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RESEARCH

A novel acidic laminarinase derived from Jermuk hot spring metagenome

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Abstract

Laminarinase, an enzyme with a specific affinity for laminarin—a complex polysaccharide found in the cell walls of brown algae and select marine organisms—was investigated in this study. We cloned and characterized a gene encoding a putative glycoside hydrolase family 16 (GH16) laminarinase derived from the Jermuk hot spring metagenome. The resulting product, named Jermuk-LamM, represents a novel 1,3-β-D-glucanase with 48.1% amino acid sequence similarity to previously characterized GH16 family members catalogued in the NCBI database. To date, this stands as the sole described endo-1,3-β-D-glucanase from the Fidelibacterota phylum, which was recently reclassified from *Marinimicrobia*. Jermuk-LamM, identified as an acidic laminarinase, exhibits optimal enzymatic activity at pH 5.0 and a temperature of 55 °C, maintaining its function for a duration of at least 7 h. Jermuk-LamM is an enzyme that efficiently hydrolyzes both soluble and insoluble (1,3)-β-D-glucans, as well as (1,3;1,4)-β-D-glucans, with a marked preference for laminarin. This enzymatic activity facilitates the valorization of macroalgal biomass by predominantly producing monosaccharides and disaccharides. These hydrolysis products can subsequently be converted into energy carriers such as alcohol, methane, and hydrogen. The enzyme's specific activities, coupled with its resistance to various additives, render Jermuk-LamM a promising candidate for various industrial applications, encompassing the realms of biofuel and pharmaceutical production.

Key points

- *Jermuk hot springs have significant potential as a source of novel enzymes.*
- *Jermuk-LamM has less than 50% amino acid similarity to known enzymes.*
- *It is the first enzyme characterized from the Fidelibacterota phylum.*

Keywords Laminarinase · Endo-1,3-β-D-glucanase · Metagenome · Fidelibacterota · Recombinant expression · Biochemical characterization

Introduction

Polysaccharides with β−1,3-linkages, such as laminarin, curdlan, lichenin, and β-glucan, have been found in animals, plants, algae, and microorganisms. They have diverse functional properties and play an essential role in sustaining life. In plants, β-glucans play structural roles, such as giving strength, flexibility, and elasticity, as well as having roles in the transport of water, nutrients, and other small molecules (Chang et al. 2021). They are also cell-wall components

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of brown algae (Salmeán et al. 2017). Lichenin is a type of β-glucan derived from lichen and is a component of the mycobiont cell wall in *Cetraria islandica* (Iceland moss). It is composed of β-D-glucopyranose units linked in a linear manner by β−1,4- and β−1,3-glycosidic bonds (Perlin and Suzuki 1962; Bacic et al. 2009) and plays a central role in the formation of symbiotic relationships between algae and fungi in lichens. Lichenin-like β-glucans have also been identified in the cell wall of *Aspergillus fumigatus*, where they contribute to structural rigidity (Fontaine et al. 2000; Chang et al. 2021). Curdlan has diverse applications including food, cosmetics, and pharmaceuticals (Lovegrove et al. 2017; Pramanik et al. 2024). In *Agrobacterium tumefaciens*, it plays an important role in biofilm formation that protects against predation, starvation, and desiccation (Matthysse 2018). Laminarin is the primary storage polysaccharide of macroalgae (Øverland et al. 2019), consisting of glucose units linked by β−1,3-linkages with glucose branches attached with β−1,6-linkages (Graiff et al. 2016). Furthermore, it is also a component of the cell wall in most fungal species (Ruiz-Herrera and Ortiz-Castellanos 2019). Curdlan is unbranched and consists of β−1,3-linked glycosyl residues with a chain length of about 135 residues (Harada et al. 1968), often with intra- or interchain 1,6-linkages (McIntosh et al. 2005); it is an extracellular and capsular polysaccharide produced by a variety of soil-dwelling bacteria belonging to the *Rhizobium*, *Agrobacterium*, *Alcaligenes*, *Cellulomonas*, and *Bacillus* genera (Karnezis 2003; McIntosh et al. 2005; Aquinas et al. 2022). The other known source of polysaccharides of this type is β-glucan, a major component of endosperm cell walls and the subaleurone layer of Gramineae, consisting of glucose linked by both β−1,4- and β−1,3-glycosidic linkages (Rimsten et al. 2003).

Unlike starch, these polysaccharides are non-food-based sugar sources and more suitable resources for the production of biofuels, as well as for pharmaceuticals, cosmetics, and packaging material (Liao et al. 2018; Deng et al. 2023). Recently, increasing attention is being drawn to macroalgae as an ideal biomass source with high levels of carbohydrates with low to zero lignin content. Moreover, macroalgae do not compete with agricultural land use (Gao et al. 2020) and are highly productive, with over 0.2 gigatons of CO_2 sequestered globally per annum (Krause-Jensen and Duarte 2016). Therefore, it is important to design biocatalysts to develop a cost-competitive process for the utilization of macroalgae carbohydrates (Radakovits et al. 2010; Takeda et al. 2011; Divate et al. 2013). In macroalgae, laminarin content ranges from 1 to 25% of total weight located in vacuoles (Adams et al. 2011).

Laminarinase, also known as laminarin hydrolase or β−1,3-glucanohydrolase, catalyzes the hydrolysis of β−1,3-glucosidic bonds, with the exception of the characterized LamR from *Rhodothermus marinus* (Krah et al. 1998) and SCLam derived from the soil +metagenome (Alvarez et al. 2015), which are able to cleave both 1,3- and 1,4-β-D-glycosyl linkages. Laminarinases have garnered interest due to their potential applications in various industries, including biofuel production, food processing, and pharmaceuticals (Pang et al. 2005; Bamforth 2009; Zhan et al. 2012; Koteshwara et al. 2021). Laminarinases have been characterized from plants (Subroto et al. 2001), fungi (Ishida et al. 2009; Tsai et al. 2011; Santana et al. 2018; Costa et al. 2020), algae (Barras and Stone 1969), Actinobacteria (Hong et al. 2002; Fibriansah et al. 2007), Archaea (Gueguen et al. 1997), soil bacteria (Akita et al. 2005; Masuda et al. 2006; Alderkamp et al. 2007), thermophilic (Zverlov et al. 1997; Gueguen et al. 1997; Cota et al. 2011; Woo et al. 2014; Kobayashi et al. 2016; Meng et al. 2016), and marine bacteria (Krah et al. 1998; Labourel et al. 2014; Kusaykin et al. 2017; Qin et al. 2017; Yang et al. 2020). Among these laminarinases that function as endo-β−1,3-glucanases (EC 3.2.1.39 and EC 3.2.1.6) typically belong to glycoside hydrolase (GH) families 16, 17, 55, 64, 81, and 128. In contrast, laminarinases exhibiting exo-β−1,3-glucanase activity (EC 3.2.1.58) are generally associated with GH families 3 and 30. However, a laminarinase with exo-β−1,3-glucanase activity has recently been characterized within the GH16 family (Li et al. 2024).

Endo-β−1,3-glucanases hydrolyze β−1,3-glucans and release glucose and other oligosaccharides, whereas exo-β−1,3-glucanases cleave glucose and small oligomers from the non-reducing end of the substrate (Santos et al. 2020; Li et al. 2024).

Considerable attention has been devoted to the characterization and continuous improvement of laminarinases, aiming to establish a cost-competitive approach for converting laminarin into fermentable sugars (Klein-Marcuschamer et al. 2012; Kim et al. 2018).

Metagenomics is a promising approach to exploit the genetic diversity of microbial communities and to discover novel enzymes, with notable examples of the discovery of several glycoside hydrolases from different GH families (Alvarez et al. 2015; Schröder et al. 2018; Suleiman et al. 2019; Klippel et al. 2019). A metagenome obtained from the Jermuk hot springs in the Lesser Caucasus mountains of Armenia served as a source for laminarinase identification (results not shown). Jermuk hot springs are located within the geothermal system of the Lesser Caucasus mountains at 2080 m above sea level; the temperature of the water in the hot springs varies from 40 to 53 °C, pH 7.5. Different strains belonging to *Bacillus*, *Parageobacillus*, *Geobacillus*, and *Anoxybacillus* genera have already been isolated from the Jermuk hot springs, producing valuable sources of hydrolases (Panosyan et al. 2020; Burkhardt et al. 2024). This study offers an in-depth characterization of a laminarinase derived from a metagenome of Jermuk hot springs. This work describes the first characterized enzyme from the *Candidatus Marinimicrobia* which has been

reclassified to Fidelibacterota phylum (Katayama et al. 2024). The potential application of this enzyme for bioconversion of natural polysaccharides was evaluated.

Materials and methods

Reagents and substrates

Phusion® DNA Polymerase, BsaI restriction enzyme, and T4 DNA ligase were purchased from New England Biolabs (Hitchin, UK). Isopropyl β-D−1-thiogalactopyranoside (IPTG) was purchased from Merck, UK. The molecular weight marker for SDS-PAGE was purchased from Thermo Fisher Scientific (Cramlington, UK). Carboxymethyl cellulose (CMC), yeast β-glucan, and lichenin were obtained from Merck, and β-glucan (barley), curdlan (*Alcaligenes faecalis*), and azo-dyed and azurine cross-linked (AZCL) substrates from Megazyme. Standards—including laminaritetraose, laminaritriose, and laminaribiose—were purchased from Sigma, and glucose was sourced from Merck. Macroalgae was kindly provided by colleagues from the Center for Biobased Solutions (CBBS) at Hamburg University of Technology (TUHH).

Bacterial strains, plasmids, and metagenome

The metagenome DNA obtained from Jermuk hot spring was used for ORF amplification. Samples were collected from different locations of a Jermuk hot spring, coordinates (39.8411693, 45.6678023), temperature 40–45 °C, pH 7–8 (sample type: water and sediment). The collected material was transported and stored at 4 °C.

DNA from the sample was isolated using the PowerSoil Isolation Kit by MO BIO Laboratories Inc. according to the manufacturer's instructions. The obtained DNA was then subjected to Illumina HiSeq 4000 by G2L sequencing lab in Göttingen sequencing. Sequence data processing, assembly, and annotation were performed as previously described (Busch et al. 2021).

Sequence analysis, cloning of ORFs

A DLam gene from *Dictyoglomus thermophilum* was used to scan contigs for predicted homologue ORFs (ACI18772.1). Potential ORFs encoding putative endo-β−1,3-D-glucanases were identified, and reannotated contigs were compared to the NCBI (BLASTx) database. To identify the most related nucleotide and amino acid, sequences for the identified ORFs, BLASTn, BLASTp, and PSI-BLAST analyses were performed. The prediction of functional and structural domains, catalytic sites, and signal sequences was performed with applications such as Conserved Domain Database (CDD) (Marchler-Bauer et al. 2015), ScanProsite (De Castro et al. 2006), SignalP 6.0 (Teufel et al. 2022), and SMART (Letunic et al. 2009). For multiple sequence alignment and construction of a phylogenetic tree from the GenBank database, amino acid sequences of characterized β−1,3-glucanases and β−1,3(4)-glucanases of the family GH16 were selected. Accession codes for the sequences used are detailed in Supplementary Table 1.

Protein sequence visualization was performed using Multalin (Corpet et al. 1999) and figures prepared with ESPript (Gouet 2003). The phylogenetic tree was constructed using MEGA11, by the neighbor-joining method (Tamura et al. 2021). The evolutionary history of the Jermuk-LamM was inferred by using the maximum likelihood method and JTT matrix-based model (Jones et al. 1992). The highest log likelihood is − 17,122.20. Initial tree(s) for the heuristic search were obtained automatically by applying neighbor-joining and BioNJ algorithms to a matrix of pairwise distances estimated using the JTT model and then selecting the topology with superior log likelihood value. This analysis involved 16 amino acid sequences.

Protein structure modeling and analysis

The Jermuk-LamM protein was modeled by AlphaFold2 (Jumper et al. 2021) using the ColabFold pipeline (Mirdita et al. 2022). Related protein structures were identified in the PDB using the advanced sequence search tools at the RCSB PDB. Both PDB models and sequences were retrieved for further analysis. Structural models were aligned and visualized using ChimeraX (Meng et al. 2023). Protein sequence alignments were prepared using Multalin (Corpet 1988) and visualized in ESPript (Robert and Gouet 2014). The theoretical molecular weight (MW) and isoelectric point (pI) were estimated using the ExPASy, ProtParam tool Compute MW/pI (De Castro et al. 2006).

Benchling was used for specific primer design, in silico PCR and assembly experiments. For cloning, Jermuk-lamM gene was amplified using LamM-F (5′**GACGGTCTCTAATG**CCCGAAGACGAATCGCCTCAGG3′) and LamM-R (5′**GTCGGTCTCTACCT**GGATTTGATCCGCTGGAAGATACGGAC3′) primers (primer extending sequences are indicated in bold) within the following PCR conditions: 98 °C for 1 min followed by 30 cycles of 98 °C for 30 s, 65 °C for 30 s, and 72 °C for 1 min, followed by a final elongation at 72 °C for 10 min, using the metagenomic DNA as template. After examination by electrophoresis, the amplified product was purified with the QIAquick PCR Purification Kit, then assembled via one-pot Golden Gate cloning (Engler et al. 2008) and CIDAR MoClo system (Iverson et al. 2016) using a modified pET28 with CIDAR MoClo compatible restriction

sites and overhang sequences. The assembled plasmid was used to transform *Escherichia coli* TOP10 cells. Recombinant plasmid DNA was extracted from insert-positive clones and, after Sanger sequence confirmation, used to transform *E. coli* C43(DE3) or *E. coli* BL21(DE3).

Expression of the *Jermuk-lamM* gene and enzyme purification

For protein expression, *E. coli* C43(DE3) harboring the plasmid pET28GGLacZ::*Jermuk-lamM* was grown in LB media supplemented with 35 µg/mL kanamycin at 37 °C with shaking at 160 rpm. Gene expression was induced at OD_{600} 0.5–0.6 by adding IPTG to a final concentration of 1 mM. Cells were harvested by centrifugation at 9000 × *g* at 4 °C for 20 min. The resulting cell pellet was stored at − 20 °C.

For purification, 0.2 g cells were resuspended per 1 mL lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8) and disrupted by three passages through a French pressure cell with a constant pressure of 1000 psi (French pressure cell press, SLM Aminco). Cell debris was removed by centrifugation (20,000 × *g*, 4 °C, 30 min), and the supernatant was loaded onto a 1-mL Ni–NTA Superflow column (QIAGEN). Proteins were eluted by an increasing imidazole gradient (250 mM) according to the manufacturer's instructions. Eluted fractions were pooled, washed three times with buffer A (50 mM Na-phosphate buffer, pH 7.2, 150 mM NaCl) by ultrafiltration in an Amicon filter unit (Amicon Ultra-15, 1000 MWCO, Merck Millipore). For the final purification via size exclusion chromatography, protein solutions were loaded onto a Sephacryl S-100 column previously equilibrated with buffer A. Protein fractions containing the purified enzymes were pooled and stored at 4 °C.

Protein samples were analyzed by SDS-PAGE (12.5%) (Laemmli 1970). Protein bands were visualized by staining with Coomassie Blue G-250. Protein concentration was determined according to the Bradford (1976) assay, with bovine serum albumin as standard.

Characterization of Jermuk-LamM

Unless otherwise noted, the standard assay was carried out at 65 °C for 10 min in 500 µL reaction mixture using 0.25% (*w/v*) laminarin from *Laminaria digitata* (Merck) as substrate in 50 mM sodium phosphate buffer pH 6.0 (adjusted at assay temperature) and 5.0 ± 0.5 µg (0.11 mg/mL) enzyme sample. Additionally, controls without enzyme were performed for all measurement series. The hydrolytic activities of the purified enzymes were detected by measuring the liberation of reducing sugars with 3,5-dinitrosalicylic acid (DNS) according to Miller (1959) with glucose as standard. In brief, after the enzyme reaction, 500 µL reaction mixture was mixed with 500 µL DNS reagent (1% (*w/v*) DNS, 30% (*w/v*) potassium sodium tartrate, 0.4 M NaOH) and incubated for 5 min at 100 °C. Samples were subsequently cooled on ice to room temperature, and absorbance was measured at 546 nm. All measurements were done in triplicates. One unit of enzyme activity was defined as the amount of enzyme required to release 1 µmol of reducing sugars per minute.

The influence of temperature on enzyme activity was examined by performing the standard assay at temperatures from 30 to 80 °C. To investigate the temperature sensitivity, the enzyme samples were pre-incubated with a concentration of 0.1 mg/mL in the same range of temperature for 10 min. Subsequently, they were stored on ice, and residual activities were measured by using the standard assay. To investigate the temperature stability at selected temperatures, Jermuk-LamM was pre-incubated at 55 and 60, respectively, and samples were taken at time intervals up to 24 h. Residual activities were measured by using the standard assay. To determine the influence of the pH on enzyme activity, a standard assay was performed using Britton-Robinson buffer (50 mM) in a range of pH 2–10 in the reaction mixture (Britton and Robinson 1931). The influence of metal ions on enzyme activity was analyzed by using a standard assay after incubating the enzyme in the presence of 5 mM $AlCl_3$, $CaCl_2$, $CoCl_2$, $CrCl_3$, $CuCl_2$, $FeCl_2$, $FeCl_3$, KCl, $MgCl_2$, NaCl, $NiCl_2$, RbCl, $SrCl_2$, or $ZnCl_2$ for 1 h at room temperature. Metal solutions were prepared freshly before each experiment. The influence of additives such as 3-((3-cholamidopropyl) dimethylammonio)−1-propanesulfonate (CHAPS), SDS, Triton X-100, Tween 20, Tween 80, guanidine hydrochloride, urea, dithiothreitol (DTT), β-mercaptoethanol, EDTA, iodoacetic acid, Pefabloc, cetyltrimethylammonium bromide (CTAB), and sodium azide was examined by the standard assay procedure followed by 1-h incubation at room temperature. All additives were tested at a final concentration of 5 mM under standard conditions. A control experiment was performed for each metal and additive in the absence of enzyme.

For analyzing the substrate spectrum, the specific activities of Jermuk-LamM were measured using different substrates. All substrates were used at a final concentration of 0.25% (*w/v*). Additionally, 0.1% and 0.5% concentrations were tested for laminarin, barley β-glucan, and lichenin, while only 0.1% for amorphous curdlan was tested.

In case of curdlan, undissolved and dissolved (amorphous) forms were tested. To achieve an amorphous type of curdlan, 0.2 g of the substrate was first solubilized in 6 mL 0.6 M NaOH and subsequently neutralized with HCl to a concentration of 0.5% (*w/v*) and pH 6.0 in 50 mM sodium phosphate buffer. A control experiment was conducted for each substrate.

Determination of hydrolysis products

The hydrolytic products were analyzed by high-performance liquid chromatography (HPLC) and by thin layer

chromatography (TLC). For determination of the hydrolysis products, 0.25% (*w/v*) substrate was incubated with Jermuk-LamM (0.1 U) in standard reaction mixtures at 55 °C for 18 h. After the inactivation of the enzyme samples at 100 °C for 10 min, samples were centrifuged (20,000 × *g*, 10 min, 4 °C), and the supernatant was filtered using a 0.22-µm membrane filter unit. Hydrolysis products were analyzed by high-performance liquid chromatography (HPLC) under the following conditions: Aminex HPx-42A column (Bio-Rad), 80 °C, 0.6 mL/min flow rate, water as mobile phase, HPLC 1260 Infinity II LC System (Agilent Technologies) with RI detector. Laminaritetraose, laminaritriose, laminaribiose, and glucose were used as standards.

To monitor the progress of the laminarin hydrolysis reaction, a substrate containing 0.5% laminarin was incubated with Jermuk-LamM (0.1 U) in standard reaction mixtures at 55 °C for 8 h. Samples were collected before the reaction started and at 1, 4, and 8 h. The reaction products were analyzed using TLC (Soghomonyan et al. 2024) to identify the hydrolytic products. Glucose, laminaribiose, and laminaritriose were used as standards at a concentration of 10 mM.

Macroalgal biomass hydrolysis

Fucus vesiculosus, which is also known as black tang, was used as a natural substrate for Jermuk-LamM. Macroalgal biomass was thoroughly washed with fresh water to remove salts, sand, and other impurities. The washed biomass was air-dried and ground into particles of 0.06–0.1 cm using a mill. The resulting material was used as a substrate for subsequent pre-treatments and enzymatic hydrolysis. Pre-treatment of the biomass was conducted to disrupt the cell walls and enhance enzyme accessibility. This was achieved using physical, chemical, or combined physical–chemical methods.

Ultrasonication was employed as a physical method. A suspension of 10 mg/mL biomass in water was prepared and sonicated on ice for 5 min with 30-s on/off cycles at a power output of 60 watts.

For chemical pre-treatment, the same amount of biomass was treated with NaOH (0.1 M) (alkaline treatment) and Na_2CO_3 (0.1 M) (mild alkaline treatment). Water also was used as a natural treatment. Each treatment was performed in duplicate, with one sample undergoing ultrasonication and the other serving as a non-sonicated control. All samples were incubated in their respective chemical solutions/water overnight at room temperature before ultrasonication.

Following pre-treatment, solubilized sugars were precipitated by using ethanol. A 1:1 solution of 70% ethanol was added to the treated biomass, and the mixture was stored at − 20 °C for 12 h. Precipitated material was collected by centrifugation, and the pellet was air-dried at room temperature for 6 h to allow ethanol evaporation.

Finally, the pre-treated biomass was suspended in a sodium phosphate buffer (50 mM), and enzymatic hydrolysis was initiated by adding 0.1 U of enzyme per reaction. The reactions were performed in a thermoshaker at a temperature of 55 °C with a shaking speed of 400 rpm for 24 h. The amount of reducing sugars was measured by the DNS method (Miller 1959). After 24 h, the reaction products were analyzed using TLC (Soghomonyan et al. 2024) to identify and characterize the hydrolytic products. Glucose, laminaribiose, and laminaritriose were used as standards at a concentration of 10 mM. Controls without enzyme were included for all reaction conditions to account for non-enzymatic degradation.

Data plotting and analysis

All numerical data was plotted using GraphPad Prism software version 10.4.1. For the comparison of effectiveness of physical and chemical treatment of macroalgal biomass, statistical tests were performed using the built-in Kolmogorov–Smirnov test in the software.

Nucleotide sequence accession number

The sequence of *Jermuk-lamM* was deposited in GenBank (accession number OK490392).

Results

A total of 68 ORFs were identified in the Jermuk metagenome dataset as potential laminarinase candidates. After bioinformatic analyses of these sequences with selection based on low sequence identity, ten of them were chosen for cloning. Initial plate tests revealed that while two of the cloned sequences exhibited activity against the tested substrates (AZCL-curdlan, AZCL-barley β-glucan, AZCL-pachyman), only one, designated as Jermuk-LamM, demonstrated specificity towards substrates containing only β−1,3-linkages. Recombinant Jermuk-lamM was subsequently selected for further analysis. The putative enzyme showed activity on agarose plates using AZCL-curdlan, AZCL-barley β-glucan, and AZCL-pachyman as substrates (Fig. S1).

The ORF *Jermuk-lamM* encodes a polypeptide of 287 amino acids. Analysis of the full-length Jermuk-LamM sequence using the SignalP 6.0 server predicts that the protein functions as a Sec-secreted membrane-anchored lipoprotein, with an N-terminal signal peptide (MPKLYFYLAGALLALNLLMTC), predicted to be cleaved between Thr20 and Cys21, with the resulting N-terminal cysteine acting as the membrane anchor. A linker sequence connects this signal peptide to the catalytic domain, which is predicted to belong

to the GH16 family (Fig. S2). A theoretical molecular mass of 30.5 kDa and a pI of 5.33 were calculated for the mature enzyme.

Sequence searches of the NCBI nucleotide and protein databases show that the Jermuk-LamM had less than 75% identity with all glycoside hydrolase sequences deposited. The closest homologue to the Jermuk-LamM was a GH16 family protein from *Candidatus.*

Marinimicrobia bacterium (GenBank: HDP67611.1) with 66.9% identity and 100% sequence coverage; other high identity matches include 67.2% to a GH16 family protein candidate from division KSB1 bacterium (GenBank MBD3289618.1) and 65.84% over 98% coverage to a GH16 family protein of Calditrichaeota bacterium (GenBank: RMF58681.1). All these protein sequences are from uncultured marine bacteria, and none of the protein products has been characterized to date. Phylogenetic analysis of selected related GH16 protein sequences belonging to sub-family 3 shows that the Jermuk-LamM is clearly within this sub-family and that it forms a cluster with the endo-1,3(4)-beta-glucanase originating from *Acetivibrio thermocellus* and is more distantly related to endo-beta-1,3-glucanase from the hyperthermophilic Archaeon *Pyrococcus furiosus* DSM 3638 and laminarinase of the thermophilic bacterium *Thermotoga maritima* MSB8 (Fig. 1). Interestingly, in contrast to Jermuk-LamM, the endo-1,3(4)-beta-glucanase (Lic16A) of *Acetivibrio thermocellus* (previously known as *Clostridium thermocellum*) is a multi-domain protein consisting of a number of structural modules including S-layer homology domains, multiple CBM4 domains, and a catalytic GH16 domain (Fuchs et al. 2003).

Phylogenetic tree produced using amino acid sequences of the GH16 domains from laminarinase enzymes related to the Jermuk-LamM protein. The evolutionary history was inferred by using the maximum likelihood method and JTT matrix-based model. The tree is drawn to a scale, with

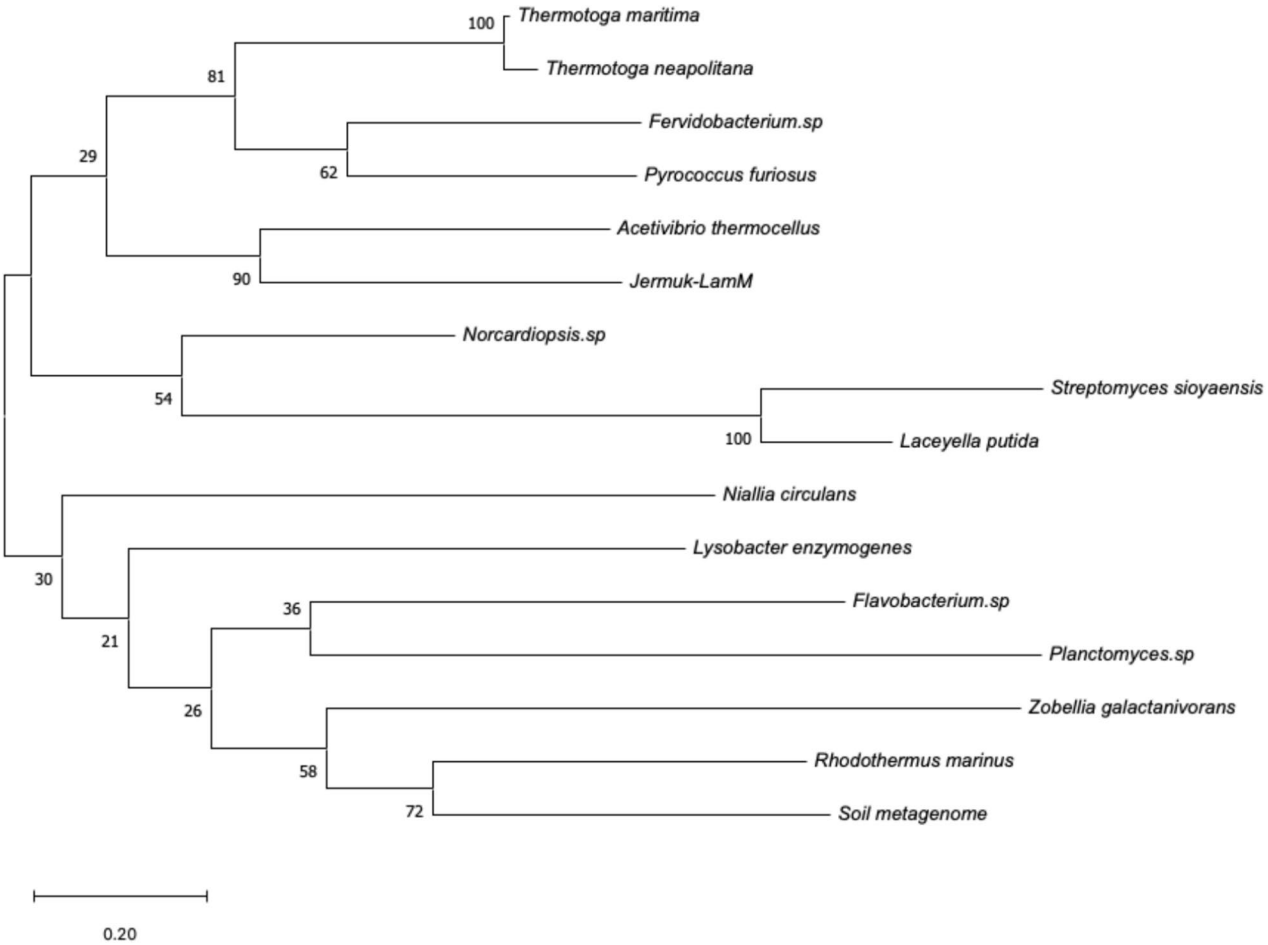

Fig. 1 Phylogenetic tree of selected laminarinase enzymes. Phylogenetic tree produced using amino acid sequences of the GH16 domains from laminarinase enzymes related to the Jermuk-LamM protein. The evolutionary history was inferred by using the Mmaximum Llikelihood method and JTT matrix-based model. The tree is drawn to a scale, with branch lengths measured in the number of substitutions per site (next to the branches). The tree with the highest log likelihood (−−17,122.20) is shown. Glycoside hydrolase domain sequences and their accession codes are shown in Supplementary Table 1

branch lengths measured in the number of substitutions per site (next to the branches). The tree with the highest log likelihood (− 17,122.20) is shown. Glycoside hydrolase domain sequences and their accession codes are shown in Supplementary Table 1.

Following on from the sequence and phylogenetic analysis, AlphaFold2 structure predictions for Jermuk-LamM gave models with high predicted local distance difference test (pLDDT) scores for the catalytic domain and low scores for the putative signal peptide and linker region (Fig. S3a). The predicted structure of Jermuk-LamM was aligned with structural homologues from the PDB, including enzymes from *T. maritima* (TmLamCD, 3AZZ, 50% sequence identity), *Pyrococcus furiosus* (pfLamA, 2VY0, 46% identity), alkaliphilic *Nocardiopsis* sp. strain F96 (BglF, 2HYK, 51% identity), and *Rhodothermus marinus* (RmLamR, 3ILN, 43% identity) (Fig. S3b). Sequence alignment of the identified structural homologues and other characterized laminarinase enzymes shows key conserved residues: the structural metal site, the active site catalytic triad, and ligand positioning residues (Fig. 2).

Despite the high level of structural conservation and retention of key catalytic residues to produce an active site with an open cleft (Figs. 2 and 3a, b), key differences were observed in terms of specific structural elements with functional significance. Notably, one of these elements has been described as a flexible loop with the sequence "GASIG" in TmLamCD, which has a divergent sequence in the Jermuk-LamM and adopts a different conformation in the predicted structure of Jermuk-LamM (Fig. S3b).

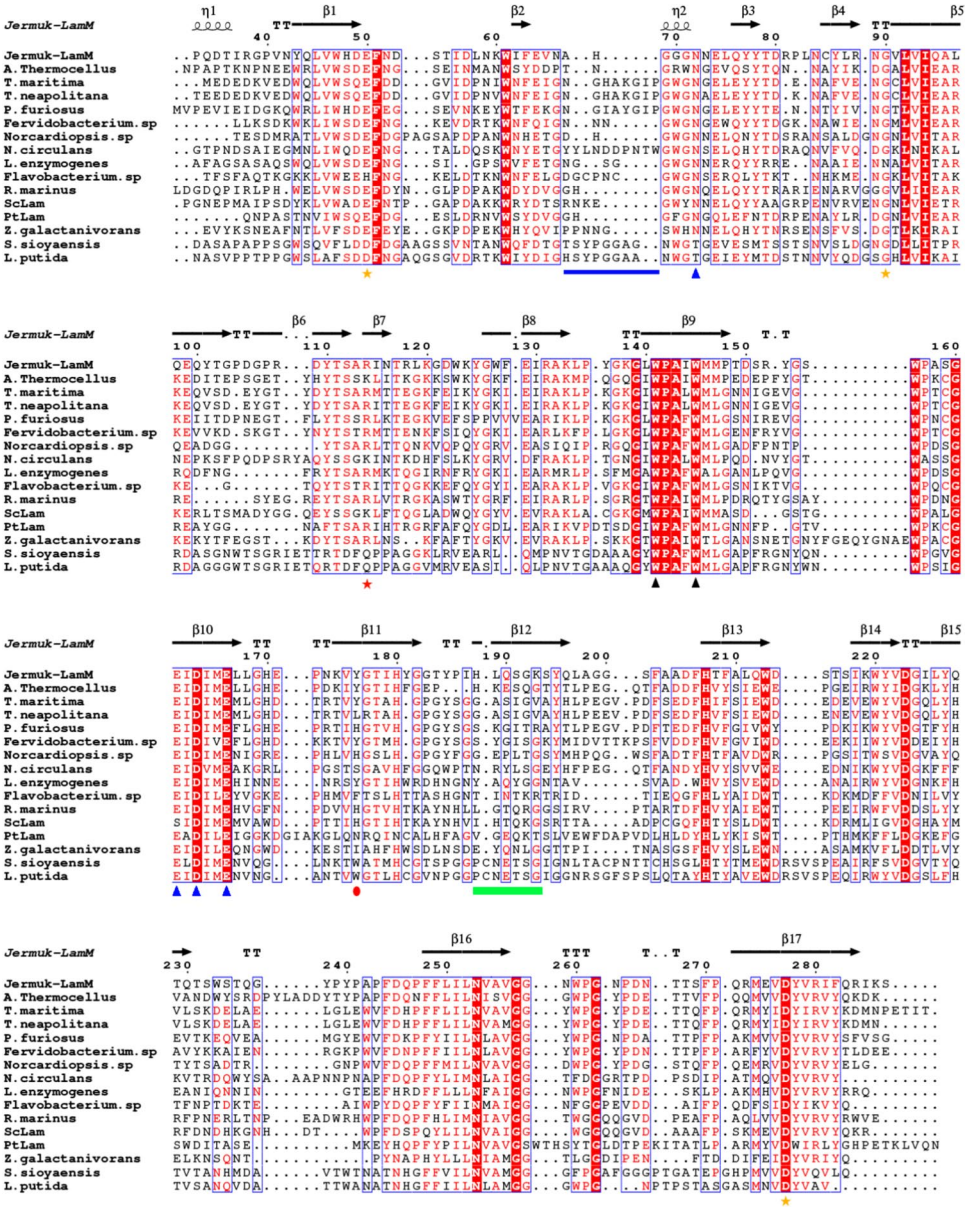

Fig. 2 Annotated sequence alignment of Jermuk-LamM homologues. Multiple sequence alignment of the catalytic domain of Jermuk-LamM with characterized laminarinase enzymes and structural homologues (T. maritima, 3AZZ; P. furiosus, 2VY0; Norcardia sp., 2HYK; R. marinus, 3ILN). Sequence alignment was performed using Multalin and visualised with ESPript. Secondary structure elements from the Jermuk-LamM model are shown above the alignment. Strictly conserved residues are shown with a red background, while partially conserved residues are shown with red text. Conserved residues involved in metal coordination are highlighted with orange asterisks. Conserved ligand positioning residues are highlighted as black triangles, and the catalytic triad is highlighted as blue triangles. A conserved arginine residue is highlighted with a red star. The GASIG flexible loop of TmLamCD is highlighted with a green line, and the "Kink" peptide insertion is highlighted in blue. Residues predicted to be involved in endo-/exo -specificity are highlighted with a red oval. Sequences and accession codes for the proteins are available in Supplementary Table 1

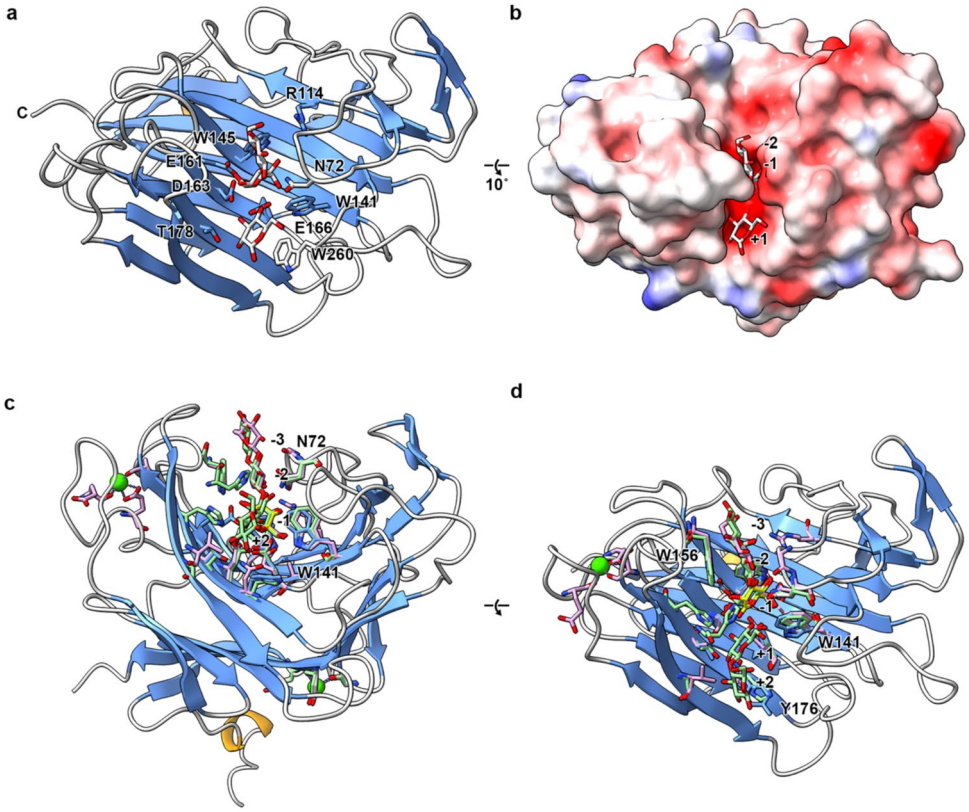

Fig. 3 Structural modeling of the Jermuk-LamM active site. **a** Conserved active site residues of Jermuk-LamM. A cartoon model of the overall structure is shown, with active site residues shown as sticks; an overlay of product molecules from the *P. furiosus* SCLam structure (6XQM) is shown with white carbons to highlight putative ligand interactions, and laminaribiose is shown in the -1/-2 site and glucose in the +1 site. **b** Calculated electrostatic surface of the Jermuk-LamM model showing the negatively charged ligand binding cleft with docked and modeled ligands from 6XQM. **c** Cartoon illustration of the Jermuk-LamM fold with conserved ligand binding residues shown as sticks. Residues from the Jermuk-LamM are in blue, those from 6XQH in green including docked 1,3-beta-D-cellobiosyl-glucose and cellobiose; and in pink laminaritriose ligand from 8XPK. The gluconolactone inhibitor from the *T. maritima* enzyme (3AZZ) is shown with yellow sticks (**d**), orthogonal view of **c** shown. The figure is prepared using ChimeraX

This loop had been demonstrated to act with Trp232 in the *T. maritima* laminarinase in the regulation of endo- or exo-activity of the enzyme and linked to a preference to release laminaritrioses in long-chain carbohydrate hydrolysis; this residue is conserved in the LamM laminarinase (Trp260) (Fig. 3a). Arginine 85 of the *T. maritima* laminarinase has been shown to have a critical role in β−1,3-glucan substrate selection and is conserved for the Jermuk-LamM (Arg114) (Jeng et al. 2011) (Fig. 3c, d). Both the *T. maritima* and *P. furiosus* homologues have a sequence insertion of six residues between residues 66 and 67 of the Jermuk-LamM (Fig. 2); this insertion forms a kinked loop at the entrance to the catalytic cleft, which is important for substrate preference in the *P. furiosus* enzyme and ensured higher activity on laminarin than lichenin (Ilari et al. 2009). This peptide is notably missing in the Jermuk-LamM, as well as the two other homologues analyzed.

Recombinant expression and purification of Jermuk-LamM

The ORF Jermuk-lamM without signal peptide was amplified with attached restriction recognition sites omitting start and stop codons and ligated into the modified vector pET28, which includes a 6xHis tag encoding region. The expression of the Jermuk-LamM construct in *E. coli* BL21(DE3) using common expression conditions (such as induction by 1 mM IPTG for 4 h at 37 °C) resulted in the formation of inclusion bodies. To overcome this, several parameters such as the concentration of IPTG, induction time, and temperature, as well as different expression hosts were tested. The expression conditions were optimized, resulting in an increase in optimal soluble protein production. Under these conditions, approximately 10% of the total induced protein was in its soluble form when expressed in *E. coli* C43(DE3) at 30 °C for 24 h.

The obtained protein harboring a C-terminal 6xHis affinity tag was purified via affinity chromatography and subsequent size exclusion chromatography. Jermuk-LamM was purified to homogeneity with a 228.73 U/mg specific activity and a final yield of total activity 13.6% (Table S2). The SDS-PAGE revealed a molecular weight of approximately 30 kDa, which was in accordance with the predicted molecular weight of 29.8 kDa (without signal peptide) (Fig. S4).

Substrate specificity of Jermuk-LamM

The activity of Jermuk-LamM was tested against several complex carbohydrates in three different concentrations at 65 °C (Table 1). The highest activity (228.0 ± 9.4 U/mg) of the enzyme was measured towards laminarin at a substrate concentration of 0.5%. This was comparable to the activity (217 ± 8.1 U/mg) measured at 0.25% concentration but was 2.3 times higher than that observed at 0.1% concentration. Compared to that, its highest activity towards amorphous curdlan was 74 ± 17.39 U/mg at the substrate concentration of 0.25% which was about twice as high as it was at the concentration of 0.1%. For undissolved curdlan, the highest activity was 35 ± 21.59 U/mg. The reason for the relatively low activity of the enzyme against curdlan is unclear, but it may be due to poor dispersal of the substrate in the assay. In comparison to β−1,3-glucans, the specific activities of Jermuk-LamM towards mixed-linked glucans such as barley β-glucan and lichenin were 128 ± 8.6 U/mg and 27 ± 3.55 U/mg, respectively, at the substrate concentration of 0.25%. The specific activity towards lichenin was lower than that for the other tested substrates; however, the enzyme exhibits little to no activity against yeast β-glucan. Hydrolysis of the β−1,4-glucan substrates (CMC, xyloglucan, and xylan) was not observed, further supporting the hypothesis that this enzyme acts primarily as β−1,3-glucanase.

Glycan hydrolysis pattern and kinetics

The hydrolysis products of laminarin, curdlan, barley β-glucan, and lichenin were investigated by HPLC analysis and have been compared with standards (Fig. 4 and Fig. S5). Hydrolysis of laminarin (Fig. 4a) mainly resulted in glucose

Table 1 Substrate specificity of Jermuk-LamM

Substrate (structure, linkage)	Specific activity (U/mg)		
Substrate concentration	0.1%	0.25%	0.5%
Laminarin (less branched, ~ 80% β−1,3: ~ 20% β−1,6)	97.6 ± 6.0	217.7 ± 8.1	228.0 ± 9.4
Curdlan*	46.6 ± 2.5	74.2 ± 17.4	-
Curdlan (linear, only β−1,3-linkage)	-**	35.5 ± 21.6	-
Barley β-glucan (linear, ~ 30% β−1,3: ~ 70% β−1,4)	30.0 ± 1.0	128.3 ± 8.6	154.4 ± 14.9
Lichenin (linear, ~ 30% β−1,3: ~ 70% β−1,4)	7.4 ± 0.5	27.2 ± 3.6	29.2 ± 1.7
Yeast β-glucan (branched, ~ 90% β−1,3: ~ 10% β−1,6)	-	3.8 ± 1.5	-
Xyloglucan (branched, ~ 80% β−1,4: ~ 20% α−1,6)	-	n.d.***	-
Xylan (branched, ~ 90% β−1,4: ~ 10% α−1,2)	-	n.d	-
CMC^+ (linear, only β−1,4-linkage)	-	n.d	-

*Amorphous curdlan, diluted in NaOH and neutralized. **Not tested. ***Not detectable

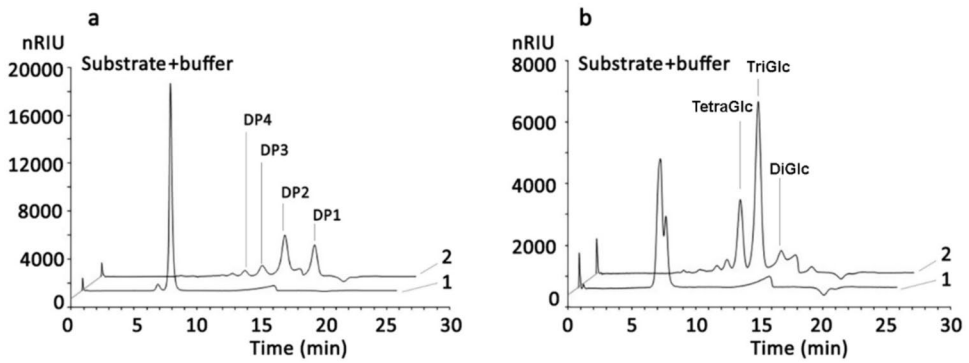

Fig. 4 HPLC analysis of products observed from polysaccharide degradation. Hydrolysis products from laminarin (**a**) and barley β-glucan (**b**) after 18 h of incubation at 55 °C. DP: degree of polymerization, DP1: glucose, DP2: laminaribiose, DP3: laminaritriose, DP4: laminaritetraose, DP5: laminaripentaose, DiGlc: disaccharide, TriGlc: trisaccharide, TetraGlc: tetrasaccharide. 1: substrate; 2: substrate + Jermuk-LamM

(DP1) and laminaribiose (DP2), glucose-mannitol units (as laminarin from brown algae can have mannitol as a terminal unit (Manns et al. 2014)), and to a lesser extent laminaritriose (DP3) and higher oligosaccharides when compared with standards (Fig. S4c). For curdlan, hydrolysis produced only glucose (DP1) and laminaribiose (DP2) consistent with its structure consisting exclusively of β−1,3-linkages (Fig. S5b/S5c). The degradation pattern of mixed-linkage polysaccharides differed. After hydrolysis of barley β-glucan (Fig. 4b) and lichenin (Fig. S5a), trisaccharide was the major product, and tetrasaccharide and other oligosaccharides were detected to a lesser extent when compared with standards (Fig. S5c). The shift in product distribution observed with barley β-glucan and lichenin suggests that the enzyme specifically hydrolyzes β−1,3-glycosidic linkages in mixed-linkage glucans.

An 8-h laminarin hydrolysis reaction was carried out, and a time course of the reaction product formation was studied. The results indicate that at the early stages of the reaction, a mixture of oligosaccharides with a degree of polymerization greater than DP2, along with glucose and laminaribiose, was produced. Over time, oligosaccharides were progressively hydrolyzed, resulting in an increase in the concentration of glucose and laminaribiose (Fig. S6). Considering the presence of various oligosaccharides during the initial stages of the hydrolytic reaction, along with the fact that the final products of both laminarin and curdlan hydrolysis are predominantly mono- and disaccharides, it can be concluded that Jermuk-LamM functions as an endo-acting β−1,3-glucanase.

Effects of pH and temperature on Jermuk-LamM activity

The effect of pH on the activity of the Jermuk-LamM was analyzed in a range of pH 2 to 9 (Fig. 5). Jermuk-LamM showed activity in a range between pH 4.5 and 7.5. The maximum activity was detected around pH 6.

To examine the temperature activity range of the Jermuk-LamM, its activity was measured in a temperature range from 30 to 80 °C. The maximum activity of the enzyme was measured at 65 °C (Fig. 6a). To examine the temperature sensitivity of the enzyme, residual activities were measured after 10 min of incubation at different temperatures ranging from 30 to 80 °C (Fig. 6b). The enzyme lost 50% of its initial activity after 10 min incubation at 65 °C. To analyze the temperature stability at selected temperatures, the enzyme was pre-incubated at 55 °C and 60 °C for 24 h (Fig. 6c). Residual activity was obtained in relation to the initial activity prior to pre-incubation. The enzyme exhibited more than 50% residual activity over 24 h at 55 °C. At 60 °C, the enzyme had a half-life time of 3 h.

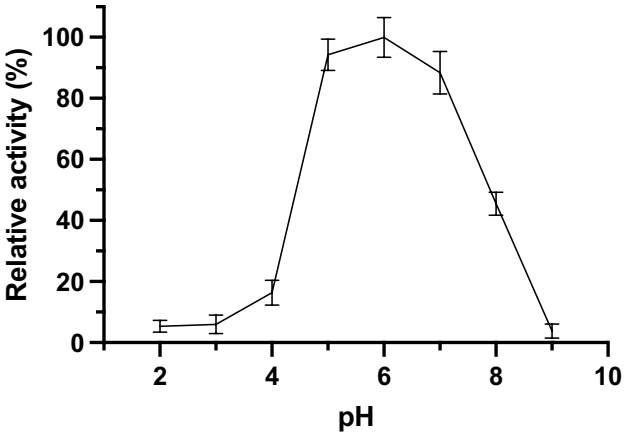

Fig. 5 Influence of pH on the activity of recombinant Jermuk-LamM towards laminarin. Data are shown as mean and standard deviation of the mean of three technical replicates. Connecting line shown for better visualization of the enzyme activity range

The influence of metals and common small molecule additives on the activity of the enzyme Jermuk-LamM was assessed. Among the tested metal ions (5 mM), only Fe^{2+} and Zn^{2+} led to significant inhibition of 86% and 60%, respectively. Fe^{3+}, Al^{3+}, and Cu^{2+} reduced the activity of the enzyme by 30%. In contrast, Mn^{2+} had a positive effect on enzyme activity (Fig. 7a). Inhibition was observed by the addition of 5 mM SDS, CTAB, and sodium periodate (Fig. 7b). The reducing agents dithiothreitol (DTT) and β-mercaptoethanol showed little effect on the enzyme activity at 5 mM concentration.

Degradation of macroalgae biomass

To assess the effectiveness of different substrate pre-treatment methods, a combination of physical (ultrasonication), chemical, and combined approaches was evaluated. For the data presented here, the results demonstrate that ultrasonication combined with chemical treatments does not significantly increase the liberation of reducing groups when compared to water alone. The highest concentration of reducing sugars reached 29 mM after 24 h of enzymatic treatment (Fig. 8).

When considering the end products after enzymatic treatment, the main products of hydrolyses are mono-, di-, and trisaccharides (Supplementary Fig. 7).

Discussion

In the light of current environmental challenges, such as climate change and the depletion of fossil fuel reservoirs, a global transition to biodegradable and renewable products is highly demanded. Recently, the degradation of macroalgae

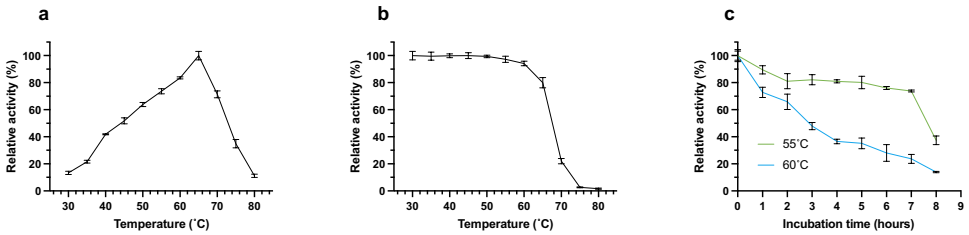

Fig. 6 Temperature activity profile of Jermuk-LamM. **a** Activity of the enzyme at temperatures ranging from 30 to 80 °C. **b** Activity remaining after the 10-min incubation between the temperature range of 30–80 °C. **c** Influence of temperature (55 °C green, 60 °C blue) on the stability of Jermuk-LamM

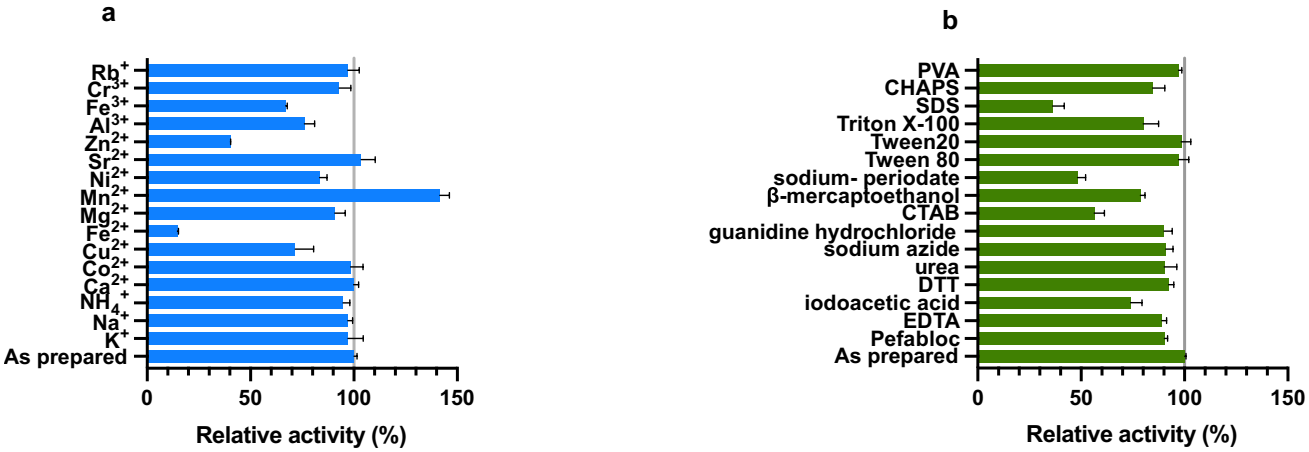

Fig. 7 Effect of cations and chemical compounds on the activity of Jermuk-LamM. The activity of the recombinant Jermuk-LamM enzyme was assessed after 1 h of incubation at room temperature in the presence of 5 mM of different metals (**a**) and additives (**b**). A blank experiment was conducted for each metal and additive. A specific activity of 217 U/mg obtained without additives was defined as 100% activity, and relative activities were calculated from this

biomass, as a renewable source of energy, has become attractive, and intensive efforts are underway to develop valorization technologies (Rocher et al. 2021; Tong et al. 2023). Consequently, high-value bioactive compounds can be extracted from macroalgae biomass, finding utility as feed and food additives (Gregersen et al. 2021; Herrera Barragán et al. 2022). Additionally, a large variety of bioactive compounds originating from algae find application in both the prevention and treatment of various diseases, as well as in the pharmaceutical and cosmetics industries (Negreanu-Pirjol et al. 2022; Hempel et al. 2023). Furthermore, simple monomers can be derived from the polysaccharides and proteins within macroalgal biomass, serving as a valuable source of fermentable nutrients (Biris-Dorhoi et al. 2020). In order to make macroalgae biomass applicable for these purposes, effective and sustainable processing methods must be developed in which the role of enzymes is vital (Manns et al. 2016).

A novel β−1,3-glucanase was identified in a metagenome sampled from the Jermuk hot springs in Armenia. A sequence-based metagenomics approach was used to identify putative 1,3-β-glucanase sequences from the Jermuk metagenome. NCBI BLAST search results indicate that the ORF Jermuk-lamM has a marine microbial origin showing the highest identity to *Candidatus* Fidelibacterota bacterium. This strain, which was previously named *Marinimicrobia* bacterium,is an uncultivated bacterium and widespread in sub-euphotic areas and particularly abundant in minimum oxygen zones (Bertagnolli et al. 2017). The information on Fidelibacterota and their metabolism is meager. According to available genome information, *Marinimicrobia* play an important role in the cycling of sulfur and nitrogen (Huang and Wang 2020). Based on our knowledge, this is the first β−1,3-glucanase characterized among the Fidelibacterota phylum and could provide useful initial information towards revealing their metabolic functions.

Jermuk hot springs are characterized by a relatively high concentration of dissolved minerals, the presence of hydrogen sulfide (H_2S), and gas bubbles of unknown composition (Saghatelyan et al. 2021). The presence of Fidelibacterota in the Jermuk hot spring probably is attributed to their participation in the biogeochemical cycling of sulfur and nitrogen.

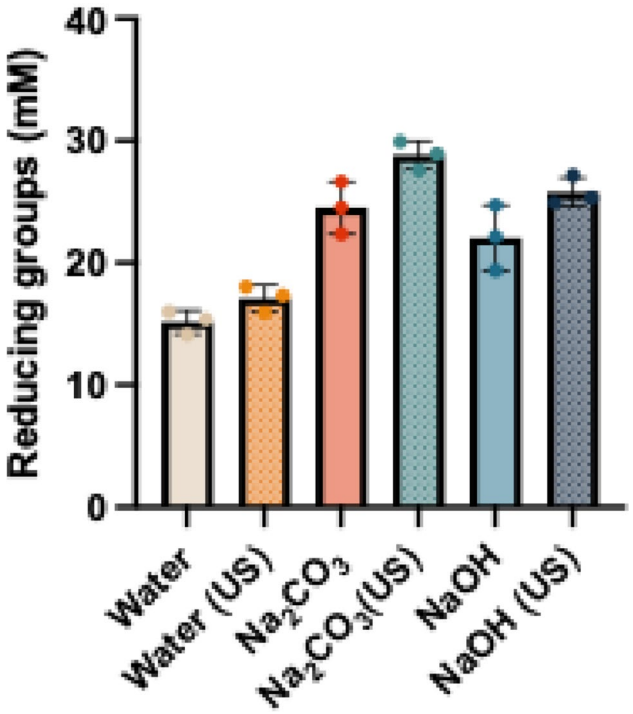

Fig. 8 Effectiveness of physical, chemical, and combined pre-treatment methods on enzymatic hydrolysis efficiency and reducing sugar release. All samples were kept in their respective solutions overnight prior to ultrasonication. The enzymatic treatment was performed for 24 h at 55 °C. Data represents three technical replicate experiments. Release of reducing sugars was measured by the DNS method

Given the geographical position and location of the Jermuk hot springs, it is plausible to hypothesize that micro- and macroalgae, as well as lichens, may be present, although no scientific research has been conducted in this area to date.

Jermuk-LamM exhibits approximately 50% sequence identity to other characterized laminarinases within the glycoside hydrolase family 16 (GH16). Members of this family active against β−1,3-linkages generally show preferences either for β−1,3-glucans (laminarinase EC 3.2.1.39) or mixed-linkage glucans (β−1,3(4)-glucanase (EC 3.2.1.6)). Although Jermuk-LamM clusters phylogenetically with the endo-1,3(4)-β-glucanase from *Acetivibrio thermocellus* (Fig. 1), their substrate specificities differ markedly. The *A. thermocellus* enzyme displays over sevenfold higher specific activity towards mixed-linkage glucans compared to laminarin (Fuchs et al. 2003), whereas Jermuk-LamM shows a pronounced preference for laminarin (Table 1).

The substrate specificity profile of Jermuk-LamM indicates that the enzyme specifically targets and hydrolyzes β−1,3-linkages closely resembling that of FLamA and FLamB from thermophilic *Fervidobacterium* species. Despite sharing less than 43% sequence identity, these enzymes exhibit a similar substrate preference order: laminarin > curdlan > barley β-glucan > lichenin. However, notable differences exist in the hydrolysis products (Burkhardt et al. 2019). Jermuk-LamM hydrolyzes laminarin predominantly into mono- and disaccharides (Fig. 4). In contrast, when acting on mixed-linkage glucans, Jermuk-LamM produces larger oligosaccharides, including trisaccharides, tetrasaccharides, and higher molecular weight fragments (Fig. 4, Fig. S5). The time-dependent product release profile indicates that oligosaccharides with a degree of polymerization greater than 3 were released early in the reaction. These were subsequently degraded into glucose and laminaribiose, which became the predominant products as the reaction progressed. This pattern suggests that the enzyme exhibits an endo-acting laminarinase nature (Fig. S6). This behavior is akin to PtLam from Planctomycetota (Li et al. 2024) which also exhibit strong affinity for laminarin, producing monomers and dimers as its main hydrolysis products. The critical role of the amino acid Asn176 in determining the exo-acting nature of *Pt*Lam has been demonstrated by mutating Asn176 to histidine, resulting in the production of oligosaccharides upon laminarin hydrolysis, indicating a shift from exo- to endo-activity (Li et al. 2024). In Jermuk-LamM, sequence alignment shows that Asn176 corresponds to Tyr176, which may form productive pi-stacking interactions with sugars in the +2 site. In the *P. furiosus* laminarinase, substitution of Asn176 to histidine shifts its activity from an exo-active enzyme and enhances associated transglycosylase activity (Ilari et al. 2009). In our sequence alignment (Fig. 2), this residue is generally aromatic in character, with the Jermuk-LamM having a tyrosine residue at this position (Tyr176), which could stabilize substrate binding at the +2 subsite to favor the release of disaccharide products (Fig. 3). Based on our sequence and structural analysis of the Jermuk-LamM coupled with Hiden Markov Profile searching using the dBCAN web server (Zheng et al. 2023), as well as biochemical characteristics, substrate specificity, and product pattern, we propose that this protein is likely to be an endo-acting laminarinase belonging to sub-family 3 of the GH16 family.

Laminarinase enzymes show a diverse domain organization, with some family members having accessory CBM domains, or S-layer-like domains. It is suggested that substrate preference is guided by the presence of carbohydrate-binding domains and loop sequences around the active site to enhance the binding of β−1,3-glucans (Jeng et al. 2011; Jeng et al. 2011; Labourel et al. 2014, 2015; Yang et al. 2020). The Jermuk-LamM does not contain any carbohydrate-binding domain and lacks the flexible loop at the entrance to its active site (Supplementary Fig. 3). In some examples of this enzyme family, a disulfide bond between two cysteine amino acids regulates the substrate preference (Qin et al. 2017). With respect to laminarin as a preferred substrate, Jermuk-LamM was found to be similar to LamA

(Gueguen et al. 1997), FLamA and FLamB (Burkhardt et al. 2019), ULam111 (Qin et al. 2017), *Zg*LamA (Labourel et al. 2014), and PtLam (Li et al. 2024).

Regarding the temperature maxima of these enzymes, with 65 °C Jermuk-LamM has a temperature maximum close to other examined β−1,3-glucanases, such as β-glucanase (60 °C) of *Bacillus halodurans* C-125 (Akita et al. 2005), BglF (70 °C) of *Nocardiopsis* sp. strain F96 (Masuda et al. 2006), and laminarinase (65 °C) of *Clostridium thermocellum* (Schwarz et al. 1988). Therefore, we describe Jermuk-LamM as an enzyme with moderate thermal tolerance, comparable to other mesophilic-to-moderately thermophilic β−1,3-glucanases. Concerning the pH profile, the enzyme retains at least 80% of its activity in a pH range from 5 to 7, with maximum activity at pH 6. Similar results have been obtained for 1,3-β-glucanase (pH 5.5) from *Streptomyces sioyaensis* (Hong et al. 2002), LamR (pH 5.5) from *Rhodothermus marinus* ITI278 (Krah et al. 1998), LamA (pH 6) from *Pyrococcus furiosus* (Gueguen et al. 1997), and laminarinase (pH 6.5) from *Clostridium thermocellum* (Schwarz et al. 1988). The temperature and pH profile of Jermuk-LamM is in good agreement with the temperature (40–45 °C) and pH (7–8) of the native environment, suggesting that the catalytic properties of Jermuk-LamM are well aligned with the environment from which the metagenome sample was taken.

To further characterize the biochemical properties of Jermuk-LamM, we examined its sensitivity to common surfactants and inhibitors. CTAB, a known inhibitor of laminarinase, has also shown an inhibitory effect on Jermuk-LamM, presumably by blocking the catalytic cleft through hydrophobic interactions (Jeng et al. 2011). Regarding inhibition by SDS, the decrease in activity could be attributed to the disruption of hydrophobic interactions and the induction of protein denaturation. The inhibition of Jermuk-LamM activity by sodium periodate can be explained by its known oxidizing ability. It can oxidize amino acids and alter the architecture of the active site of the enzyme (Clamp and Hough 1965). Among metals, Zn^{2+} has shown an inhibitory effect on Jermuk-LamM, similar to the inhibition observed for FLamA and FLamB from *Fervidobacterium* sp. (Burkhardt et al. 2019), *Pt*Lam from marine Planctomycete bacterium (Li et al. 2024), and Bgn2115 from *Bacillus halodurans* C-125 (Akita et al. 2005). Conversely, heavy metals such as Co^{2+}, Ni^{2+}, Cr^{2+}, and Cu^{2+} strongly inhibited FLamA and FLamB and *Pt*Lam, but exhibited only a slight inhibitory effect on Jermuk-LamM (Burkhardt et al. 2019; Li et al. 2024).

Based on the experimental findings, Jermuk-LamM exhibits strong potential for industrial applications, particularly in the valorization of *Fucus vesiculosus* biomass, which contains up to 7% laminarin as a reserve polysaccharide (Choulot et al. 2025).

Remarkably, the enzyme is capable of hydrolyzing raw macroalgal biomass without requiring prior physical or chemical pre-treatment (Fig. 8), offering a cost-effective and environmentally friendly approach by minimizing processing steps and avoiding chemical contaminants. Furthermore, enzymatic hydrolysis with Jermuk-LamM yielded a mixture of fermentable sugars, including mono-, di-, and trisaccharides, underscoring its efficacy in the bioconversion of laminarin-rich algal feedstocks (Supplementary Fig. 7).

Thus, due to its substrate specificity and hydrolytic activity to produce fermentable sugars, Jermuk-LamM can be successfully applied for macroalgae biomass processing. An essential advantage of this enzyme lies in its resilience to the most common transition metal ions, detergents, and other chemicals. This characteristic broadens the potential applications of this enzyme across diverse industrial conditions. The characteristics of Jermuk-LamM not only give a possibility for an application in different industries but also make this enzyme a promising candidate for enzymatic cocktail formulation.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00253-025-13557-4.

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Author contributions Study conceptualization: AP, AK, GA Investigation: Cloning and characterization of enzyme: AP, TS, MK, HG; HPLC analyses: ChB; Structural analysis: JMW, FC. Writing – Original Draft: AP Writing – Review and Editing: AP, AK, GA, ChB, FC, JMWStudy conceptualization: AP, AK, GA Investigation: Cloning and characterization of enzyme: AP, TS, MK, HG; HPLC analyses: ChB; Structural analysis: JMW, FC. Writing – Original Draft: AP Writing – Review and Editing: AP, AK, GA, ChB, FC, JMW.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval Not applicable.

Competing interests The authors declare no competing interests.

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