Structural characterization of two solute-binding proteins for \(N, N'\)-diacetylchitobiose/\(N, N', N''\)-triacetyldichitotoriose of the gram-positive bacterium, \textit{Paenibacillus} sp. str. FPU-7

Takahumi Itoh *, Misaki Yaguchi, Akari Nakaichi, Moe Yoda, Takao Hibi, Hisashi Kimoto *

Department of Bioscience and Biotechnology, Fukuoka Prefectural University, 4-1-1 Matsukakuenjojyusima, Eiheiji-cho, Yoskida-gun, Fukuoka 810-1195, Japan

**ARTICLE INFO**

**Keywords:**
- ABC transporter
- Chitin oligosaccharide
- Paenibacillus
- Solute binding protein
- Two-component regulatory system

**ABSTRACT**

The chitinolytic bacterium \textit{Paenibacillus} sp. str. FPU-7 efficiently degrades chitin into oligosaccharides such as \(N\)-acetyl-D-glucosamine (GlcNAc) and disaccharides (GlcNAc)\(_2\) through multiple secretory chitinases. Transport of these oligosaccharides by \(P.\) str. FPU-7 has not yet been clarified. In this study, we identified \(nagB1\), predicted to encode a sugar solute-binding protein (SBP), which is a component of the ABC transport system. However, the genes next to \(nagB1\) were predicted to encode two-component regulatory system proteins rather than transmembrane domains (TMDs). We also identified \(nagB2\), which is highly homologous to \(nagB1\). Adjacent to \(nagB2\), two genes were predicted to encode TMDs. Binding experiments of the recombinant NagB1 and NagB2 to several oligosaccharides using differential scanning fluorimetry and surface plasmon resonance confirmed that both proteins are SBPs of (GlcNAc)\(_2\) and (GlcNAc)\(_3\). We determined their crystal structures complexed with and without chitin oligosaccharides at a resolution of 1.2 to 2.0 Å. The structures shared typical SBP structural folds and were classified as subcluster D-I. Large domain motions were observed in the structures, suggesting that they were induced by ligand binding via the "Venus flytrap" mechanism. These structures also revealed chitin oligosaccharide recognition mechanisms. In conclusion, our study provides insight into the recognition and transport of chitin oligosaccharides in bacteria.

**Introduction**

Chitin is produced by arthropods and fungi as a polysaccharide for the cytoskeleton and it is a linear polymer of \(N\)-acetyl-D-glucosamine (GlcNAc) linked by  \(\beta\)-1,4 glycosidic bonds (Aam et al., 2010). Polysaccharides are not only an important source of carbon and nitrogen in marine and terrestrial ecosystems, but are also an important natural resource for the production of a variety of chemicals, and their degradation products, chitin oligosaccharides, are used in several biomedical applications (Kato et al., 2003). The biodegradation of chitin has been explored using various chitinolytic bacteria such as \textit{Serratia marcescens} (Fuchs et al., 1986), \textit{Bacillus circulans} WL-12 (Watanabe et al., 1990), \textit{Paenibacillus} sp. (Itoh et al., 2013), \textit{Streptomyces coelicolor} A3 (Saito et al., 1999), and \textit{Vibrio harveyi} (Montgomery and Kirchman, 1993). The chitin-degrading enzymes of these organisms have also been well studied (Bhattacharya et al., 2007; Daihya et al., 2006; Eijssink et al., 2010). The glycoside hydrolases (GHs) that act on chitin can have an \textit{endo}-mode of action (endochitinases) or an \textit{exo}-mode of action (exochitinases), and these enzymes act synergistically to release oligosaccharides from insoluble chitin. In addition to GHs, lytic polysaccharide monoxygenases oxidatively cleave chitin (Vaaje-Kolstad et al., 2013). The degraded oligosaccharides are then imported through the bacterial cell membranes, and in the bacterial cytosol or periplasmic space, oligomers are hydrolyzed to GlcNAc by \(N\)-acetyl-D-glucosaminidases (GlcNAcases) (Itoh and Kimoto, 2019; Vaaje-Kolstad et al., 2013). The obtained GlcNAc is then transformed into the common metabolic intermediate, fructose-6-phosphate, to be further metabolized via glycolysis in the cytosol (Nathaf et al., 2003).

Although a number of chitin-degrading bacteria have been studied, as mentioned above, the only known bacterial transport systems for

**Abbreviations:** a.a., amino acid; ABC, ATP-binding cassette; DSF, differential scanning fluorimetry; GlcN, D-glucosamine; GlcNAc, \(N\)-acetyl-D-glucosamine; GH, glycoside hydrolase; OD600, optical density at 600 nm; PDB, Protein Data Bank; PTS, phosphoenolpyruvate phosphotransferase system; r.m.s.d., root mean-square deviation; RI, response unit; SBP, solute binding protein; Se-Met, selenomethionine; TMD, transmembrane domain.

* Corresponding author.

E-mail addresses: ito-t@fpu.ac.jp, kimoto@fpu.ac.jp (T. Itoh).

https://doi.org/10.1016/j.yjssbx.2021.100049
Received 28 May 2021; Accepted 28 May 2021
Available online 10 June 2021
2590-1524/© 2021 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
chitin degradation products, GlcNAc and (GlcNAc)_2, are those identified in Streptomyces sp. and Vibrioaceae. In bacteria, phosphoenolpyruvate phosphotransferase systems (PTS) are utilized to import monosaccharides while ATP-binding cassette (ABC) transporters import di-, tri-, and oligosaccharides. In S. olivaceoviridis and S. coelicolor A3(2), GlcNAc uptake is mainly achieved by the PTS and ABC transporter NgeEFG (Saito and Schrempf, 2004; Wang et al., 2002; Xiao et al., 2002), which also transports (GlcNAc)_2. In S. coelicolor A3(2), the ABC transporter DasABC transports (GlcNAc)_2, which is hydrolyzed by the GlcNAcCase DasD in the cytosol (Saito et al., 2007). The gram-negative cholera pathogen Vibrio cholerae can also use chitin as a source of nutrients. The chitin oligosaccharides produced by the chitinases of V. cholerae are transported across the outer membrane via chitoporin to the periplasm, where the saccharides are further broken down into GlcNAc and (GlcNAc)_2. The resulting monomers and dimers are then transported across the inner membrane by specific transporters, such as PTS and ABC transporters, respectively (Hayes et al., 2017; Hunt et al., 2008; Meibom et al., 2004).

The bacterial ABC transporters for uptake (ABC importers) consist of five proteins: a solute binding protein (SBP), two transmembrane domains (TMDs), and two nucleotide-binding domains (ATPases). The free energy of ATP hydrolysis of ATPases is used to import various molecules across the cell membrane, whereas SBP is responsible for specifically trapping ligands and passing them through the pathway formed by the two TMDs (Ter Beek et al., 2014).

Recently, we isolated the gram-positive bacterium Paenibacillus sp. str. FPU-7 from the soil as a highly chitinolytic bacterium that can degrade insoluble chitin flakes (Itoh et al., 2013). This bacterium contains at least six extracellular chitinases (ChiA, B, C, D, E, and F), which are catalytically inhibited by GlcNAc, and one cell surface-expressed multi-modal chitinase (ChiW) for efficient chitin degradation (Itoh et al., 2014, 2013, 2016). ChiW hydrolyzes chitin into (GlcNAc)_2 on the cell surface, and the expression of ChiW is induced by (GlcNAc)_2. P. str. FPU-7 can then hydrolyze (GlcNAc)_2 into GlcNAc via GlcNAcase (PsNagA), a GH3 enzyme found in the cytosol (Itoh et al., 2019). However, the mechanism by which chitin oligosaccharides are transported into P. str. FPU-7 cells across the cell membrane remains unclear.

In this study, we aimed to elucidate the full extent of (GlcNAc)_2 transport in P. str. FPU-7 and identified two proteins (NagB1 and NagB2) in the draft genome of P. str. FPU-7 as SBPs of chitin oligosaccharides. These proteins are not homologous to the previously known SBPs of (GlcNAc)_2 in S. olivaceoviridis, S. coelicolor A3(2), or V. cholerae (sequence identity < 20%). The amino acid (a.a.) sequences and the properties of NagB1 and NagB2 were also different from the chitin-binding domains (or proteins) that work in concert with chitinolytic enzymes. We also determined the affinities of these SBPs for (GlcNAc)_2 and (GlcNAc)_3 using surface plasmon resonance and its recognition mechanisms using the crystal structures of the SBPs in the complex formed with the oligosaccharides. Altogether, the present study reports the biochemical and structural features of the SBPs NagB1 and NagB2 that mediate the capture of (GlcNAc)_2 and (GlcNAc)_3 within Paenibacillus spp.

Materials and methods

Chemicals and reagents

Saccharides (GlcNAc)_2, (GlcNAc)_3, and (GlcNAc)_4 were purchased from Tokyo Chemical Industry (Tokyo, Japan). Other chemicals and reagents were obtained from analytical grade and purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan) or Sigma-Aldrich (St. Louis, MO, USA), unless otherwise noted.

Amino acid sequence analysis

Sequence analysis was performed using the Pfam database (El-Gebali et al., 2019), using the basic local alignment search tool (BLAST) (Altschul et al., 1990) and ClustalW (Larkin et al., 2007). The N-terminal signal peptides were predicted using the SignalP 5.0 server (http://www.cbs.dtu.dk/services/SignalP/) (Almagro Armenteros et al., 2019). Protein palmitoylation sites were predicted using the CSS-Palm 4.0 server (http://csspalm.biocuckoo.org/index.php) (Ren et al., 2008).

Cloning of nagB1 and nagB2 genes from P. str. FPU-7

The nagB1 gene (DBJ/EMBL/GenBank database accession number: LC622162) was subcloned into the expression vector pET21b (Novagen, Madison, WI, USA). Colony PCR was performed using KOD-Plus-Neo polymerase (Toyobo, Osaka, Japan) and a single colony of P. str. FPU-7 as template. Synthetic oligonucleotides with NdeI and XhoI restriction sites were then added to their termini as forward and reverse primers (Table S1). To express a stable recombinant protein, facilitate purification, and obtain good quality crystals, the primers were designed to add a six-histidine tag (LEHHHHHH) to the C-terminus and delete the N-terminal signal peptide (Met1–Ser21) and 27 a.a. residues (Cys22–Phe48). These residues were selected by comparing them to the disordered region of the crystal structure of NagB2, as described in the results section. In contrast, the nagB2 gene (DBJ/EMBL/GenBank database accession number: LC622163) was prepared without the predicted N-terminal signal peptide (Met1 to Ala20) and subcloned into the expression vector pET21b (Novagen) with a six-histidine tag at the C-terminus (LEHHHHHH) (Table S1). The PCR products were ligated into the vectors using the In-Fusion HD Cloning Kit (Takara Bio, Kusatsu, Japan). Successful plasmid construction was confirmed by DNA sequencing using the ABI PRISM 3130xL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Purification of NagB1 and NagB2 recombinant proteins

The expression vector was transformed into competent Escherichia coli BL21(DE3) cells (Novagen) or T7 Express Crystal Cells (New England Biolabs Inc., Ipswich, MA, USA). E. coli BL21(DE3) transformants were grown in Luria-Bertani medium (0.5 L) containing 50 µg/mL ampicillin at 37 °C. For the expression of a NagB2 derivative with selenomethionine (Se-Met), E. coli T7 Express Crystal was aerobiologically cultured in minimal medium supplemented with 25 µg/mL of Se-Met. When the optical density of the culture measured at 600 nm (OD600) reached 0.6, the cultures were supplemented with 1.0 mM IPTG, followed by incubation at 20 °C for 20 h. The cultured cells were collected by centrifugation at 6000 × g at 4 °C for 5 min. The harvested cells were ultrasonically disrupted in 20 mM sodium phosphate buffer (pH 7.4) and 1.0 mM PMSF. Clear solutions were obtained after centrifugation at 15,000 × g for 40 min. The proteins were then fractionated with (NH₄)₂SO₄ from 30% to 70% saturation. The precipitants were dissolved in sodium phosphate buffer containing 20 mM imidazole, and applied to a nickel-immobilized metal affinity column (HisTrap HP 5 mL column; Cytiva, Tokyo, Japan). The column-absorbed proteins were eluted with a linear gradient of imidazole (0.02–0.15 M) in sodium phosphate buffer (50 mL). Ammonium sulfate was added to the solution containing proteins to reach 30% saturation. The proteins were further purified by hydrophobic interaction chromatography using a HiTrap Butyl HP 5 mL column (Cytiva) equilibrated with 20 mM Tris buffer (pH 7.5) and 1.2 M (NH₄)₂SO₄ and eluted with a linear gradient of (NH₄)₂SO₄ (1.2–0 M) in the same buffer. The eluted proteins were dialyzed at 4 °C overnight in 10 mM HEPES buffer (pH 7.4) and used as purified protein sources.

Protein concentrations were determined by UV spectrophotometry using the theoretical molar extinction coefficients of 43,890 and 42,400 M⁻¹ cm⁻¹ for NagB1 and NagB2, respectively, according to the ExPASy ProtParam tool server (http://web.expasy.org/protparam/) (Gasteiger et al., 2003). The purity of the proteins was assessed by 12% SDS-PAGE followed by CBB R-250 staining.
**Differential scanning fluorimetry**

The differential scanning fluorimetry (DSF) assay mixtures contained 20 mM Tris (pH 7.5), 0.25 mg/mL protein, and SYPRO Orange Protein Gel Stain dye (5 μM). Assays were performed in triplicate. The melting curves were calculated as the difference between the average TmD values measured with and without the ligand.

**Surface plasmon resonance (SPR)**

The affinities of NagB1 and NagB2 for (GlcNAc)2 and (GlcNAc)3 were evaluated using the Biacore X100 Plus Package (Cytiva). Both proteins were diluted in 10 mM sodium acetate buffer (pH 5.5) to 0.1 mg/mL. The proteins were covalently immobilized onto CMS sensor chips using the Amine Coupling Kit (Cytiva) to a level of approximately 3000 response units (RU). Sensorgrams were recorded at 25 °C in 10 mM HEPES (pH 7.4), 0.15 M NaCl, 3 mM EDTA, and 0.005% (v/v) surfactant P20. Assays were performed in the range of 0–10 μM saccharides. The equilibrium dissociation constants (Kd) and other kinetic parameters were calculated by fitting a one-site binding model using the Biacore X100 evaluation software.

**Culture conditions for P. str. FPU-7 and analysis of bacterial transportation of (GlcNAc)2**

To obtain an efficient cell yield, P. str. FPU-7 was grown at 30 °C with shaking in a medium containing 1.0% (v/v) lipopolysaccharide N and 0.5% (w/v) NaCl at pH 7.5 (lipopolysaccharide N medium) (Itoh et al., 2013). For the (GlcNAc)2 transportation analysis, P. str. FPU-7 cells were grown in lipopolysaccharide N medium supplemented with 2 mM (GlcNAc)2. Aliquots (100 μL) were taken from the medium at appropriate time intervals and centrifuged at 12,000 × g for 5 min. The supernatants were filtered using Ultrafree-CL centrifugal filter devices with 0.45 μm pore size membranes (Merck Millipore, Billerica, MA, USA). The filtrated culture media (5 μL) were analyzed using the LC-20AD HPLC system (Shimadzu, Kyoto, Japan) equipped with a TSKgel Amide-80 column (2.0 × 250 mm; Tosoh Co., Tokyo, Japan). (GlcNAc)2 was eluted with a mobile phase containing 77% (v/v) acetonitrile and detected at 210 nm wavelength.

**RNA extraction and quantitative real-time PCR (qPCR)**

P. str. FPU-7 cells were grown at 30 °C with shaking in lipopolysaccharide N medium with or without 2 mM (GlcNAc)2. When the OD600 reached 0.5, P. str. FPU-7 cells were collected from the culture medium (4 mL) by centrifugation at 6,000 × g for 5 min at 4 °C. Total RNA from cultured bacteria was extracted using the FastGene RNA extraction kit (NIPPOGenetics, Tokyo, Japan). Complementary DNA (cDNA) was synthesized by random hexamer priming using the ReverTra Ace qPCR RT Kit (Toyobo). Gene expression was subsequently detected in triplicate using the THUNDERBIRD SYBR qPCR Mix kit (Toyobo) on a qTOWER G thermal cycler (Analytik Jena AG, Jena, Germany). All quantification data were normalized to the 16S rRNA gene of P. str. FPU-7. The primers used are shown in Table S1.

**Immunoblot analysis of NagB1 and NagB2 of P. str. FPU-7**

Rabbit anti-NagB1 and NagB2 antisera were raised against the recombinant proteins and collected by Eurofins Genomics (Tokyo, Japan). P. str. FPU-7 cells were grown at 30 °C with shaking in lipopolysaccharide N medium with or without 2 mM (GlcNAc)2. When OD600 reached 0.5, P. str. FPU-7 cells were collected from the culture medium (0.5 mL) by centrifugation at 6,000 × g for 5 min at 4 °C. The cells were then ultrasonically disrupted in 100 μL of 20 mM sodium phosphate buffer (pH 7.4). The remaining bacterial debris and non-lysed cells were removed by centrifugation at 15,000 × g for 10 min at 4 °C. Protein concentrations were determined using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA) with bovine serum albumin as the standard, based on the Bradford method (Bradford, 1976). Protein samples were separated by SDS-PAGE and transferred onto a PVDF membrane (Immobilon-P, PVDF, 0.45 μm, Merck Millipore) by electroblotting. After transfer, the PVDF membrane was blocked with Blocking One (Nacalai Tesque, Inc., Kyoto, Japan) for 1 h. The membrane was washed with 25 mM Tris (pH 7.4), 137 mM NaCl, and 0.05% (v/v) Tween 20. Then, the rabbit anti-NagB1 or anti-NagB2 (diluted 1:5,000 in Can Get Signal Solution 1, Toyobo) was added, and the blot was incubated for 1 h. Next, affinity isolated alkaline phosphatase-conjugated swine anti-rabbit immunoglobulins (Agilent, Santa Clara, CA, USA) was added at a 1:5000 dilution in Can Get Signal Solution 2 (Toyobo) and incubated for 1 h. Bands were visualized using the BCPIP-NBT solution kit (Nacalai Tesque).

**Crystallization and X-ray diffraction**

Purified proteins were concentrated using Amicon Ultra concentrators with a 10,000 molecular weight cutoff membrane (Merck Millipore) to a final concentration of 10 mg/mL. Protein solutions for crystallization were prepared to contain 10 mM (GlcNAc)2. Initial screening for the crystallization of NagB1 and NagB2 was performed using commercially available crystallization kits, Crystal Screen 1 and 2 (Hampton Research, Aliso Viejo, CA, USA), and Wizard Classic Crystallization Screens 1 and 2 (Emerald BioSystems, Bedford, MA, USA), with the sitting-drop vapor-diffusion method in 96-well plates at 20 °C. The crystallization conditions were further refined using a 24-well plate and performed using the sitting-drop vapor-diffusion method at 20 °C. The drop (4 μL) consisted of 2 μL of protein solution and 2 μL of reservoir solution (0.5 mL) containing 20% (w/v) PEG4000, 0.2 M sodium acetate, and 0.1 M Tris (pH 8.5) for the crystallization of NagB1 with (GlcNAc)2 and 20% (w/v) PEG3350 and 0.1 M MES (pH 6.0) for that of NagB2 with or without (GlcNAc)2 and (GlcNAc)3.

Single crystals were soaked in crystallization solutions supplemented with 30% (w/v) glycerol for NagB1 or 30% (w/v) PEG400 for NagB2, followed by cooling in a stream of cold nitrogen gas. X-ray diffraction images were acquired for the native and Se-Met derivative crystals at −173 °C under a nitrogen gas stream with a MAR MX 225HS or 225HE detector (Rayonix, LLC., Evanston, IL, USA) and synchrotron radiation (λ = 0.85 or 1.0 Å for the native crystal or 0.9790 Å for the Se-Met derivative crystal) at the BL-26B1 or BL-38B1 stations of SPring-8 (Japan) and then integrated and scaled using HKL-2000 (Otwinowski and Minor, 1997) (Table 1).

**Structure determination and refinement**

Phase determination and initial model building were performed using the processed Se-Met anomalous dataset of NagB2 (Table 1) and the SHELX(CDE) (Sheldrick, 2015) programs implemented in the HKL2MAP program package. The partial model obtained was manually rebuilt using Coot ver. 0.9 (Winn et al., 2011). The model was refined using the Phenix.refine program in the PHENIX package (Adams et al., 2010) against the native NagB2 dataset (Table 1). Several rounds of refinements and manual model building were performed for the 1.8 Å resolution.
dataset. Water molecules were automatically incorporated where the $F_o - F_c$ and $2F_o - F_c$ electron density maps showed densities greater than 3.0 $\sigma$ and 1.0 $\sigma$, respectively. A number of water molecules were manually identified based on the density maps. The crystal structures of NagB1 and NagB2 complexes with (GlcNAc)$_2$ or (GlcNAc)$_3$ were determined by the molecular replacement method using the Phaser program (McCoy et al., 2007) in the PHENIX package, employing the structure of the oligosaccharide-unbound NagB2 structure as a search model. The oligosaccharide-bound structures were refined and manually rebuilt using Phenix.refine and Coot (Table 1). Initially, GlcNAc residues were manually placed and linked with $p$-1,4-linkages based on the interpretation of electron density shapes in the $2F_o - F_c$ and $F_o - F_c$ maps. Several rounds of refinement were conducted, followed by manual model building to improve the model. Structural similarity was screened using the Protein Data Bank (PDB) (Berman et al., 2000) and the DALI program (Dietmann et al., 2001). Large-scale hinge bending motions were analyzed using the DynDom web server (Hayward and Lee, 2002). Structural alignments were conducted by superimposition using a secondary structure matching program (Krippin and Henrick, 2004) in Coot. The conservation of the a.a. sequence on the surface of the structure was estimated using the ConSurf server (Ashkenazy et al., 2016). The server performed a three-iteration CSI-BLAST (Angermüller et al., 2012) search and selected 150 homologous sequences (E-value score, $8.9 \times 10^{-22}$ to $3.9 \times 10^{-48}$) from the UniRef90 database (Suzek et al., 2015) for multiple sequence alignment. The electrostatic potential was calculated using APBS and PDB2PQR (Unni et al., 2011). Structural figures were prepared using PyMol 2.5 (Schrödinger, New York, NY, USA).

### Accession numbers

The nucleotide sequence data of nagB1 and nagB2 are available in the DDBJ/EMBL/GenBank databases under the accession numbers LC622162 (nagB1) and LC622163 (nagB2). The coordinates and structure factors of NagB1 and NagB2 are available in the PDB under the accession numbers 7EHQ (NagB1/(GlcNAc)$_2$), 7EHQ (NagB2/(GlcNAc)$_2$), and 7EHQ (NagB2/(GlcNAc)$_3$).

### Results

**Production and purification of recombinant NagB1 and NagB2**

The bacterium P. str. FPU-7 can degrade chitin to (GlcNAc)$_2$ in the culture medium (Itoh et al., 2015). We confirmed that nagB1 and nagB2 are highly expressed in E. coli BL21 (DE3) cells and purified recombinant NagB1 and NagB2 using Ni-NTA agarose columns (QIAGEN, Hilden, Germany). The recombinant NagB1 and NagB2 were separated from the expression vector and other impurities by size exclusion chromatography (GE Healthcare, Baie d'Urfe, QC, Canada). The purified recombinant NagB1 and NagB2 were characterized by SDS-PAGE and Western blotting.
(denoted as nagB1), which was the most promising candidate for a chitin oligosaccharide-binding protein on the cell membrane able to import the saccharides (Fig. 1A). A gene homologous to nagB1 (nagB2) was also found in the draft genome (Fig. 1B), and their a.a. sequences were highly similar (a.a. sequence identity = 46.4%) (Supplementary Fig. S1). Both a.a. sequences showed identity with those of other SBPs for saccharides such as the xylooligosaccharide-binding protein XBP1 of *Caldanaerobius polysaccharolyticus* (identity = 27% for NagB1 and 28% for NagB2) (Han et al., 2012) and the arabinoxyloligosaccharide-binding protein BIAXBP of *Bifidobacterium animalis* subsp. *Lactis* BI-04 (identity = 26% for NagB1 and 27% for NagB2) (Ejby et al., 2013). Signal peptide sequences were predicted at their N-terminal regions (21 a.a. residues, Ser21–Met1) and 27 a.a. residues (Cys22 to Phe48) was prepared by genetic engineering. The 27 deleted residues corresponded to the disordered membrane domains (TMDs) of ABC transporter proteins (Fig. 5). Two-component regulatory sensor histidine kinase NagA (β-N-Acetylhexosaminidase) and an ABC transporter were predicted to encode a two-component regulatory sensor histidine kinase and a response regulator.

### Expression of NagB1 and NagB2 in *P. str. FPU-7*

*P. str. FPU-7* is unable to grow well in minimal media such as M9 (Itoh et al., 2013). Thus, the bacterium was cultured in a medium containing 1.0% (w/v) hipolypepton N and 0.5% (w/v) NaCl supplemented with or without 2 mM (GlcNAc)2. Hipolypepton N is made from soybean hydrolysate and it is considered almost free of (GlcNAc)2. Bacterial growth was slightly faster in the (GlcNAc)2-containing medium than in the medium without disaccharide. As the bacteria grew, the disaccharide in the medium was consumed and completely disappeared after 21 h (Fig. 4A). The expression of each gene was similar in the presence and absence of (GlcNAc)2. The expression of nagB1 in the medium without (GlcNAc)2 was 0.73 ± 0.13 fold and that of nagB2 was 0.42 ± 0.20 fold compared with that in the medium with (GlcNAc)2. Bacterial cell extracts were then immunoblotted with each antibody to confirm whether both nagB1 and nagB2 proteins were produced (Fig. 4B–D). Unfortunately, both antibodies reacted against both proteins (Fig. 4C, D, lanes 3–6), and it was not possible to identify whether only one or both proteins were produced. However, at least one protein was produced in sufficient amount with the addition of (GlcNAc)2 (Fig. 4C, D, lane 1) or without it (Fig. 4C, D, lane 2). When 5 μg of protein from the cell extract was loaded per lane (Fig. 4B-D, lanes 1 and 2), the presence of more than 100 ng of the binding protein was confirmed (Fig. 4C, D, lanes 3 and 5).

### Overall structure of NagB1

The crystal structure of NagB1 in complex with (GlcNAc)2 (NagB1/(GlcNAc)2) was determined at 2.0 Å (Fig. 5A, Table 1). To date, no ligand-free form of NagB1 has been obtained. The asymmetric unit of NagB1/(GlcNAc)2 contains two polypeptide chains (A and B). There were no disordered regions, except for the nine C-terminal residues, including the hexa-histidine tag of both chains and four residues (Asp299–Lys302) of chain B. The two polypeptide chains were virtually identical and formed the same folded structure with a root mean square deviation (r.m.s.d.) of 0.57 Å for all Cα atoms. The overall structure of NagB1 adopts a typical SBP-fold (Bertansson et al., 2010) consisting of...
two globular α/β domains: N-terminal domain I (residues Thr49–Thr162 and Ser330–Arg395; Fig. 5 A blue ribbon model) and C-terminal domain II (residues Glu167–Met324 and Ala399–Lys445; Fig. 5 A orange ribbon model) and three hinge regions composed of antiparallel β-strands between the two domains (residues Ala163–Val166, Ile325–Tyr329, and Gln396–Pro398; Fig. 5 A cyan ribbon model). Domain I contains five β-strands and seven α-helices, whereas domain II contains five β-strands and 12 α-helices. The ligand-binding site is buried at the interface between domains.

The DALI server indicated that the NagB1/(GlcNAc)_2 structure was similar to those of various sugar SBPs such as the glucose binding protein (PDB: 5DVI) of Pseudomonas putida CSV86 (Pandey et al., 2016), with a

Table 2

| Kinetics of chitin oligosaccharide binding to NagB1 and NagB2. |     |     |     |     |
|---------------------------------------------------------------|-----|-----|-----|-----|
| Protein           | Ligand | k_a × 10^5 (M^-1 s^-1) | k_d (s^-1) | K_D (µM) | χ_a^2 |
| NagB1             | (GlcNAc)_2 | 7.81 ± 0.11 | 0.135 ± 0.001 | 0.173 | 0.112 |
|                   | (GlcNAc)_3 | 1.40 ± 0.05 | 0.414 ± 0.008 | 2.97 | 0.135 |
| NagB2             | (GlcNAc)_2 | 7.91 ± 0.11 | 0.150 ± 0.001 | 0.190 | 0.120 |
|                   | (GlcNAc)_3 | 3.83 ± 0.08 | 0.428 ± 0.004 | 1.12 | 0.162 |

* Averaged squared residual per data point (χ^2 = Σ (y_i - ˆy_i)^2/(n-p)), where y_i is the fitted value at each data point, ˆy_i is the experimental value at the point, n is the number of data points, and p is the number of fitted parameters.
Z-score of 40.3 (r.m.s.d. for the 396 Cα atoms) in media without (GlcNAc)3 was bound, this angle changed to 56.8° (Fig. 6D) between the closed and open forms (Fig. 6E) between the closed and open forms. When (GlcNAc)3 was bound, the angles were 27.1° between the closed and open forms (Fig. 6E) between the closed and open forms. When (GlcNAc)3 was bound, the angles were 27.1° between the closed and open forms (Fig. 6E) between the closed and open forms. When (GlcNAc)3 was bound, the angles were 27.1° between the closed and open forms (Fig. 6E) between the closed and open forms. When (GlcNAc)3 was bound, the angles were 27.1° between the closed and open forms (Fig. 6E) between the closed and open forms. When (GlcNAc)3 was bound, this angle changed to 56.8° (Fig. 6D) between the closed and open forms and 30.2° (Fig. 6E) between the closed and open forms. When (GlcNAc)3 was bound, the angles were 58.6° between the closed and open forms and 32.1° between the closed and semi-closed forms.

Architecture of the ligand binding sites of NagB1 and NagB2

In the crystal structures, the bound oligosaccharides were confined at the binding sites in an extended conformation (Fig. 6A–C) and were well defined in the density maps (Fig. 5A, C, D). The oligosaccharides were bound to the binding sites with many water molecules: 29 molecules in NagB1/(GlcNAc)2, 28 molecules in NagB2/(GlcNAc)2, and 34 molecules in NagB2/(GlcNAc)3 (Fig. 6A–C). The pyrophosphate chains of NagB1 and NagB2 superimposed well (Fig. 5E), and both proteins recognized the bound oligosaccharide in a similar manner. The bound GlcNAc units were numbered starting at 1 from the non-reducing end. In both (GlcNAc)2 bound structures of NagB1 and NagB2 (Fig. 6A, B), the first GlcNAc interacted with the tryptophan residue Trp213 of NagB1 or Trp217 of NagB2 and formed hydrogen-bonding networks with surrounding residues. The O3, O4, O6, and O7 atoms of the first GlcNAc formed hydrogen bonds with Arg95, Asp401, and Ser330 of NagB1 and Arg96, Asp408, and Ser334 of NagB2. Other residues such as Asp91 and Glu167 of NagB1 and Asp92 and Glu168 of NagB2 interacted with GlcNAc mediated by water molecules. In NagB2/(GlcNAc)2, Lys416 interacted with the first GlcNAc via a water molecule. In contrast, Lys416 was not conserved in NagB1 (Fig. S1). The residue was substituted with Arg409, and the residue space was filled with Arg409 and Phe96 in NagB1. The second GlcNAc unit formed a CH/π interaction with Trp292 of NagB1 or Trp296 of NagB2 (Fig. 6A, B). The O1, O6, O7, and N2 atoms of the second GlcNAc formed hydrogen bonds with Arg64, Asp92, and Asn331 of NagB1 or Arg373, Asp92, and Asn335 of NagB2. Thr55 of NagB1 and Ile56, Asn57, and Gly165 of NagB2 interacted with the second GlcNAc through water molecules. Other residues, such as Phe115 and Phe165 of NagB1 and Phe116 and Phe166 of NagB2, hydrophobically interacted with (GlcNAc)2. In NagB2/(GlcNAc)2, Arg64 interacted with the second GlcNAc. In NagB2, this residue was substituted with Thr65 (Fig. S1), but the spatially closer Arg373 played an important role in recognizing the second GlcNAc. In the (GlcNAc)3 bound structure of NagB2 (Fig. 6C), the first and second GlcNAc units interacted with the proteins in a similar manner to the (GlcNAc)2 bound structures (Fig. 6A, B). However, the binding of the third GlcNAc moved the structural folds of two regions (Ser299–Asp305 and Gly365–Val374) outward, slightly widening the active-site groove, and causing the side-chain of Arg373 to shift in the opposite direction. The O1, O6, and N2 atoms of the third GlcNAc unit formed hydrogen bonds with Thr65, Asn57, and Thr296 of NagB2. The residues Arg59, Glu163, Gln280, Ser299, and Asp371 of NagB2 interacted with the third unit through water molecules (Fig. 6C).

The two domains formed similar closed ligand-binding structures in NagB1 and NagB2. These conformational changes occurred through rigid body rotation, similar to other SBPs (Sharff et al., 1992). In contrast, in the asymmetric unit of the ligand-free crystal of NagB2, the two pyrophosphate chains showed two different opening structures. The inter-domain rotation angle between the two chains was 27.1° and belonged to an open form (chain A) and a semi-closed form (chain B). When (GlcNAc)2 was bound, this angle changed to 56.8° (Fig. 6D) between the closed and open forms and 30.2° (Fig. 6E) between the closed and semi-closed forms. When (GlcNAc)3 was bound, the angles were 58.6° between the closed and open forms and 32.1° between the closed and semi-closed forms.

Conservation of the a.a. Residues

The conservation of the a.a. residues of both proteins was analyzed
using the ConSurf server, based on the multiple sequence alignment with 150 a.a. sequences (Fig. 7A, B). The homologous sequences were automatically selected from the UniRef90 database (Suzek et al., 2015) using the server with CSI-BLAST (Angermüller et al., 2012). Almost all sequences (a.a. sequence identity at approximately 40–80%) belonged to uncharacterized bacterial proteins, mainly from gram-positive bacterial genera such as Paenibacillus, Cohnella, Brevibacillus, and Geobacillus. In the binding sites, the residues surrounding the first GlcNAc were highly conserved (Fig. 7A). However, the residues around the second and third units were less conserved than those around the first unit, which was similar to what was observed when comparing NagB1 and NagB2. On the other hand, the surface of the binding site was negatively charged regardless of a.a. conservation (Fig. 7C). In addition to the binding site, several surface regions and one alpha-helix in domain II in particular were highly conserved (Fig. 7B), suggesting that these regions play an important role in binding to other proteins, such as the transmembrane domains (TMDs) of ABC transporters.

Discussion

P. str. FPU-7 degrades chitin by constitutively producing multiple chitinases (Itoh et al., 2013). In addition to these extracellular enzymes, ChiW induced by (GlcNAc)_2 is expressed on the cell surface and hydrolyzes chitin to (GlcNAc)_2 (Itoh et al., 2013, 2016). P. str. FPU-7 has recently been found to hydrolyze (GlcNAc)_2 to two GlcNAc residues in the cytosol via GlcNAcase PsNagA (Itoh et al., 2019). However, whether the disaccharides were transported across the P. str. FPU-7 membrane remained unclear. Many bacteria import oligosaccharides using ABC transporters, which are the most common class of sugar transporters in bacteria. SBPs, a component of ABC transporters, are responsible for trapping ligands that are imported through a specific pathway formed by two TMDs, and mainly define the specificity and affinity of transporters (Berntsson et al., 2010; ter Beek et al., 2014). SBPs that import (GlcNAc)_2 have been found in gram-positive S. coelicolor A3(2) (Saito et al., 2007) and gram-negative V. harveyi (Hayes et al., 2017), and their various properties have been elucidated. In the present study, the nagB1 gene, which was predicted to encode the SBP of sugar, was located next to the nagA (GlcNAcase) gene. However, no genes encoding the TMD of the ABC transporter were found in the vicinity of the nagA and nagB1 genes. Instead, a nagB1-homolog, nagB2, with two adjacent TMDs was identified (Fig. 1A and B). These two TMDs had a.a. sequences with moderate similarity (approximately 20–33%) to identity to those of trehalose (Liu et al., 2020), maltose (Oldham et al., 2007), and alginate ABC transporters (Maruyama et al., 2015), and were presumed to be components of a sugar ABC transporter. However, both NagB1 and NagB2 proteins showed a low a.a. sequence similarity with the previously known (GlcNAc)_2 SBPs of S. coelicolor A3(2) and V. harveyi (a.a. sequence identity < 20%), making it difficult to identify both proteins as SBPs of (GlcNAc)_2. Thus, we prepared and characterized both NagB1 and NagB2 proteins and confirmed that these proteins were SBPs of chitin oligosaccharides. We then analyzed their functions and structures, and clarified the SBP mechanism recognizing (GlcNAc)_2.

Sequence analysis using the CSS-Palm server predicted that the NagB1 and NagB2 proteins were anchored on the cell membrane via post-translational modification by palmitic acid. Although these proteins specifically bound chitin-oligosaccharides such as (GlcNAc)_2 and (GlcNAc)_3 (Fig. 2), they recognized (GlcNAc)_2 more tightly than (GlcNAc)_3 (Table 2). On the cell surface, ChiW degrades chitin to disaccharides, and it is more convenient for disaccharides rather than trisaccharides to be recognized by both proteins. We confirmed that the NagB1 and NagB2 proteins were expressed in P. str. FPU-7 by gene expression and western blot analyses (Fig. 4). Both genes were expressed in the presence or absence of (GlcNAc)_2. Although the NagB1- or NagB2-derived polyclonal antibodies could not distinguish between NagB1 and NagB2, at least one of the proteins was indeed expressed in the bacterium, suggesting that NagB1 or NagB2 helped to capture the oligosaccharides on the cell surface.

The recognition mechanism of chitin oligosaccharides was clarified by determining the crystal structures of these proteins. Both structures were similar and belong to the SBP structural fold superfamily (Davidson et al., 2008; Rice et al., 2014; Scheepers et al., 2016). The SBP structural fold is classified into six clusters (A–F), and SBPs for sugar transport mainly belong to clusters B and D (Berntsson et al., 2010). Clusters B and D are further classified into subclusters B-I to B-V and D-I to D-IV (Scheepers et al., 2016). The structures of NagB1 and NagB2 were classified as subcluster D-I and contained two a/b domains connected by a hinge region consisting of three strands, with the substrate-binding pocket located between the two domains (Fig. 5). The binding
sites of the oligosaccharides are large aqueous cavities that can accommodate up to three sugar residues, as revealed by the crystal structures. There seems to be no place for the fourth saccharide to bind, which is consistent with what would be expected from the DSF results with several saccharides. The kinetic parameters exhibited by both NagB1 and NagB2 for (GlcNAc)_2 showed similar K_d values of approximately 200 nM. The a.a. residues for the recognition of (GlcNAc)_2 on the binding site are also well conserved between both proteins. However,
the a.a. residues around the third saccharide are less conserved. The difference in $K_d$ values for $\text{(GlcNAc)}_3$ is probably due to this divergence.

Consistent with the results of the DALI search, the NagB1 and NagB2 structures were superimposed on SBPs of other sugars such as glucose, xylooligosaccharide, raffinose, and galactose (Fig. 8A). Although these SBPs have low a.a. sequence similarity (approximately 19–24% identity), two tryptophan residues such as Trp213 and Trp292 of NagB1 were well conserved in their binding sites (Fig. 8B-E), except for the SBP of galactose (Fig. 8F). In addition to the two tryptophan residues, one tyrosine residue (e.g., Tyr292 of NagB1) was also well conserved in the proteins (Fig. 8B, D, E), except for the SBPs of glucose and galactose (Fig. 8C, F). These residues belong to C-terminal domain II and form a platform for the sugar rings with CH/π interactions. Although the overall structures were very similar, the structures of the two loops of N-terminal domain I were different for each protein (e.g., Gln56–Arg64 and Val89–Val93 of NagB1), which resulted in different wall structures at the binding sites (Fig. 8). It was suggested that each protein forms a different wall structure and specifically recognizes hydroxy groups and other functional groups of sugars through this wall structure.

Upon binding to the ligand, SBP undergoes a conformational change from an open conformation to a closed conformation. This change is known as the “Venus flytrap” mechanism (Berntsson et al., 2010; Mao et al., 1982; Tirado-Lee et al., 2011; Vyas et al., 1988). The structure of NagB2 also changed significantly upon ligand binding, and the rotation angle was comparable to that of previously known structures (Fig. 6D). Although the structure of NagB1 in its ligand-free form has not yet been determined, considering the conservation of a.a. sequences and three-dimensional structures, it is likely that NagB1 undergoes the same structural changes as NagB2.

Although the genes nagB1 and nagB2 of SBPs for chitin oligosaccharides were constitutively expressed in the presence or absence of $\text{(GlcNAc)}_2$, functions other than substrate binding are unknown. The neighboring genes of nagB1 were predicted to encode a two-component regulatory system (histidine kinase and response regulator) (Fig. 1A). In contrast, the neighboring genes of nagB2 were predicted to encode the TMD of the oligosaccharide ABC transporter (Fig. 1B). Some SBPs function as part of ABC transporters or two-component regulatory systems (Scheepers et al., 2016). In V. cholerae, the SBP for $\text{(GlcNAc)}_2$ functions not only as the SBP of the disaccharide-importing ABC transporter but also as a component of the two-component
regulatory (signal transduction) system together with a sensor histidine kinase and a response regulator that controls the expression of target genes such as chitinases (Li and Roseman, 2004). Thus, NagB1 may trap (GlcNac)₂ and induce the expression of chitinase genes, such as ChiW, via a phosphorylation relay in the system. Identifying the target genes that are induced is a challenge for the future. NagB2 may be involved in transporting (GlcNac)₂ across the membrane as a typical SBP of the ABC transporter with two predicted TMDs. However, there was no ATP-hydrolyzing enzyme (ATPase) in the nearby genes. In the genome of Bacillus subtilis, there are at least seven incomplete ABC sugar importers lacking genes encoding ATPases; MsmX at another locus has been proposed as a multitask ATPase, and the six incomplete transporters share this enzyme as a component (Leisico et al., 2020; Quentin et al., 1999). Similar multitask ATPases have also been found in streptococci (Marion et al., 2011; Tan et al., 2015). Thus, the chitin oligosaccharide uptake system (ABC transporter) of P. str. FPU-7 may utilize multiskewed ATPase. On the other hand, although the two proteins may have different functions, they may complement each other’s functions because NagB1 and NagB2 are similar in their a.a. sequences, conformations, and ligand binding abilities.

In conclusion, we identified and characterized Paenibacillus spp. NagB1 and NagB2 proteins as SBPs for chitin oligosaccharides. These findings will facilitate our understanding of the structural and functional aspects of chitin oligosaccharide transport and regulatory systems in bacteria.

**Funding**

This work was supported in part by a Grant-in-Aid for Scientific Research (C) (TI, Grant Numbers 16 K08114 and 19 K06340) from the Japan Society for the Promotion of Science.

**CRediT authorship contribution statement**

Takafumi Itoh: Conceptualization, Funding acquisition, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing - original draft. Misaki Yaguchi: Investigation, Writing - review & editing. Akari Nakaichi: Investigation, Writing - review & editing. Moe Yoda: Investigation, Writing - review & editing. Takao Hibi: Investigation, Formal analysis, Supervision, Validation, Writing - review & editing. Hisashi Kimoto: Conceptualization, Resources, Supervision, Project administration, Writing - review & editing.

(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

We would like to thank the beamline staff (Spring-8 beamline BL26B1, BL26B2, and BL38B1) for their help with data collection.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yjbis.2021.100049.

References

Aam, B.B., Heggett, E.B., Norberg, A.L., Serlie, M., Vårum, K.M., Ejikijin, V.G., 2010. Production of chitinoligosaccharidases and their potential applications in medicine. Mar. Drugs 8, 1482–1517.

Adams, P.D., Afonine, P.V., Bunkoczi, G., Chen, V.B., Davis, I.W., Echols, N., Headd, J.J., Hung, L.W., Kapral, G.J., Grosse-Kunstleve, R.W., McCoy, A.J., Moriarty, N.W., Oeffner, R., Read, J.R., Richards, D.C., Richards, J.S., Terwilliger, T.C., Zwart, P.H., 2010. PHENIX: A comprehensive Python-based system for macromolecular structure solution. Acta Crystallogr. D Biol. Crystallogr. 66, 2124–2128.

Almagro Armenteros, J.J., Tsirigos, K.D., Pandey, S., Modak, A., Phale, P.S., Bhaumik, P., 2016. High resolution structures of Pseudomonas putida CSV86 reveal structural basis of its substrate specificity. Acta Crystallogr. F Struct. Biol. Commun. 70, 350–353.

Itoh, T., Hijii, T., Sugimoto, I., Suzuki, F., Fujii, Y., Taketo, A., Kimoto, H., 2014. Crystallization and preliminary X-ray analysis of the catalytic domain of Paenibacillus sp. strain FPPU-7 cell-surface-expressed chitinase ChW. Acta Crystallogr. F Struct. Biol. Commun. 70, 350–353.

Itoh, T., Hijii, T., Fujii, Y., Sugimoto, I., Fujiwara, A., Suzuki, F., Iwaski, Y., Kim, J.K., Taketo, A., Kimoto, H., 2013. Cooperative degradation of chitin by extracellular and cell-surface-expressed chitinases from Paenibacillus sp. strain FPPU-7. Appl. Environ. Microbiol. 79, 7482–7490.

Itoh, T., Fujii, T., Suzuki, F., Sugimoto, I., Fujisawa, A., Inaka, K., Tanaka, H., Ohta, K., Fujii, Y., Taketo, A., Kimoto, H., 2016. Crystal structure of chitinase ChW from Paenibacillus sp. strain FPPU-7 reveals a novel type of bacterial cell-surface-expressed multi-modular enzyme machinery. PloS One 11, e0167310.

Kato, Y., Onishi, H., Machida, Y., 2003. Application of chitin and chitosan derivatives in the pharmaceutical field. Curr. Pharm. Biotechnol. 4, 303–309.

Krisinel, E., Herrick, K., 2004. Secondary-structure matching (SSM), a new tool for fast protein structure alignment in three dimensions. Acta Crystallogr. D Biol. Crystallogr. 60, 2256–2268.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948.

Leisico, F., Goinhdo, L.M., Gonzales, I.C., Silva, S.P., Carneiro, B., Romao, M., Santos-Silva, T., de Sá-Nogueira, I., 2020. Multitask ATPases (NBDs) of bacterial ABC importers type I and their interspecies exchangeability. Sci. Rep. 10, 19564.

Li, X., Germain, S., 2004. The chitinolytic cascade in Vibrio is regulated by chitin-oligosaccharidases and a two-component chitin catabolic sensor/kinase. Proc. Natl. Acad. Sci. U S A 101, 627–631.

Licht, A., Bulut, H., Schefl, F., Daumke, O., Wehmeier, U.F., Schneider, W., Ehrlich, G., 2009. Crystal structures of the heat-shock protein MscS displaying an exclusive substrate preference for (β-d-galactopyranose. J. Mol. Biol. 406, 92–105.

Liu, F., Liang, J., Zhang, B., Gao, Y., Yang, X., Hu, T., Yang, H., Xu, W., Gubbay, J., Rao, Z., 2020. Structural basis of trehalose recycling by the ABC transporter LpqY-SugABC. Sci. Adv. 6, 1–7.

Mao, B., Pear, M.R., McCammon, J.A., Quichoio, F.A., 1982. Hinge-bending in L-arabinose-binding protein. The ‘Venus’s-flytrap’ model. J. Biol. Chem. 257, 1139–1153.

Marion, C., Atten, A.E., Woodaga, S.A., King, S.J., 2011. Identification of an ATPase, MsmK, which energizes multiple carbohydrate ABC transporters in Strepptococcus pneumoniae. Infect. Immun. 79, 4193–4200.

Maruyama, Y., Itoh, T., Kaneko, A., Nishitani, Y., Mikami, B., Hashimoto, M., Murata, K., 2015. Structure of a bacterial ABC transporter involved in the import of an acidic polysaccharide alginite. Structure 23, 1643–1654.

McCoy, A.J., Grosse-Kunstleve, R.W., Adams, P.D., Winn, M.D., Storoni, L.C., Read, R.J., 2007. Phaser crystallographic software. J. Appl. Crystallogr. 40, 658–674.

Meibom, K.L., Li, X.B., Nielsen, A.T., Wu, C.Y., Roseman, S., Schoolnik, G.K., 2004. The Vibrio cholerae chitin utilization program. Proc. Natl. Acad. Sci. U S A 101, 15247–15252.

Montgomery, M.T., Kirchan, D.L., 1993. Role of chitin-binding proteins in the specific attachment of the marine bacterium Vibrio harveyi to chitin. Appl. Environ. Microbiol. 59, 373–379.

Nordaas, R., Drieder, D., Willimak, A., Mahr, K., Niederweis, M., Tiggesmeier, F., 2003. The phosphotransferase system of Streptomyces coelicolor is biased for N-acetylglucosamine metabolism. J. Bacteriol. 185, 7019–7023.

Oldman, L.M., Khare, D., Quichoio, F.A., Davidson, A.L., Chen, J., 2007. Crystal structure of a catalytic intermediate of the maltose transporter. Nature 450, 515–521.

Otwinowski, S., Minor, W., 1997. Processing of X-ray diffraction data collected in oscillation mode. Methods Enzymol. 276, 307–326.

Pandey, S., Modak, A., Phale, P.S., Bhamsub, P., 2016. High resolution structures of periplasmic glucose-binding protein of Pseudomonas putida CSV86 reveal structural basis of its substrates pecificity. J. Biol. Chem. 291, 7844–7857.

Quentin, Y., Fichant, G., Denizot, F., 1999. Inventory, assembly and analysis of ABC transporters. J. Mol. Biol. 287, 467–483.

Rao, Z., 2020. Structural basis of trehalose recycling by the ABC transporter LpqY-SugABC. Sci. Adv. 6, 1–7.

Rao, Z., 2020. Structural basis of trehalose recycling by the ABC transporter LpqY-SugABC. Sci. Adv. 6, 1–7.

Saito, A., Schrempf, H., 2004. Mutational analysis of the binding affinity and transport activity of the degrader Paenibacillus sp. str. FPU-7 cell-surface-expressed chitinase ChiW. Acta Crystallogr. F Struct. Biol. Commun. 70, 350–353.
Sharff, A.J., Rodseth, L.E., Sparlin, J.C., Quiocho, F.A., 1992. Crystallographic evidence of a large ligand-induced hinge-twist motion between the two domains of the maltodextrin binding protein involved in active transport and chemotaxis. Biochemistry 31, 10657–10663.

Sheldrick, G.M., 2015. Crystal structure refinement with SHELXL. Acta Crystallogr. C Struct. Chem. 71, 3–8.

Suzek, B.E., Wang, Y., Huang, H., McGarvey, P.B., Wu, C.H., UniProt, C., 2015. UniRef clusters: a comprehensive and scalable alternative for improving sequence similarity searches. Bioinformatics 31, 926–932.

Tan, M.F., Gao, T., Liu, W.Q., Zhang, C.Y., Yang, X., Zhu, J.W., Teng, M.Y., Li, L., Zhou, R., 2015. ManK, an ATPase, contributes to utilization of multiple carbohydrates and host colonization of Streptococcus suis. PLoS One 10, e0130792.

Tirado-Lee, L., Lee, A., Rees, D.C., Pinkett, H.W., 2011. Classification of a Haemophilus influenzae ABC transporter HI1470/71 through its cognate molybdate periplasmic binding protein, MolA. Structure 19, 1701–1710.

Tomoo, K., Miki, Y., Morioka, H., Seike, K., Ishida, T., Benishi, S., Miyamoto, K., Hasegawa, T., Yamano, A., Hamada, K., Tsujibo, H., 2017. Crystal structure of the solute-binding protein BxIE from Streptomyces thermoviolaceus OPC-520 complexed with xylobiose. J. Biochem. 161, 493–501.

Unni, S., Huang, Y., Hanson, R.M., Tobias, M., Krishnan, S., Li, W.W., Nielsen, J.E., Baker, N.A., 2011. Web servers and services for electrostatics calculations with APBS and PDB2PQR. J. Comput. Chem. 32, 1488–1491.

Vasjek-Kolstad, G., Horn, S.J., Serlie, M., Eijsink, V.G., 2013. The chitinolytic machinery of Serratia marcescens: a model system for enzymatic degradation of recalcitrant polysaccharides. FEBS J. 280, 3026–3045.

Vyas, N.K., Vyas, M.N., Quiocho, F.A., 1988. Sugar and signal-transducer binding sites of the Escherichia coli galactose chemoreceptor protein. Science 242, 1290–1295.

Wang, F., Xiao, X., Saito, A., Schrempf, H., 2002. Streptomyces olivaceoviridis possesses a phosphotransferase system that mediates specific, phosphoenolpyruvate-dependent uptake of N-acetylglucosamine. Mol. Genet. Genomics 268, 344–351.

Watanabe, T., Suzuki, K., Oyanagi, W., Ohnishi, K., Tanaka, H., 1990. Gene cloning of chitinase A1 from Bacillus circulans WL-12 revealed its evolutionary relationship to Serratite chitinase and to the type III homology units of fibronectin. J. Biol. Chem. 265, 15659–15665.

Winn, M.D., Ballard, C.C., Cowtan, K.D., Dodson, E.J., Emsley, P., Evans, P.R., Keegan, R. M., Krissinel, E.B., Leslie, A.G., McCoy, A., McNicholas, S.J., Murshudov, G.N., Pannu, N.S., Potterton, E.A., Powell, H.R., Read, R.J., Vagin, A., Wilson, K.S., 2011. Overview of the CCP4 suite and current developments. Acta Crystallogr. D Biol. Crystallogr. 67, 235–242.

Xiao, X., Wang, F., Saito, A., Majka, J., Schlosser, A., Schrempf, H., 2002. The novel Streptomyces olivaceoviridis ABC transporter Ngc mediates uptake of N-acetylglucosamine and N,N’-diacetyldichitobiose. Mol. Genet. Genomics 267, 429–439.