS-----AAFIKNLKTALDNGDISINDVDKAVARVLTA   367
Mesoflavibacter_sabulilitoris        YDVTSLALKAGLDMDMAGDSPNIK-----ASFTHALKGALDQGRISMEDIDTAVARVLTA   367
Meridianimaribacter_sp._CL38_GH3b    YDVTSLALKAGLDMDMAGDSPNIK-----ASFTHALKGALDQGRISVEDIDTAVARVLTA   367
Flavobacterium_sediminis             TEASLKAVEAGADMDMESHL-----------YVYELAKLVWEGKVDEKLIDDAVRRILRV   359
Olleya_aquimaris                     KHAAEIAANAGSDMDMESYL-----------YVEELAGLVKEGKVDEKLIDDAARRILRV   366
Kordia_zhangzhouensis                RQAAIIAARAGSDLDMEGHV-----------YIQELVQLVQEGVVQESIIDDAVRRILKV   352
Winogradskyella_vidalii              KEAAKLAITAGSDMDMEGHV-----------YIKALADLINEGTVDVQLLNDAVRRILRV   360
Flavobacteriaceae_bacterium_144Ye    RQAAQIAANAGSDMDMEGYV-----------YIQELAQLVKDGVVKESILDDAVRRILKV   361
Meridianimaribacter_sp._CL38_GH3a    RQAAQIAANAGSDMDMEGYV-----------YIQELAQLVKDGVVKESILDDAVRRILKV   361
Meridianimaribacter_flavus           RQAAQIAANAGSDMDMEGYV-----------YIQELAQLVKDGVVKESILDDAVRRILKV   361
Chaetomium_thermophilum              --TGVASAVAGLDMTMPGDT---VFNSGLSFWGANLTVAVLNGTLPAYRLDDMAMRIMAA   347
Cochliobolus_heterostrophus          --SGVSSTLAGLDMSMPGDRNDIPLVLGNSYWMYEQTRSVLNGSVPVDRVNDAVTRILAT   341
                                           :  ** *: * .                     : :* :    ::  . *:: .

Mangrovimonas_xylaniphaga            KFKLGLFEDP-----------------YKYCDLERPK-----KDVFTNENRAFARRVSAE   401
Winogradskyella_litoriviva           KYQVGLFEDP-----------------YKYCNEERAK-----NDIYTQENRDYARLVGAE   405
Mesoflavibacter_sabulilitoris        KYQLGLFDDP-----------------YKYCSEERAK-----NETYTQKNRDYARLVGAE   405
Meridianimaribacter_sp._CL38_GH3b    KYQLGLFDDP-----------------YKYCSEERAK-----NETYTQENRDYARLVGAE   405
Flavobacterium_sediminis             KFELGLFDDP-----------------YRYCDEKREQ-----KVIGSKENREAVLDMAKK   397
Olleya_aquimaris                     KFELGLFDDP-----------------YKYLDEAREK-----EVIGSQEIHDAALDMAKK   404
Kordia_zhangzhouensis                KFELGLFDNP-----------------YTYCDETNEK-----ENIGHTKHHEAVLDVAKK   390
Winogradskyella_vidalii              KYELGLFDNP-----------------YRYGNKRDEE-----KRTGSQSHRESVLDMAKK   398
Flavobacteriaceae_bacterium_144Ye    KFELGLFEDP-----------------YRYCDDDREK-----EWTYHESYREAVLDMAKK   399
Meridianimaribacter_sp._CL38_GH3a    KFELGLFEDP-----------------YRYCDDDREK-----EWTYHESYREAVLDMAKK   399
Meridianimaribacter_flavus           KFELGLFEDP-----------------YRYCDDDREK-----EWTYHESYREAVLDMAKK   399
Chaetomium_thermophilum              FFKVRGTQDVDLDPINFSFWTLDTYGPIHWGAKEG-HQQINFHVDVREDHSRLIREIAAK   406
Cochliobolus_heterostrophus          YFQMGQDQNY--PRPNFDTNTQNAEGPLYPGALISPSGVVNEFVDVQGNHAEVAREVARD   399
                                      :::   ::                                       .       :. .

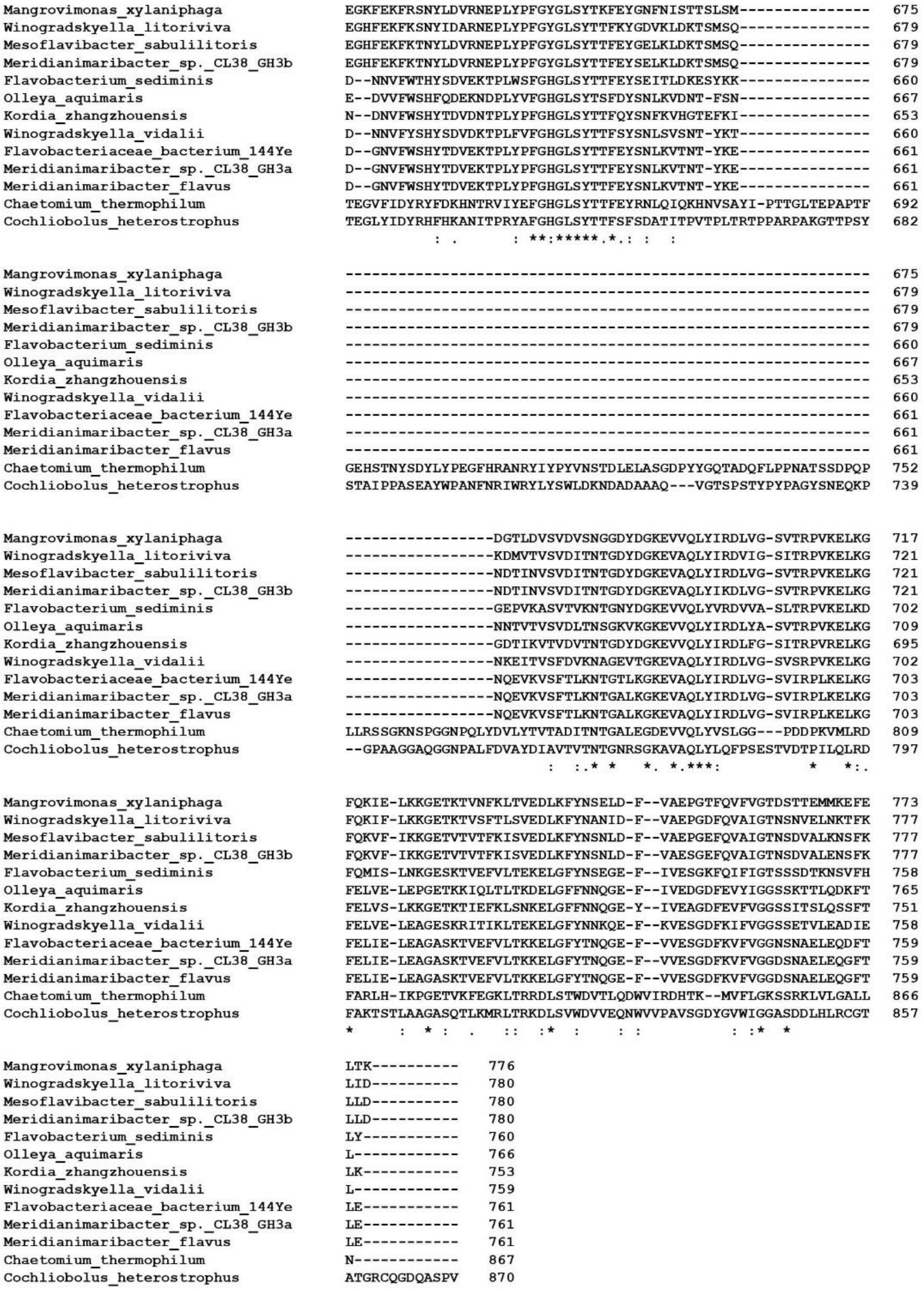
Mangrovimonas_xylaniphaga            SMVLLKNDNNLLPLKKSG-TIALIGPLANTAVNMAGTWSVATKQDKSNPVLEGLKTVVGD   460
Winogradskyella_litoriviva           STVLLKNENQTLPLKKSG-TIAVIGPLAKASNNMAGTWSVSTDHENCISVWDGLQQTVGD   464
Mesoflavibacter_sabulilitoris        SSVLLKNDNQLLPLKKSG-TIAVIGPLAKANSNMAGTWSVSTDHQSSISVWDGLQQTVGN   464
Meridianimaribacter_sp._CL38_GH3b    SSVLLKNDNQLLPLKKSG-TIAVIGPLAKANSNMAGTWSVSTDHKSSISVWDGLQQTVGN   464
Flavobacterium_sediminis             SIVLLKNDKQLLPLPKTGKRIALIGALAADKTSPLGSWRIASDDETAVSVLEGMQQYTGN   457
Olleya_aquimaris                     SIVLLKNENNLLPLKKEGQKIALIGALAADKTSPLGSWRIAAKDNTAVSVLEGLNQYTGN   464
Kordia_zhangzhouensis                SIVLLKNQGNLLPLQKEGQKIALIGPLAADKNSPLGSWRIASDDHTAVSVLEGMQQYTGN   450
Winogradskyella_vidalii              SIVLLKNEGDLLPLKKKGQRIALIGDLANDKSSPLGSWRLASDDETAVSVLEGMQKYKGN   458
Flavobacteriaceae_bacterium_144Ye    SIVLLKNDNHLLPLKKNGQTIALIGDLADDKSSPLGSWRIASDDDTAVSVLEGMSFYKGN   459
Meridianimaribacter_sp._CL38_GH3a    SIVLLKNDNNLLPLKKNGQTIALIGDLADDKSSPLGSWRIASDDDTAVSVLEGMSSYKGN   459
Meridianimaribacter_flavus           SIVLLKNDNNLLPLKKNGQTIALIGDLADDKSSPLGSWRIASDDDTAVSVLEGMSSYKGN   459
Chaetomium_thermophilum              GTVLLKN-EGALPLDKPK-FLAVIGEDAGPNHNGPNS----CDDRGCVGGTLAMGWGSGT   460
Cochliobolus_heterostrophus          AITLLKNQDNILPLAASA-PLKIFGTDAEKNPDGINS----CADQGCNKGTLGMGWGSGS   454
                                     . .****    ***      : ::*  *    .  .:      .  .     .:    *

Mangrovimonas_xylaniphaga            QANILYAKGSNVDYDVDYEKRITMFGKEIPRDNKSDKELLNEALAVAAKADVVVAAIGES   520
Winogradskyella_litoriviva           DVNLLYAKGSNVDYDFELEKRATMFGKTIPRDNRTDQQMIDEAVAVAKKSDVILATIGES   524
Mesoflavibacter_sabulilitoris        DVNLLYAKGSNVDYDLDLEKRATMFGKDIPRDGRTDQQMLNEALAIAKKSDVIVATIGES   524
Meridianimaribacter_sp._CL38_GH3b    DANLLYAKGSNVDYDLDLEKRATMFGKDIPRDGRTDQQMLDEALAIAKKSDVIVATIGES   524
Flavobacterium_sediminis             RLV--YEKGADVTI------GSVSFIQEV-QINTTDKSSFEAAKKTAQESDIVVMVLGEH   508
Olleya_aquimaris                     TVT--YTKGADVSI------GKEEFAFEI-KVNTTDKSGFSKAIQTAKQADVVVMVLGEH   515
Kordia_zhangzhouensis                TLT--HHKGVELVV------GNTAFIEEV-QINTTNRTGIDEAVAAAQTVDVVVMVLGEH   501
Winogradskyella_vidalii              TLN--FSNGPEVFK------GTSTFLKEL-DINTTDYSGFEEAVETAKNADVVVMVLGEH   509
Flavobacteriaceae_bacterium_144Ye    TLN--FSRGPEVFE------GTSTFLQEV-KINTTDTTGFDEAIATAKTADVVVMVLGEH   510
Meridianimaribacter_sp._CL38_GH3a    TLN--FSRGPEVFE------GTSTFLQEV-KINTNDTSGFDEAIATAKAADVVVMVLGEH   510
Meridianimaribacter_flavus           TLN--FSRGPEVFE------GTSTFLQEV-KINTTDTSGFDEAIATAKAADVVVMVLGEH   510
Chaetomium_thermophilum              -ANFPYLVTPDAALQAQAIKDGSRY----ESVLSNHAMETIRKVVSQDNVTAVVFVNANS   515
Cochliobolus_heterostrophus          -ARYPYMDSPIDGFKARGA----NY----QFFNTDN---FPGNSNPSPNDTAIVFVTADS   502
                                          .                  :          .                :: . .:

Mangrovimonas_xylaniphaga            AE--LS--G--ESSSVTNLQIPQAQRDLLHALLQTGKPVVMVLFTGRPLAI-VEE-NEKV   572
Winogradskyella_litoriviva           SE--LS--G--ESSSRTDIGIPQVQKDLLNALLKTGKPVVLVLFTGRPLTL-VEE-SENV   576
Mesoflavibacter_sabulilitoris        AE--FS--G--ESSSRTDLGIPQVQKDLLQALLKTGKPVVLVLFTGRPLTL-VEE-SETV   576
Meridianimaribacter_sp._CL38_GH3b    AE--FS--G--ESSSRTDLGIPQVQKDLLQALLKTGKPVVLVLFTGRPLTL-VEE-SETV   576
Flavobacterium_sediminis             GF--QT--G--EARSRTELGLPGVQQELLEEVYKVNPNIVLVLNNGRPLAL-PWA-AKHI   560
Olleya_aquimaris                     GF--MS--G--EGRSRTDLGLPGVQQELLEAVYKVNKNIVLVLNNGRPLTI-TWA-DKNI   567
Kordia_zhangzhouensis                GF--QS--G--EGRSRTHLDLPGLQQELLEAVYAVNQNIVLVLNNGRPLAI-EWA-TEHI   553
Winogradskyella_vidalii              GF--QS--G--EGRSRTNLELPGLQQELLQKVYEVNQNIVLVLNNGRPLAI-TWA-AEHI   561
Flavobacteriaceae_bacterium_144Ye    GF--QS--G--EGRSRTNLDLPGLQQELLEEVYKVNPNVVLVLNNGRPLAI-EWA-SEHI   562
Meridianimaribacter_sp._CL38_GH3a    GF--QS--G--EGRSRTNLDLPGLQQELLEEVYKVNTNVVLVLNNGRPLAI-EWA-SEHI   562
Meridianimaribacter_flavus           GF--QS--G--EGRSRTNLDLPGLQQELLEEVYKVNPNVVLVLNNGRPLAI-EWA-SEHI   562
Chaetomium_thermophilum              GEGYITVDGNRGDRN--NLTLWNGGDELIKNVASWCSNTIVVIHSVGPVLLTDWYDHPNI   573
Cochliobolus_heterostrophus          GENYITVEDNPGDRTSANLNLWHNGDRLIQDVAAKYSNVVVVVHTVGVILMNEWHDLPSV   562
                                     .    :  .     .  .: :      *:. :       ::*: .   : :        :

Mangrovimonas_xylaniphaga            PAILNVWFPGSEAGLAISDVLFGDVNPSGKLTATFPMNVGQ--VPIFYNHKNTGRPLSNK   630
Winogradskyella_litoriviva           PSILNVWFPGSEAGLSISDVLFGAVNPSGKLTATFPRNVGQ--VPLFYNHKNTGRPLGNS   634
Mesoflavibacter_sabulilitoris        PAILNVWFPGSEAGLSISDVLFGDVNPSGKLTATFPRNVGQ--VPLFYNHKNTGRPLGND   634
Meridianimaribacter_sp._CL38_GH3b    PAILNVWFPGSEAGLSISDVLFGDVNPSGKLTATFPRNVGQ--VPLFYNHKNTGRPLGND   634
Flavobacterium_sediminis             PAIVEAWQLGTETGNAVAQVLYGDYNPSGKLPMSFPRNVGQ--CPIYYNYYATGRPIDK-   617
Olleya_aquimaris                     PAIVEAWQLGTQSGHAIAQVLYGDYNPSGKLPMTFPRNVGQ--VPIYYNYKNTGRPVLPG   625
Kordia_zhangzhouensis                PAIVEGWQLGTQSGNAIAQVLYGDYNPSGKLPMTFPRNVGQ--IPIYYNYKSTGRPVDT-   610
Winogradskyella_vidalii              PAIVEAWQLGTETGNAVAQVLYGDYNPSGKLPMTFPRNVGQ--MPIYYNYKNTGRPIDK-   618
Flavobacteriaceae_bacterium_144Ye    PAIVEAWHLGTESGNAIANVLYGDYNPSGKLPMSFPRNVGQ--APIYYNNYPTGRPTDK-   619
Meridianimaribacter_sp._CL38_GH3a    PAIVEAWHLGTESGNAIANVLYGDYNPSGKLPMSFPRNVGQ--APIYYNNYPTGRPTDK-   619
Meridianimaribacter_flavus           PAIVEAWHLGTESGNAIANVLYGDYNPSGKLPMSFPRNVGQ--APIYYNNYPTGRPTDK-   619
Chaetomium_thermophilum              TAILWAGLPGQESGNAITDVLYGKVNPAGRSPFTWGATREGYGADVLYDPDDARVPQQNF   633
Cochliobolus_heterostrophus          KAIVFAHLPGQEAGNSLMQVLYGDVSPSGHLPYTLPNAEDDFGNSVKLVGYQLGQPQDTF   622
                                      :*:     * ::* :: :**:*  .*:*:   :           :         *
```

continued



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Mangrovimonas_xylaniphaga                EGKFEKFRSNYLDVRNEPLYPFGYGLSYTKFEYGNFNISTTSLSM---------------   675
Winogradskyella_litoriviva               EGHFEKFKSNYIDARNEPLYPFGYGLSYTTFKYGDVKLDKTSMSQ---------------   679
Mesoflavibacter_sabulilitoris            EGHFEKFKTNYLDVRNEPLYPFGYGLSYTTFEYGELKLDKTSMSQ---------------   679
Meridianimaribacter_sp._CL38_GH3b        EGHFEKFKTNYLDVRNEPLYPFGYGLSYTTFEYSELKLDKTSMSQ---------------   679
Flavobacterium_sediminis                 D--NNVFWTHYSDVEKTPLWSFGHGLSYTTFEYSEITLDKESYKK---------------   660
Olleya_aquimaris                         E--DVVFWSHFQDEKNDPLYVFGHGLSYTSFDYSNLKVDNT-FSN---------------   667
Kordia_zhangzhouensis                    N--DNVFWSHYTDVDNTPLYPFGHGLSYTTFQYSNFKVHGTEFKI---------------   653
Winogradskyella_vidalii                  D--NNVFYSHYSDVDKTPLFVFGHGLSYTTFSYSNLSVSNT-YKT---------------   660
Flavobacteriaceae_bacterium_144Ye        D--GNVFWSHYTDVEKTPLYPFGHGLSYTTFEYSNLKVTNT-YKE---------------   661
Meridianimaribacter_sp._CL38_GH3a        D--GNVFWSHYTDVEKTPLYPFGHGLSYTTFEYSNLKVTNT-YKE---------------   661
Meridianimaribacter_flavus               D--GNVFWSHYTDVEKTPLYPFGHGLSYTTFEYSNLKVTNT-YKE---------------   661
Chaetomium_thermophilum                  TEGVFIDYRYFDKHNTRVIYEFGHGLSYTTFEYRNLQIQKHNVSAYI-PTTGLTEPAPTF   692
Cochliobolus_heterostrophus              TEGLYIDYRHFHKANITPRYAFGHGLSYTTFSFSDATITPVTPLTRTPPARPAKGTTPSY   682
                                                   : .      : **:*****.*.: :  :

Mangrovimonas_xylaniphaga                ------------------------------------------------------------   675
Winogradskyella_litoriviva               ------------------------------------------------------------   679
Mesoflavibacter_sabulilitoris            ------------------------------------------------------------   679
Meridianimaribacter_sp._CL38_GH3b        ------------------------------------------------------------   679
Flavobacterium_sediminis                 ------------------------------------------------------------   660
Olleya_aquimaris                         ------------------------------------------------------------   667
Kordia_zhangzhouensis                    ------------------------------------------------------------   653
Winogradskyella_vidalii                  ------------------------------------------------------------   660
Flavobacteriaceae_bacterium_144Ye        ------------------------------------------------------------   661
Meridianimaribacter_sp._CL38_GH3a        ------------------------------------------------------------   661
Meridianimaribacter_flavus               ------------------------------------------------------------   661
Chaetomium_thermophilum                  GEHSTNYSDYLYPEGFHRANRYIYPYVNSTDLELASGDPYYGQTADQFLPPNATSSDPQP   752
Cochliobolus_heterostrophus              STAIPPASEAYWPANFNRIWRYLYSWLDKNDADAAAQ---VGTSPSTYPYPAGYSNEQKP   739


Mangrovimonas_xylaniphaga                -----------------DGTLDVSVDVSNGGDYDGKEVVQLYIRDLVG-SVTRPVKELKG   717
Winogradskyella_litoriviva               -----------------KDMVTVSVDITNTGDYDGKEVAQLYIRDVIG-SITRPVKELKG   721
Mesoflavibacter_sabulilitoris            -----------------NDTINVSVDITNTGDYDGKEVAQLYIRDLVG-SVTRPVKELKG   721
Meridianimaribacter_sp._CL38_GH3b        -----------------NDTINVSVDITNTGDYDGKEVAQLYIKDLVG-SVTRPVKELKG   721
Flavobacterium_sediminis                 -----------------GEPVKASVTVKNTGNYDGKEVVQLYVRDVVA-SLTRPVKELKD   702
Olleya_aquimaris                         -----------------NNTVTVSVDLTNSGKVKGKEVVQLYIRDLYA-SVTRPVKELKG   709
Kordia_zhangzhouensis                    -----------------GDTIKVTVDVTNTGDYDGKEVVQLYIRDLFG-SITRPVRELKG   695
Winogradskyella_vidalii                  -----------------NKEITVSFDVKNAGEVTGKEVAQLYIRDLVG-SVSRPVKELKG   702
Flavobacteriaceae_bacterium_144Ye        -----------------NQEVKVSFTLKNTGTLKGKEVAQLYIRDLVG-SVIRPLKELKG   703
Meridianimaribacter_sp._CL38_GH3a        -----------------NQEVKVSFTLKNTGALKGKEVAQLYIRDLVG-SVIRPLKELKG   703
Meridianimaribacter_flavus               -----------------NQEVKVSFTLKNTGALKGKEVAQLYIRDLVG-SVIRPLKELKG   703
Chaetomium_thermophilum                  LLRSSGKNSPGGNPQLYDVLYTVTADITNTGALEGDEVVQLYVSLGG---PDDPKVMLRD   809
Cochliobolus_heterostrophus              --GPAAGGAQGGNPALFDVAYDIAVTVTNTGNRSGKAVAQLYLQFPSESTVDTPILQLRD   797
                                                                :  :.* *   *. *.***:          *   *:.

Mangrovimonas_xylaniphaga                FQKIE-LKKGETKTVNFKLTVEDLKFYNSELD-F--VAEPGTFQVFVGTDSTTEMMKEFE   773
Winogradskyella_litoriviva               FQKIF-LKKGETKTVSFTLSVEDLKFYNANID-F--VAEPGDFQVAIGTNSNVELNKTFK   777
Mesoflavibacter_sabulilitoris            FQKVF-IKKGETVTVTFKISVEDLKFYNSNLD-F--VAEPGEFQVAIGTNSDVALKNSFK   777
Meridianimaribacter_sp._CL38_GH3b        FQKVF-IKKGETVTVTFKISVEDLKFYNSNLD-F--VAESGEFQVAIGTNSDVALENSFK   777
Flavobacterium_sediminis                 FQMIS-LNKGESKTVEFVLTEKELGFYNSEGE-F--IVESGKFQIFIGTSSSDTKNSVFH   758
Olleya_aquimaris                         FELVE-LEPGETKKIQLTLTKDELGFFNNQGE-F--IVEDGDFEVYIGGSSKTTLQDKFT   765
Kordia_zhangzhouensis                    FELVS-LKKGETKTIEFKLSNKELGFFNNQGE-Y--IVEAGDFEVFVGGSSITSLQSSFT   751
Winogradskyella_vidalii                  FELVE-LEAGESKRITIKLTEKELGFYNNKQE-F--KVESGDFKIFVGGSSETVLEADIE   758
Flavobacteriaceae_bacterium_144Ye        FELIE-LEAGASKTVEFVLTKKELGFYTNQGE-F--VVESGDFKVFVGGNSNAELEQDFT   759
Meridianimaribacter_sp._CL38_GH3a        FELIE-LEAGASKTVEFVLTKKELGFYTNQGE-F--VVESGDFKVFVGGDSNAELEQGFT   759
Meridianimaribacter_flavus               FELIE-LEAGASKTVEFVLTKKELGFYTNQGE-F--VVESGDFKVFVGGDSNAELEQGFT   759
Chaetomium_thermophilum                  FARLH-IKPGETVKFEGKLTRRDLSTWDVTLQDWVIRDHTK--MVFLGKSSRKLVLGALL   866
Cochliobolus_heterostrophus              FAKTSTLAAGASQTLKMRLTRKDLSVWDVVEQNWVVPAVSGDYGVWIGGASDDLHLRCGT   857
                                         *     :  * :  .   ::  :*  :    : :          : :*  *

Mangrovimonas_xylaniphaga                LTK----------   776
Winogradskyella_litoriviva               LID----------   780
Mesoflavibacter_sabulilitoris            LLD----------   780
Meridianimaribacter_sp._CL38_GH3b        LLD----------   780
Flavobacterium_sediminis                 LY-----------   760
Olleya_aquimaris                         L------------   766
Kordia_zhangzhouensis                    LK-----------   753
Winogradskyella_vidalii                  L------------   759
Flavobacteriaceae_bacterium_144Ye        LE-----------   761
Meridianimaribacter_sp._CL38_GH3a        LE-----------   761
Meridianimaribacter_flavus               LE-----------   761
Chaetomium_thermophilum                  N------------   867
Cochliobolus_heterostrophus              ATGRCQGDQASPV   870
```

Figure 4. Multiple sequence alignment of Bgl3a and Bgl3b with β-glucosidase of other species. β-glucosidases of *Cochliobolus heterostrophus* (Genbank Accession: AAB82946.1) and *Chaetomium thermophilum* (ABR57325.2) were used for motifs and active site identification, respectively. Region of motifs were drawn with black lines, and the active site was shown in red box. Asterisks indicates fully conserved amino acids; colon indicates strongly similar properties between group and full stop indicate weakly similar properties between groups.

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