Journal of Applied Microbiology, 2025, 136(8), lxaf187
https://doi.org/10.1093/jambio/lxaf187
Advance access publication date: 23 July 2025
Research Article

JOURNAL OF APPLIED MICROBIOLOGY
OXFORD UNIVERSITY PRESS

Cultivation of marine chitinolytic bacteria reveals the sponge-associated *Motilimonas* isolate Spo1_1 as an efficient degrader of insoluble chitin

Etienne Dechamps[1], Arthur Salengros[1], Laurence Meunier[1], Séverine Chevalier[1], Johan Danguy[1], Sophie-Luise Heidig[2,3], Jean-François Flot[2,3], Tina Keller-Costa[4,5], Rodrigo Costa[4,5], Isabelle F. George[1,*]

[1]Laboratory of Ecology of Aquatic Systems, Brussels School of Bioengineering, Université libre de Bruxelles (ULB), 1050 Brussels, Belgium
[2]Evolutionary Biology and Ecology, Université libre de Bruxelles (ULB), 1050 Brussels, Belgium
[3]Interuniversity Institute of Bioinformatics in Brussels – (IB)[2], 1050 Brussels, Belgium
[4]Institute for Bioengineering and Biosciences (iBB) and Institute for Health and Bioeconomy (i4HB), Instituto Superior Técnico (IST), Universidade de Lisboa, 1049-001 Lisbon, Portugal
[5]Department of Bioengineering, Instituto Superior Técnico (IST), Universidade de Lisboa, 1049-011 Lisbon, Portugal

*Corresponding author. Laboratory of Ecology of Aquatic Systems, Brussels School of Bioengineering, Université Libre de Bruxelles, Campus de la Plaine CP221, Boulevard du Triomphe, 1050 Brussels, Belgium, E-mail: Isabelle.George@ulb.be

Abstract

Aims: Culture-independent studies in the literature suggest that marine habitats hold diversified chitin-degrading microbial communities. This study was conducted to isolate novel chitinolytic bacteria from two bacteria-rich marine biotopes, namely sponges and sediments, and compare the efficiency with which those strains degrade different forms of chitin.

Methods and Results: Bacterial colonies were isolated from chitinolytic consortia derived from the microbiota of the marine sponge *Hymeniacidon perlevis* of and its surrounding sediment collected at Audresselles beach, France. Many isolates (49%) produced a halo of chitin degradation on colloidal chitin agar plates, including isolates belonging to two genera (*Motilimonas*, *Pseudophaeobacter*) yet unknown as chitin degraders. However, 83% of the positive isolates degraded poorly insoluble chitin powder in liquid cultures. Nine isolates were further tested for colloidal chitin degradation in liquid cultures and exhibited contrasting results. One isolate, *Motilimonas* Spo1_1, exhibited the strongest chitinolytic activity in liquid culture containing insoluble chitin powder (i.e. 37% of degradation). The analysis of its genome and that of other *Motilimonas* spp. revealed an arsenal of genes for chitin degradation. Genomic analyses suggest that Spo1_1 is a new species within the genus *Motilimonas*, we propose the name *Motilimonas chitinivorans*.

Conclusions: *Motilimonas* Spo1_1 largely outperformed all 70 other strains in terms of its insoluble chitin degradation capabilities, including strains belonging to the well-known chitinolytic genera *Vibrio* and *Pseudoalteromonas*. Those results encourage further studies on the potential of *Motilimonas* spp. to eliminate chitinous waste. More generally, they confirm that marine habitats are a reservoir of chitinolytic microbes yet to be discovered.

Impact Statement

This study is the first to experimentally prove the chitin degradation ability of an isolate of the genus *Motilimonas*.

Keywords: marine bacteria; insoluble chitin powder; colloidal chitin; chitin degradation; *Motilimonas*; chitinases

Introduction

Chitin is a polysaccharide of N-acetyl-glucosamine (GlcNAc), in which the monomers are linked by β(1–4) glycosidic bonds. It is considered the second most abundant polymer in the world after cellulose (Elieh-Ali-Komi et al. 2016). Indeed, chitin is a structural component of different organisms, and it can be found in the exoskeleton of crustaceans, insects, molluscs, in the cell wall of fungi and in some algae (Gooday 1990, Dhole et al. 2021, Zhang et al. 2000). Chitin exists in different structural forms (α, β, and γ) depending on the orientation of the micro-fibrils. The α form is the most prevalent one in nature and is dominant in the shell of crustaceans, among others. Its antiparallel chains make it more crystalline (due to intra-sheet hydrogen bonds) and less susceptible to swelling (i.e. penetration of molecules into the crystal lattice) than the β and γ forms (Rinaudo 2006, Kaya et al. 2017).

The annual production of chitinous material in the world has been estimated to amount to 10^{10}–10^{11} tons per year (Gooday 1990). In the seafood industry (e.g. shellfish processing industry), large amounts of chitinous shell waste are not valorised. For example, in 2018, 10^6 tons (live weight) of marine crustaceans were captured (Mathew et al. 2021). In shrimps, 50%–70% of the raw material weight is waste (Gortari and Hours 2013). Such waste is burned, discharged at sea, or left rotting when no controlled disposal is available, causing environmental problems (Xu et al. 2013). Another way to handle the shell waste is to process it industrially

Received 30 October 2024; **revised** 26 June 2025; **accepted** 22 July 2025

to extract chitin and its derivatives. Chitin, chitosan (i.e. the N-deacetylated product of chitin), and chito-oligosaccharides (COS, i.e. the oligomers of chitin) are biocompatible and biodegradable. These molecules have many different applications, but the majority concerns chitosan and COS due to their lower degree of polymerisation, acetylation, and crystallinity. In those molecules, the reduction of intra-chain hydrogen bonds results in facilitated contacts between functional chemical groups and the environment, increasing their solubility and biological properties (Roy et al. 2017, Hou et al. 2023). Chitin and its modified products are used for food preservation, as absorbents in wastewater treatment, in biomedicine, in agriculture, in cosmetics and toiletries, etc. (reviewed in Rinaudo 2006, Hou et al. 2023, Kumari et al. 2023). However, the extraction of those molecules is done by using strong chemicals (acid and/or alkali solutions) for demineralization, deacetylation, and deproteinization, which, if not disposed properly, causes environmental issues. Using biological methods based on biocatalysts, including enzymes from chitinolytic microorganisms, to transform chitinous waste would be more sustainable and less polluting (Gohel et al. 2006). Similarly, bacterial chitinases have multiple applications. First, they can be used to treat chitinous wastes enzymatically and release chitosan, COS and GlcNAc (Sakai et al. 1998, Rathore and Gupta 2015). Second, they can be used as biocontrol agents against pathogenic fungi, or for other applications, for example, to increase the extraction of tannase proteins produced by *Aspergillus niger* (reviewed in Rathore and Gupta 2015, Dhole et al. 2021).

Chitinases are glycosyl hydrolases (GH) that cleave the β(1–4) glycosidic bonds between GlcNAc residues. They belong to different GH families, but the most studied ones are the GH18, 19, and 20 families (Dhole et al. 2021, Lau and Furusawa 2024). Chitinases are found in various organisms such as bacteria, fungi, viruses, arthropods, mammals, and plants (Kumari et al. 2023). They are divided into two major categories, endochitinases and exochitinases. Endochitinases (EC3.2.1.14) cleave randomly the chitin micro-fibrils at internal sites producing multimers of GlcNAc that can then be attacked by the exochitinases, thus multiplying the number of cleavage sites. Exochitinases "lato *sensu*" include the categories EC3.2.1.200 and EC3.2.1.201 that cleave progressively the reducing or the non-reducing ends of chitin molecules releasing dimeric residues (chitobiose) (Dhole et al. 2021). They also include β-*N*-acetylhexosaminidases (EC3.2.1.52) that cleave the terminal non-reducing residues, resulting in monomeric residues of GlcNAc (Beygmoradi et al. 2018). These degradation products can then be assimilated by the cells and enter metabolic pathways. Another family of enzymes that contributes to the degradation of chitin (and crystalline polysaccharides in general) is the lytic polysaccharide monooxygenases (LPMOs, EC1.14.99.53). These oxidative copper enzymes cleave chitin and cellulose at internals sites as well, leaving new chain ends on the polymers (Hamre et al. 2015).

In soils and aquatic systems, bacteria are key players in the degradation of chitin (Gooday 1990, Kielak et al. 2013). For example, ocean sediments contain only traces of chitin, whereas chitin is constantly produced in the water column (mainly as carapaces of zooplankton) (Alldredge and Gotschalk 1990, Gooday 1990). This is because of the presence of chitinolytic bacteria in the water column and sediments (Zobell and Rittenberg 1937, Keyhani and Roseman 1999). In nature, it is known that the degradation of polymers such as chitin impacts microbial community structuring and metabolic interactions, opening possibilities for interspecies cross-feeding that can improve the efficiency of polymer degradation (Beier and Bertilsson 2013, Yurgel et al. 2022).

Presently, various bacterial genera are well known to degrade chitin, e.g. *Vibrio*, *Pseudoalteromonas*, *Shewanella*, *Serratia*, *Bacillus*, *Aquimarina*, *Aeromonas*, and *Streptomyces* (reviewed in Dhole et al. 2021, Raimundo et al. 2021). Moreover, in the marine environment, it was shown that sessile invertebrates such as corals and sponges host chitinolytic microbial symbionts, and that such chitinolytic consortia may differ in taxonomic composition from those of the environmental surroundings (Raimundo et al. 2021). For example, *Endozoicomonadaceae* are dominant symbionts of octocorals, whose unanticipated role in the degradation of chitin was revealed by metagenomics (da Silva et al. 2023). Further, the study of da Silva et al. (2023) confirmed the widespread occurrence of chitinase-encoding genes in 42 publicly available genomes of cultured and uncultured *Endozoicomonadaceae* strains. Such results illustrate that marine environments are a reservoir of as-yet unknown chitin-degrading bacterial taxa that remain to be discovered.

Recently, Meunier et al. (2024) selected bacterial consortia that degrade raw chitin from the tissue of the sponge *Hymeniacidon perlevis*, as well as seawater and sediment collected at the beach of Audresselles, Pas de Calais, France. Based on 16S rRNA gene sequencing, the authors characterized the composition of those communities and identified their dominant members, including *(Hal)arcobacter, Marinomonas, Motilimonas, Pseudoalteromonas,* and *Vibrio* spp. Screening of published genomes from those taxa revealed that several of them were likely to play a pivotal role in chitin hydrolysis and/or deacetylation. However, their chitinolytic degradation capabilities were not demonstrated nor quantified.

In this context, our study aimed at improving the knowledge of the phylogenetic breadth of marine chitinolytic bacteria and bioprospecting for novel, high-performing chitin degraders. To this end, we (i) screened the above-mentioned consortia in search of new chitinolytic isolates using selective media with chitin as sole carbon and nitrogen source, and (ii) compared the chitinolytic activity of the isolates in different chitin media (powdered chitin and colloidal chitin, solid vs. liquid media) in order to identify the most promising and versatile strains for future applications.

Materials and methods

Enrichment cultures

Chitinolytic consortia were obtained from two different habitats: the tissue of the intertidal marine sponge *H. perlevis* and sediment. The protocol to select those efficient multi-species chitinolytic consortia was described in Meunier et al. (2024). *Hymeniacidon perlevis* specimens (three biological replicates, Spo1, Spo2, and Spo3) and surrounding surface sediment (maximum 1 cm depth) (Sed) were collected at the beach of Audresselles, France (Lat. 50° 49′14.888 N, Long. 1° 35′ 34.354E), at low tide on the 6 June 2020 by Meunier et al. (2024). One additional specimen of *H. perlevis* (SpoA) and one sample of surrounding surface sediment (SedA) were collected at the same location

at low tide on the 20 February 2023 to extend the culture collection.

Bacterial cell suspensions were recovered from the different samples following protocols detailed in Meunier et al. (2024). Briefly, 4 g of sponge specimens were cleaned from encrusted organisms and crushed using a mortar and a pestle in 50 mL of artificial seawater (ASW) (Instant Ocean). The homogenates underwent several rounds of centrifugations, and then filtrations on 10- and 3-μm pore size membranes (Whatman, UK) to remove aggregates. The flow through was centrifuged for 20 min at 4°C and 5200 g to recover a microbial cell pellet. Sediment (1 g, wet weight) was mixed with nine mL of ASW and underwent the same round of centrifugation and filtration steps as described above. All cell pellets were resuspended in 830 μL of sterile ASW and transferred into sterile, 2-mL cryovials containing 150 μL of sterile 100% glycerol and 20 μL of 100% DMSO. These glycerol stocks were stored at −80°C until use as inoculum for the enrichment cultures.

Two chitin media were used to obtain the chitinolytic consortia by enrichment culturing: (A) a liquid marine minimal medium made of 100 mL of ASW (Instant Ocean) (33 g L^{-1}), 0.15 g of KH_2PO_4 (VWR), and 160 μL of a solution of trace elements (for details, see Table S1), supplemented with 1 g of insoluble chitin powder extracted from shrimp shells (C7170 from Sigma-Aldrich/Merck, Germany) as sole source of carbon and nitrogen (as described in Meunier et al. 2024); and (B) the same marine minimal medium but instead supplemented with 1 g of dried (at 70°C for 24 h), pounded, and sterile (autoclaved at 121°C for 20 min) shrimp shells from a local supermarket as sole source of carbon and nitrogen. The pH of both media was adjusted to 7.5–8 with NaOH before autoclaving. One hundred μL of each of the above-mentioned glycerol stocks were added to 100 mL of chitin medium and the incubation lasted for 7 days at 20°C and 85 rpm.

Preparation of colloidal chitin

Colloidal chitin was prepared from insoluble chitin powder from shrimp shells (C7170 from Sigma-Aldrich/Merck, Germany), following the protocol described in Hsu and Lockwood (1975) with modifications. 6.6 g of chitin were slowly added to 150 mL of HCL (37%) then stirred vigorously for 120 min at 30°C. Chitin was precipitated as a colloidal suspension by mixing it slowly with 2 L of MilliQ water at 4–10°C. The suspension was filtered through a coffee filter with the help of a vacuum pump. The colloidal chitin was collected from the filter and washed by resuspension in 1 L of MilliQ water before another round of filtration was applied on an 8-μm pore size filter (CAT No. 1002–070, Whatman 2, Cytiva, USA). The colloidal chitin recovered on the filter was washed again several times using the same protocol until the pH of the suspension increased up to 4. The wet colloidal chitin was then used as a C and N source in agar media. When used in liquid cultures, the colloidal chitin was dried for 24 h at 70°C and grinded into a fine powder before use.

Isolation of bacteria from the chitinolytic consortia

The solid media used to recover bacterial isolates from the chitinolytic consortia were Marine Agar (MA, i.e. Marine broth MB (Difco BD, Sparks, MD, USA) supplemented with 15 g L^{-1} of agar (VWR)), and colloidal chitin agar (CC-agar). CC-agar was prepared with ASW salts (Instant Ocean) 33 g L^{-1}; KH_2PO_4 (VWR) 1.5 g L^{-1}; yeast extract (MERCK, Darmstadt, Germany) 0.5 g L^{-1}; colloidal chitin 5 g L^{-1}, trace elements (Table S1) 1.6 mL L^{-1}; and agar 15 g L^{-1}. Its pH was adjusted to 7.5–8 with a solution of 5 M NaOH before autoclaving. Serial dilutions of the chitinolytic consortia were prepared (10^{-3}–10^{-7}) and 100 μL of each were spread on CC-agar and MA. Plates were incubated at room temperature for 1–4 weeks. We picked colonies having distinct morphotypes on MA agar and colonies producing a degradation halo on CC-agar, along with additional colonies which displayed a distinct morphology on CC-agar, although not producing a clear halo of degradation. Colonies were streaked onto MA, several times if needed until a pure isolate was obtained. Seventy-one bacterial isolates were obtained this way (Tables S2 and S3). For each isolate, one colony was then mixed with 830 μL of MB, 150 μL of glycerol 100%, and 20 μL of 100% DMSO, and these glycerol stocks were stored at −80°C until further use.

Sequencing of the 16S rRNA gene of the bacterial isolate

For each bacterial isolate, DNA was recovered by resuspending cellular material from one colony in 25 μL of sterile PCR grade water and boiling the suspension at 100°C for 15 min. PCR amplification was performed by adding three μL of DNA solution to 47 μL of PCR mix containing 25 μL of 2× Green GoTaq Green MasterMix (Promega, Madison, WI, USA), PCR grade water, and 1 μL of a 20-μM solution of each universal primer 27F (5′-GAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′; Weisburg et al. 1991). The PCR cycle steps consisted of an initial denaturation step at 94°C for 6 min 30 s, followed by 30 cycles at 94°C for 30 s, 55°C for 1 min 30 s, and 72°C for 2 min 30 s, and a final elongation step at 72°C for 5 min. PCR products were analysed by electrophoresis on a 0.8% agarose gel, and purified and Sanger-sequenced using the universal primers 27F and 1492R at Macrogen Europe (Amsterdam, The Netherlands). Quality-check and trimming of the sequences were performed with CodonCode Aligner (CodonCode Corporation, www.codoncode.com). The closest 16S rRNA sequence matches were determined using the Basic Local Alignment Search Tool (BLAST) of The National Centre for Biotechnology (NCBI) with the Reference RNA sequences (refseq_rna) database. The phylogenetic affiliation was also performed with arb-Silva "Alignment, classification and tree service" (ACT) (Quast et al. 2013). Based on the arb-Silva affiliation, a phylogenetic tree of the 16S rRNA genes was constructed using FastTree, employing the General Time Reversible (GTR) model and Gamma distribution as the rate model for likelihood calculations. These options were provided by the arb-Silva ACT service. The tree was viewed and rooted with TreeViewer. The tree was edited with Inkscape.

Degradation of colloidal chitin on agar plates

The ability to degrade colloidal chitin was tested for the 71 bacterial isolates. A loop of the frozen stock was spread on MA and the plates were incubated at room temperature until colonies developed. One colony was then picked to inoculate 1 mL of MB, and the pre-culture was incubated at 20°C and 176 rpm (Innova 44R, New Brunswick) overnight. For each isolate, biological triplicates of this preculture were prepared (each time starting from a different colony). Then the concentration of cells in the precultures was assessed by flow cytom-

etry using an Accuri C6 flow cytometer (BD, Franklin Lakes, USA) according to the procedure described in Bonal et al. (2023). Briefly, the overnight precultures were serially 10-fold diluted in 0.22 μm-filtered, autoclaved phosphate buffered saline (PBS), and cell counting was performed on one dilution with a rate of 200–2000 events s^{-1} as target. Afterwards, a volume of preculture containing 5.10^7 cells was transferred into an Eppendorf tube and set to a total volume of 1 mL with physiological water (0.9% NaCl). Ten μL of that cell suspension were spotted on CC-agar. This step was repeated on CC-agar without yeast extract (CC-WoYE-agar), to test the effect of the addition of yeast extract on the size of the degradation halo. The plates were incubated at 20°C in micro-aerobic conditions to mimic the conditions in the sponge (Lavy et al. 2016) and improve the readability of the test (Gazpak EZ Campy Container System Sachets, BD Biosciences, Sparks, USA) in an airtight jar (260 672, BD Biosciences, SpoArks, USA). After 14 days, the diameter of the chitin degradation halo around the bacterial spot was measured (Fig. S1) to calculate the surface of degradation.

Degradation of insoluble powdered and colloidal chitin in liquid cultures

The ability to degrade insoluble chitin powder in liquid cultures was tested in biological triplicates for the 35 isolates that were able to degrade colloidal chitin on agar plates. The preculture and the estimation of its cell concentration were performed as described earlier. A volume of preculture containing 5.10^7 cells was used to inoculate 100 mL of liquid medium into a 150-mL Erlenmeyer flask. This medium had the same composition as medium A used to select the chitinolytic consortia, and the incubation conditions were identical as well. After the incubation period, the culture was filtered through a pre-weighed 8-μm pore-size filter (CAT No. 1002–070, Whatman 2, Cytiva, USA) and rinsed with about 250 mL of MilliQ water. The filter with the chitin retained on it was dried at 70°C for 24 h and then weighed. The same protocol was extended to 14 days of incubation for all the isolates that showed chitin degradation after 7 days. One negative control (liquid medium without inoculation of bacteria, i.e. blank) was prepared for each batch of experiments (the 7- and 14-day experiments were done independently).

The percentage of degradation at the end of the incubation period was calculated as follows:

$$\%\ degradation = \left(\left(\frac{m_{Bt7\ or\ Bt14}}{m_{Bt0}} - \frac{m_{Ct7\ or\ Ct14}}{m_{Ct0}}\right) * 100\right),$$

where m_{Ct0}= mass of chitin added in the culture; $m_{Ct7\ or\ Ct14}$ = mass of chitin remaining after the incubation time (seven or 14 days); m_{Bt0} = mass of chitin added in the blank; and $m_{Bt7\ or\ Bt14}$ = mass of chitin remaining in the blank after the incubation time (7 or 14 days).

The same experiment was repeated for nine isolates (Spo1_1, Spo1_26, Sed_10, Spo1_17, Spo1_6, Spo1_7, Spo1_19, Spo1_22, Sed_3) by changing the chitin source: dried colloidal chitin was used instead of insoluble chitin powder. The incubation time was set to 14 days.

Statistical analyses

A Shapiro–Wilk test was performed to assess the normality of the data of chitin degradation halos on agar plates supplemented with yeast extract or without yeast extract. As the data were not normally distributed, a Kruskal–Wallis' test was conducted to determine if there was a significant difference in degradation depending on the presence yeast extract. For each condition (presence or absence of yeast extract), a Kruskal–Wallis' test was also performed to evaluate differences in degradation across all strains. The results of those tests are detailed in Table S4.

In addition, a Shapiro–Wilk test was performed to assess the normality of the data of insoluble chitin degradation in liquid cultures after 7 and 14 days. As they were not normally distributed, a Friedman test was conducted to determine if there was a significant difference in degradation after 7 and 14 days (paired data). For each group of data (7 and 14 days), a Kruskal–Wallis' test was performed to evaluate whether there was a significant difference in degradation across all strains. Additionally, for each group, a post hoc Dunn's test was conducted to identify which pairs of strains were significantly different in their degradation ability. The results of those tests are detailed in Table S5.

Finally, a Shapiro–Wilk test was performed to assess the normality of the data of insoluble and colloidal chitin degradation by the nine selected strains in liquid cultures after 14 days. As they were normally distributed, an unpaired *t*-test was performed for each strain to compare their efficiency to degrade insoluble (powdered) chitin versus colloidal chitin. The results of those tests are detailed in Table S6.

Growth on N-acetylglucosamine in liquid cultures

The ability of isolates to use GlcNAc was tested in liquid cultures by measuring their growth in a microplate with a Spectra max Plus 384 spectrophotometer (Molecular Devices, USA). The medium used had the same composition as medium A), except that GlcNAc (10 g L^{-1}) was used as the sole source of carbon and nitrogen. One hundred and ninety five μL of the medium was added to each well and 5 μL of an overnight preculture in MB (at 20°C and 176 rpm) was transferred into the well. The microplate was incubated at 20°C and 85 rpm for 7 days. An isolate was considered able to grow on GlcNAc ("+" in Table S2) if DO_{600} in stationary phase was at least the double of the value of the negative control (which was 0.1) in minimum one of the biological triplicates.

Genome sequencing of *Motilimonas* Spo1_1

We sequenced and annotated the genome of the most efficient chitin-degrading bacterium analysed in this study, *Motilimonas* isolate Spo1_1 with the purpose to search for genes involved in chitin degradation. DNA extraction of an overnight liquid culture of *Motilimonas* Spo1_1 in MB (at 20°C and 176 rpm) was performed with the kit Puregene Yeast/Bact Kit B (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA was then size-selected using the SRE kit (PacBio, USA) according to the manufacturer's instructions and sequenced using a MinION device (Oxford Nanopore Technologies, Oxford, UK). The genomic library was prepared using the Nanopore kit SQK-RBK114.24 according to the manufacturer's instructions and loaded on an R10.4.1 flow cell after a quality check using MinKNOW. The library was sequenced for 72 h. The base calling and barcoding were performed using GUPPY (Oxford Nanopore Technologies, Oxford, UK) and the final reads were collected on a FASTQ file.

De *novo* genome assembly, quality check, and genome annotation

The assembly was performed using FLYE v2.9-b1768 (Kolmogorov et al. 2019) with the –nano-hq parameter. The assembly was visualized with Bandage (Wick et al. 2015). K-mer frequency distributions and assembly completeness were assessed using the K-mer Analysis Toolkit (KAT) v2.4.2 with the "comp" parameter (Mapleson et al. 2017) and genome completeness was assessed using BUSCO v5.2.2 (Manni et al. 2021). The genome was annotated with Prokka v1.14.6 (Seemann 2014). Coding sequences were translated into amino acid sequences and functionally annotated against the Cluster of Orthologous Genes (COG) database with eggNOG-mapper (Cantalapiedra et al. 2021). The 16S rRNA genes were extracted from the genome using the Bacterial ribosomal RNA predictor v0.9 (Barrnap) (https://github.com/tseemann/barrnap) and the closest sequence match was determined using BLAST of NCBI then compared to what was inferred with the 16S Sanger sequencing previously described.

Comparison of sequencing and assembly features between *Motilimonas* Spo1_1 and other *Motilimonas* genomes available on NCBI

Five *Motilimonas* genomes were available on NCBI (*Motilimonas pumila* PLHSC7-2, *Motilimonas* sp. E26, *Motilimonas cestriensis* MKS20, *Motilimonas eburnea* YH6, *Motilimonas* sp. 1_MG-2023 G1M02). They were downloaded and annotated as described above. Information about assembly features were recovered from NCBI (G + C content, N50, mean coverage). The five genomes were annotated using Prokka v1.14.6 and the annotation was summarized in the .txt file generated by the program (number of contigs, size, number of rRNAs and tRNAs). The BUSCO analysis was performed as described above.

Phylogenetic affiliation of *Motilimonas* Spo1_1

The percentage of average nucleotide identity (ANI) value was calculated between the genomes of isolate Spo1_1 and its closest relative, *M. cestriensis* MKS20, using the OrthoANIu tool (https://www.ezbiocloud.net/tools/orthoaniu). The percentage of digital DNA–DNA hybridization (dDDH) between genomes was estimated using the Genome-to-Genome Distance Calculator (GGDC 3.0; Meier-Kolthoff et al. 2013) with formula 2.

Comparative analysis of sequences coding for putative enzymes involved in the chitin degradation pathway in the *Motilimonas* genomes

Coding sequences from the other five genomes were functionally annotated against the COG database as described above. Amino acid sequences belonging to the COG categories involved in chitin degradation (COG 3325, 3469, 3979, 3525, 1472, 3397; see Table S7 for their description) were searched for in the functionally annotated protein sequence files of those five genomes and the Spo1_1 genome. Each sequence belonging to these COG categories was then annotated for protein family (Pfam) domains known to be associated with chitin degradation (GH18; GH19; GH20; GH3; NAGidase and LPMO10) using the HMMER scan tool from EMBL-EBI (Madeira et al. 2022). A sequence was taken into account when it contained at least one of those domains (GH18; GH19; GH20; GH3; NAGidase and LPMO10). Lastly, the COG category 0363 (enzymes that participate in the catabolism of amino sugars allowing the use of glucosamine) was searched for to ascertain whether GlcNAc could be used by the isolates under study (Oliva et al. 1995). To determine if the genes encoding for GH18, GH19, and GH20 chitinases were grouped in operons, we screened the Spo1_1 genome with Operon-mapper using default parameters (Taboada et al. 2018).

Phylogenetic tree of GH18 chitinases found in the *Motilimonas* genomes

Protein sequences showing at least one GH18 or GH19 domain according to the Pfam-based annotation were included in the phylogenetic analysis of chitinase gene sequences predicted in the *Motilimonas* genomes (two trees were built separately: one for GH18 and another for GH19). To build the phylogenetic trees, only GH18 or GH19 domain sequences identified by Hmmer Pfam annotation were taken into account. The closest sequences found in the NCBI database (Blastp tool, NCBI) of each of the nine GH18 chitinase amino acid sequences identified in the Spo1_1 genome were also included in the trees, following the same procedure. For each domain sequence, a protein structure model was created using ESMfold (Lin et al. 2023) with the ESM-2 model using default parameters. The resulting dataset containing 64 GH18 and 15 GH19 models and sequences were aligned using SIMSApiper (Crauwels et al. 2024) and its "magic" presets without the initial data reduction step (—magic—dropSimilar false). SIMSApiper is a novel pipeline to generate structure-informed multiple sequence alignments of larger datasets, which is particularly useful for the study of highly divergent protein families with shared domains. The phylogenetic trees were calculated from the MSA using IQ-TREE 2 (Minh et al. 2020) with ModelFinder (Kalyaanamoorthy et al. 2017) and 10 000 ultrafast bootstrap replicates.

All calculations were carried out on the VSC (Flemish Supercomputer Center) Tier-2 general-purpose clusters provided by the Vrije Universiteit Brussel High Performance Computing (VUB-HPC).

The tree was viewed and rooted with TreeViewer. In addition, for each protein sequence (beyond the GH18 domain), the presence of protein domains, signal peptides, and active sites were searched for using HMMER Pfam and plotted next to the tree using Inkscape. Arbitrary clusters were created based on groups of minimum three sequences sharing a branch length of maximum 0.7. In cluster II, two subclusters were created based on groups of minimum three sequences sharing a branch length of maximum 0.15.

Potential chitin utilization pathway in *Motilimonas* Spo1_1

A BlastKOALA (Blast KEGG Orthology And Links Annotation) (Kanehisa et al. 2016) analysis was conducted using the annotated genome of *Motilimonas* Spo1_1 generated by Prokka. Metabolic pathways were reconstructed using KEGG Mapper. The pathways "00 520 Amino sugar and nucleotide sugar metabolism", "02 010 ABC transporters", and "0260 Phosphotransferase system (PTS)" were extracted to identify the potential hydrolytic and oxidative degradation pathways in *Motilimonas* Spo1_1.

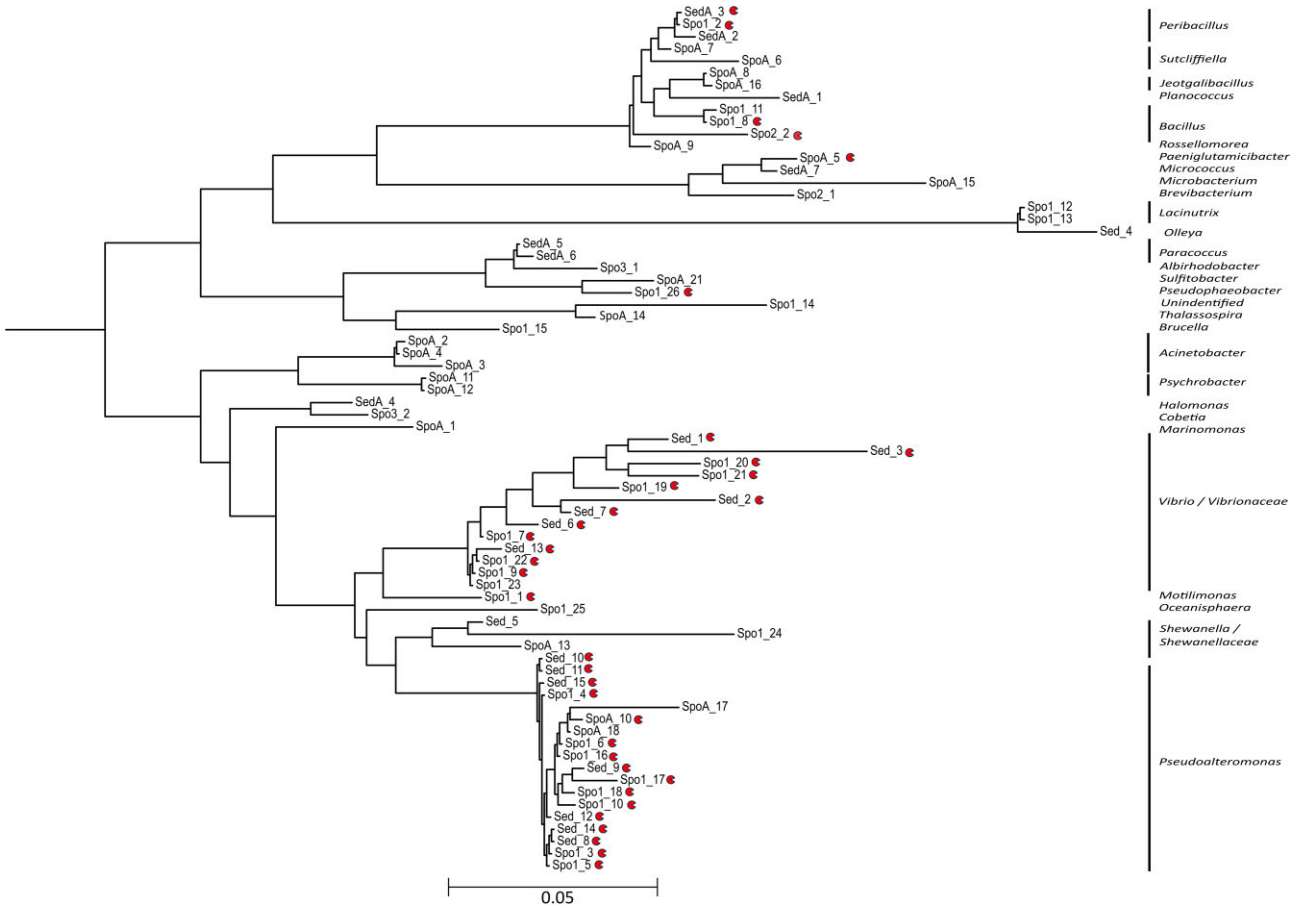

Figure 1. Phylogenetic tree of the 16S rRNA gene of the 71 isolates and their phylogenetic affiliation. The symbol "C" refers to the ability of a strain to degrade colloidal chitin on CC-agar.

Results

Phylogenetic affiliation of the bacterial isolates

A total of 71 colonies, 47 on CC-agar, 24 on MA, were isolated and identified in this study (Tables S2 and S3). Four phyla were represented, *Pseudomonadota* (52 isolates, 73% of the colonies), *Bacillota* (12 isolates, 17%), *Actinomycetota* (4 isolates, 6%), and *Bacteroidota* (3 isolates, 4%). The two most abundant genera were known chitin degraders, i.e. *Pseudoalteromonas* (18 isolates, 25%) and *Vibrio* (12 isolates, 17%) (Fig. 1, Tables S2 and S3). On CC-agar, isolates belonged to 18 genera, and two could not be affiliated to a genus (Spo1_14 and Sed_3). For Spo1_14, arb-Silva could not find an affiliation and for Sed_3 the percentage of identity to his closest relative was < 94.5%, i.e. below the threshold set to define the genus level by 2.8% (Table S2). On MA, isolates belonged to 14 genera, with 4 genera in common with those recovered on CC-agar. In total, isolates belonging to 28 different genera were obtained (Fig. 1). The phylogenetic tree of isolates obtained from sediments and sponges did not show two distinct clusters of bacterial taxa according to their original habitat (Fig. 1). However, among the genera represented by multiple strains, one was unique to the sediments (i.e. *Paracoccus*) and several were unique to the sponges (i.e. *Sutcliffiela*, *Joetgalibacillus*, *Bacillus*, *Lacinutrix*, *Acinetobacter*, and *Psychrobacter*). Most isolates showing a halo of degradation on agar plates belonged to the *Gammaproteobacteria* class.

Degradation of colloidal chitin in agar-based tests

The ability to hydrolyse colloidal chitin on agar plates was greatly dependent on the medium used to isolate the strains, as 60% of the isolates recovered on CC-agar (i.e. 28 isolates) demonstrated this ability compared to only 29% among those recovered on MA (i.e. seven isolates) (Table S2). However, among the two novel chitin degraders that were identified in our collection (Fig. 2), one was not isolated on CC-agar, but on MA (*Pseudophaeobacter* Spo1_26).

Altogether, 35 out of the 71 isolates (49%) showed chitinolytic activity on CC-agar (Fig. 2). They belonged to the genera *Pseudoalteromonas*, *Motilimonas*, *Pseudophaeobacter*, *Vibrio*, *Vibrionaceae_unknown genus*, *Bacillus*, *Peribacillus*, and *Paeniglutamicibacter*.

The isolates produced halos of degradation whose size was significantly different, both on CC-agar with yeast extract (Kruskal–Wallis test, $P < 0.0001$, Table S4B) and without yeast extract (Kruskal–Wallis test, $P < 0.0001$, Table S4C). Moreover, the ability to degrade colloidal chitin on solid medium was highly variable within a same genus: degradation halos ranged from 9.6 to 0 cm^2 for *Pseudoalteromonas*, from 6.2 to 0 cm^2 for *Vibrio*, 3.8 to 0 cm^2 for *Bacillus*, and from 3.3 to 0 cm^2 for *Peribacillus* (Fig. 2).

In addition, the halos of colloidal chitin degradation were significantly different (smaller) when the CC-agar medium was not supplemented with yeast extract (CC-WoYE-agar) (Fig. 2, Kruskal–Wallis test, $P < 0.0001$, Table S4D). This re-

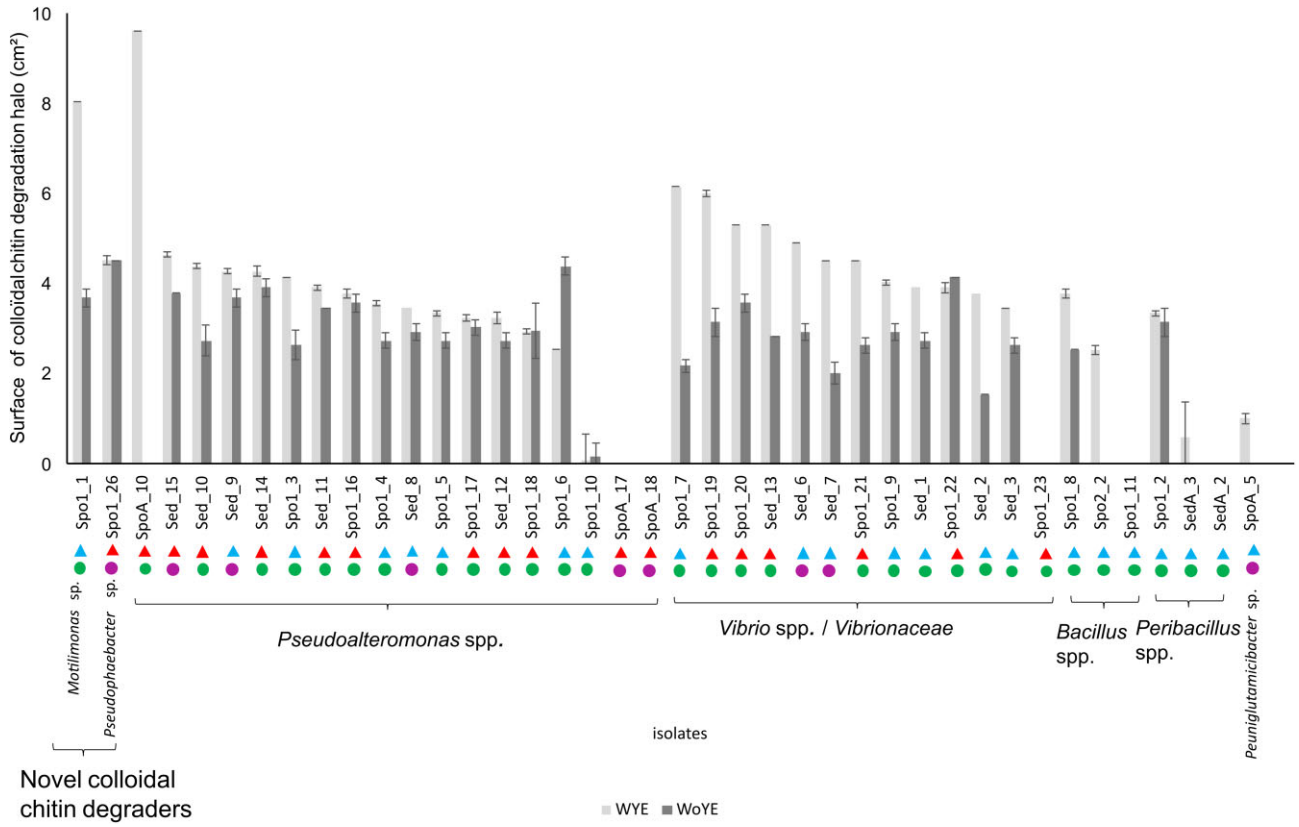

Figure 2. Degradation of colloidal chitin on agar plates supplemented or not with yeast extract, expressed as surface of degradation in cm^2. Only genera including a minimum of one strain able to degrade colloidal chitin are presented. WYE = with yeast extract; WoYE = without yeast extract. The symbols refer to the chitin source used in the liquid enrichment medium (▲ raw chitin and ▲ shrimp shells) and to the solid medium (● colloidal chitin agar and ● marine agar). The error bars correspond to the standard deviation (SD) across three biological replicates. Statistical tests performed on those data are detailed in Table S4.

duction was isolate-dependent, and some isolates were more affected than others. For example, Spo1_1 showed a 54% decrease of the halo surface, Spo1_7 a 65% decrease, and Sed_7 a 56% a decrease. The degradation halo even dropped to (almost) zero for isolates SpoA_19, SpoA_10, Spo1_10, Spo2_2, SedA_3, and SpoA_5. Conversely, other isolates were barely affected, such as isolates Spo1_16 (5.92% decrease), Spo1_17 (6.4%), or Spo1_2 (6.19% decrease).

On CC-agar, the isolates that did not degrade colloidal chitin (no halo of degradation) still formed colonies as they were growing on the yeast extract, but they did not grow on CC-WoYE-agar (see Fig. S2 for illustration). Moreover, 81% of the isolates (54 out of 67 tested) could grow on GlcNAc as sole C and N source in liquid medium, and all the isolates able to produce a degradation halo on CC-agar grew on GlcNAc (Table S2).

Degradation of insoluble chitin powder in liquid cultures after 7 and 14 days

The isolates that could degrade colloidal chitin on CC-agar (Fig. 2) were tested in liquid cultures in the presence of insoluble chitin powder as sole C and N source (Fig. 3). The percentage of chitin degradation was measured after 7 and 14 days.

All in all, percentages of insoluble chitin degradation were not significantly different after 7 and 14 days of incubation (Friedmann test, $P > 0.1$, Table S5B). Of note, the 7- and 14-day experiments were run independently. However, the ability to degrade insoluble chitin was significantly different across strains, whether after 7 (Kruskal–Wallis test, $P < 0.005$, Table S5C) or 14 days (Kruskal–Wallis tests, $P < 0.005$, Table S5E).

Insoluble chitin degradation by *Motilimonas* Spo1_1 was significantly different (higher) from that of 16 (out of 28) strains at both incubation times (Dunn tests, $P < 0.05$, Tables S5D and S5F). It degraded 37% (±2.7%) and 36% (±1%) of insoluble chitin after 7 and 14 days of incubation in the tested conditions, respectively (Fig. 3). For comparison, the most efficient degraders in the genera *Pseudoalteromonas* (isolate Spo1_5) and *Vibrio* (isolate Spo1_21) degraded both 6.8% of chitin after 7 days. For 28 isolates out of 29 (the exception was isolate Spo1_1), the percentage of insoluble chitin degradation did not exceed 10%, and for 24 isolates (83%), it did not exceed 5%.

Comparison of colloidal and powdered chitin degradation in liquid cultures after 14 days

Among the isolates that degraded insoluble, powdered chitin in liquid cultures, we selected 12 isolates to test their ability to degrade colloidal chitin. We choose the two new colloidal chitin degraders belonging to the genera *Motilimonas* and *Pseudophaeobacter* and some isolates belonging to the genera *Pseudoal teromonas* and *Vibrio* (and family *Vibrio nacea e*) (i.e. taxa known to hold chitin degraders) to investigate whether there would be any difference in the degra-

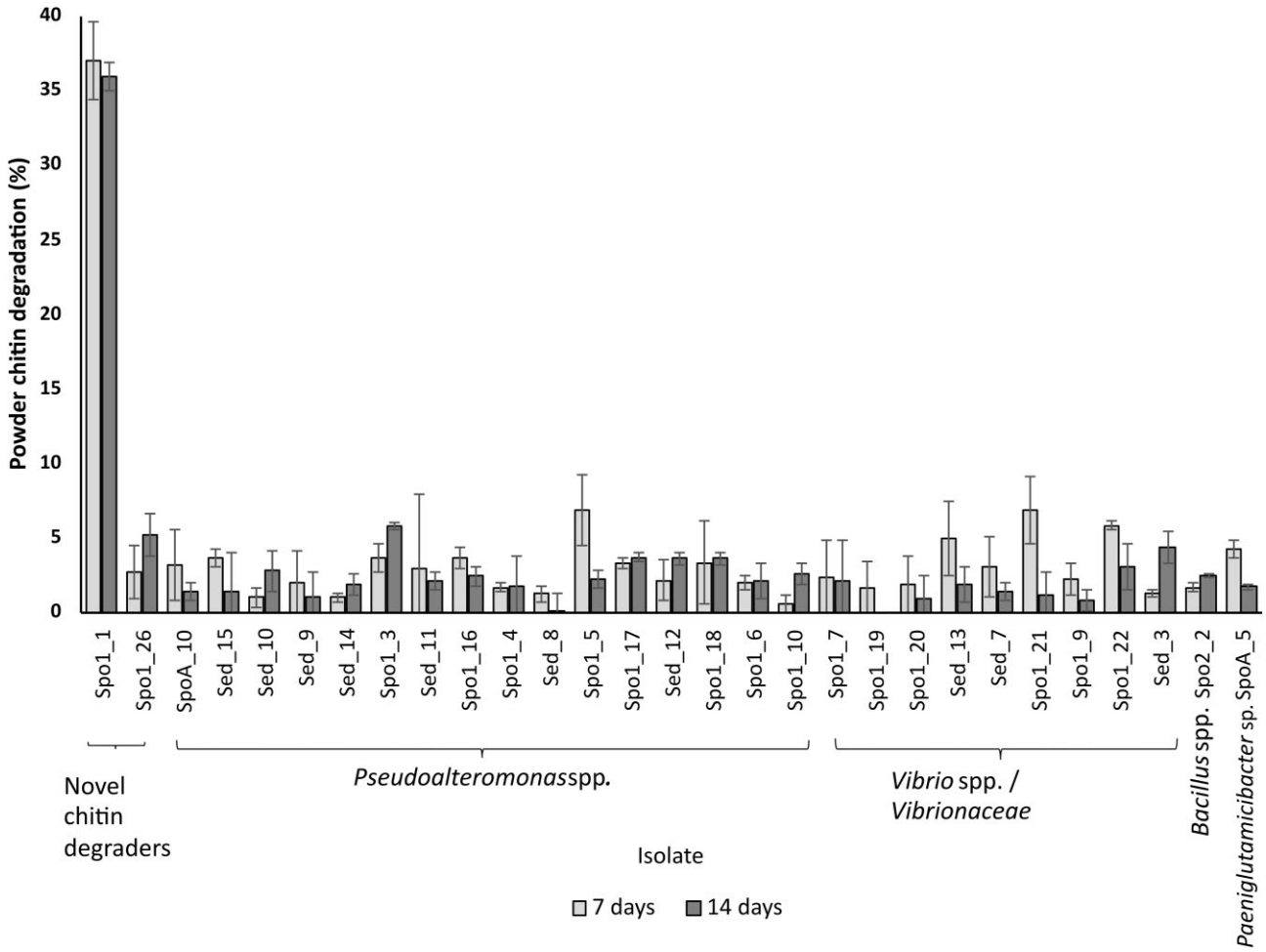

Figure 3. Mean percentage of raw chitin degradation in liquid cultures after 7 and 14 days. The error bars correspond to the SD across three biological replicates. Statistical tests performed on those data are detailed in Table S5.

dation according to the type of chitin. The nine isolates degraded colloidal chitin in liquid cultures (Fig. 4, statistical tests in Table S6). For *Mo tili monas* Spo1_1, colloidal and powdered chitin degradations averaged 35% ±2% and 36% ±1%, respectively. They were not statistically different (Student's *t*-test, $P > 0.5$). A similar observation was made for *Pseudophaeobacter* Spo1_26 and for all *Pseudoalteromonas* isolates, but their percentage of chitin degradation was overall much lower than that of Spo1_1. For the three *Vibrio* isolates and the *Vibrionaceae* bacterium (Sed_3) that we tested, colloidal chitin degradation was significantly higher than the one of powdered chitin (Student's *t*-test, $P < 0.05$). For example, the *Vibrio* isolate Spo1_7 and *Vibrionaceae* isolate Sed_3, which degraded only 2.7% and 3.9% of insoluble chitin powder, could degrade 54% and 18% of colloidal chitin.

De novo assembly and general characteristics of the *Motilimonas* sp. Spo1_1 genome

The complete genome of *Motilimonas* isolate Spo1_1 had a size of 4.75 Mb and a GC content of 43.8%, which was within the range of the other, publicly available *Motilimonas* genomes (4.54–4.81 Mb and 43.5%–46%) (Table 1). However, due to the long-read sequencing technology employed here, the Spo1_1 genome was assembled in one contig, whereas the other assemblies retrieved from public databases contained between 71 and 391 contigs. Annotation by Prokka predicted 4257 coding sequences, 28 rRNAs, and 102 tRNAs in the Spo1_1 genome. The K-mer completeness score for the genome of *Motilimonas* sp. Spo1_1 was 99.99% and could not be calculated for the other *Motilimonas* genomes retrieved from public databases since the analysis is based on the raw reads used for the assemblies. The BUSCO completeness score was 98.7% and was similar for all the assemblies regardless of the sequencing technology used (Table 1).

The closest match to the 16S rRNA gene sequence extracted from the Spo1_1 genome was *M. cestriensis* isolate MKS20 (99.94% of identity) in accordance with what was inferred from the 16S rRNA gene sequence amplified by PCR and identified using BLAST (99.79% of identity) (Table S2). The two 16S rRNA gene sequences extracted (genomic and PCR) had 100% of similarity, but as the 16S rRNA gene extracted from the genome was longer (1539 bp) than the PCR amplified 16S rRNA gene fragment (1413 bp), the percentage of identity with *M. censtriensis* was slightly different. Between the genomes of *M. cestriensis* MKS20 and isolate Spo1_1, the percentages of ANI and dDDH were 89.61% and 39.4%, respectively.

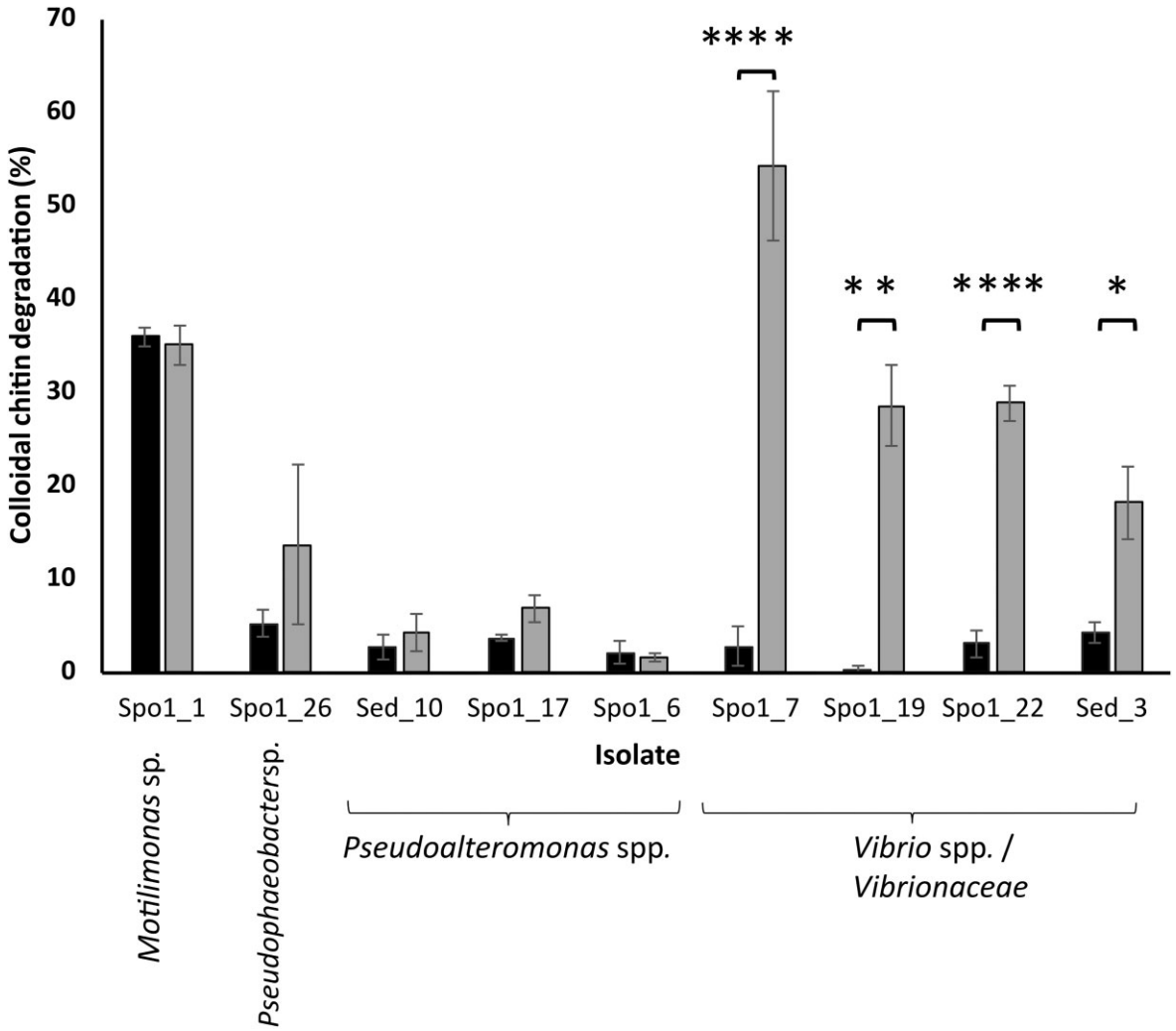

Figure 4. Comparison of colloidal and raw chitin degradation after 14 days. The error bars correspond to the SD across three biological replicates. Only significant comparisons (Student's *t*-test, detailed in Table S6) between mean colloidal and raw chitin degradation after 14 days are presented on the graph as horizontal brackets (∗$P < 0.05$, ∗∗$P < 0.01$, ∗∗∗$P < 0.001$, and ∗∗∗∗$P < 0.0001$).

Table 1. Comparison of sequencing and assembly features of the Spo1_1 genome and of the five Motilimonas genomes available in NBCI.

	Spo1_1	*Motilimonas pumila* PLHSC7-2	*Motilimonas* sp. E26	*Motilimonas cestriensis* MKS20	*Motilimonas eburnea* YH6	*Motilimonas* sp. 1_MG-2023 G1M02
Sequencing Technology	Nanopore	Illumina	Illumina	Illumina	Illumina	Illumina
Size (Mbp)	4.75	4.54	4.63	4.8	4.59	4.81
Number of contigs	1	189	84	89	71	391
N50 (Kb)	4750	124.6	140.8	170.7	178.3	88.3
Mean coverage	71	150	200	30	30	101
G + C content (%)	43.8	45.5	43.5	44	46	43.5
No. of protein-coding genes	4257	4047	4181	4325	4137	4290
No. of rRNAs	28	15	4	11	9	6
No. of tRNAs	102	76	61	87	94	87
KAT completenesss (%)	99.99	/	/	/	/	/
BUSCO completeness (C) (%)	98.7	99.2	98.7	98.7	98.9	98.7
BUSCO fragmented genes (F) (%)	0.5	0	0.5	0.5	0.3	0.5
BUSCO missing genes (M) (%)	0.8	0.8	0.8	0.8	0.8	0.8

Phylogenetic tree of putative GH18 chitinases found in the *Motilimonas* genomes

The classification of enzymes depends on the database used: the COG database forms groups of orthologous sequences and attempts a phylogenetic classification of proteins, KEGG is based on protein activities and enzymatic reaction pathways, and Pfam on the similarity of protein sequences and the definition of protein families using Hidden Markov models.

The different annotation schemes and databases can lead to somewhat different results regarding the number and types of chitinases per genome. In this study, we first looked for all protein sequences associated with the COG categories that could play a role in chitin degradation and then checked if those proteins had a glycosyl hydrolase domain associated with chitin degradation according to the Pfam database (Table S7).

Sequences of proteins involved in chitin degradation (chitinases and LPMOs) were found in all the *Motilimonas* genomes analysed. Therefore, chitin degradation appears to be a trait associated with the genus *Motilimonas* rather than some of its species (Table S7). The number of protein sequences having at least one domain corresponding to the glycosyl hydrolase families 18 and 19 (GH18 and GH19) in the COG categories 3325, 3469, and 3979 ranged from 9 (*Motilimonas* sp. E26 and *Motilimonas* sp. 1_MG-2023 G1M02) to 16 (*M. pumila* PLHSC7-2) (Table S7). All genomes had two GH20 protein domains and one NAGidase domain (except *M. pumila* PLHSC7-2), both known to be involved in β-N-acetylhexosaminidase activity. All genomes except the one of *M. pumila* PLHSC7-2 had one protein sequence identified in the COG1472 category, which includes the GH3 domain family that may have an β-N-acetylhexosaminidase activity. Finally, all genomes contained one protein sequence with an LPMO_10 domain. In summary, all *Motilimonas* genomes contained chitinase genes necessary to degrade chitin and at least one gene for glucosamine utilisation (glucosamine-6-phosphate isomerase/deaminase) indicating that they are able to use the degradation products as well.

A phylogenetic analysis of the GH18 and GH19 domain amino acid sequences of all *Motilimonas* plus the closest match (NCBI Blastp analysis) to those found in the Spo1_1 genome was performed (Fig. 5). It revealed great heterogeneity at the amino acid sequence level between the chitinase genes with a GH18 and GH19 domain in each the *Motilimonas* genomes. In almost each phylogenetic cluster, there was at least one sequence coding for a chitinase domain of each genome, suggesting that the distribution of GH18 domains of the chitinase genes into defined clusters was similar across the six *Motilimonas* genomes. Two exceptions were subclusters 2.1 and cluster III. These exceptions could be explained by the fact that the genomes of *Motilimonas sp*. E26 and *Motilimonas sp*. 1_MG-2023 G1M02 possessed less chitinase genes than the other genomes. In general, the clusters and subclusters grouped entire chitinase sequences carrying the same annotated domains beyond the GH18 domains that were used to build the tree (right panel on Fig. 5). For example, all sequences in cluster IV possessed a chiC domain. This grouping of endochitinase sequences into clusters across the different *Motilimonas* genomes was also true for those belonging to the GH19 family: sequences without a carbohydrate binding domain (CBD) formed cluster IX and were clearly separated from the sequences possessing at least one CBD (cluster VIII). In sum, 38% (30/79) of the sequences included in the tree had one or several CBDs, and most of those sequences belonged to two large clusters (II, VI, and VIII). Furthermore, the analysis of the amino sequences also showed that some sequences belonging to the GH18 family possessed at least one ChiC or one ChiA domain and that those sequences formed four distinct clusters (II, III, IV, and V) among which three were phylogenetically close (clusters III, IV, and V). All sequences (except one, *Motilimonas*_sp_E26_00 909) had a signal peptide. Finally, an active site was detected in all but two (Spo1_1_01 072 and *Motilimonas_pumila*_PLHSC7_2_1665) (Fig. 5). According to Operon-mapper, protein sequences containing a GH18, GH19, or GH20 domain were not grouped into operons in the Spo1_1 genome, except two closely related in the phylogenetic tree (Spo1_1_00 089 and Spo1_1_00 090).

Potential chitin utilisation pathway in *Motilimonas* Spo1_1

According to the metabolic pathways inferred by BlastKOALA and the presence of peptide signals in GH18 and GH19 chitinases (Fig. 5), chitin hydrolysis occurs extracellularly and in the periplasm through the action of chitinases, releasing N-acetylglucosamine (GlcNAc), chitobiose, and chitooligosaccharides. The degradation products released extracellulary cross the outer membrane into the periplasm via unidentified porin proteins (Fig. 6). In the periplasm, chitooligosaccharides are again degraded into chitobiose and GlcNAc. GlcNAc is transported across the inner membrane via the PTS NagE and enters the cytoplasm as GlcNAc-6-phosphate. Chitobiose, on the other hand, is imported into the cytoplasm through ABC sugar transporters and subsequently separated by the ChbP protein into GlcNAc-1-phosphate and GlcNAc. However, only the MsiK protein composing the ABC chitobiose transporter was found by BlastKOALA. In the cytoplasm, these molecules can then be further metabolised and integrated into various biochemical pathways (e.g. GlcNAc can be transformed into Fru-6-P through the action of three proteins, NagK, NagA, and NagB).

Except for the presence of a gene coding for an LPMO, no oxidative chitin degradation pathway was found.

Discussion

The aim of this study was to screen a collection of marine bacteria isolated from two different environments (sponge tissue and sediment) expected to be hotspots of chitin degradation (Souza et al. 2011). The 71 isolates that we recovered using different liquid and then solid media belonged to 4 phyla (*Pseudomonadota*, *Bacillota*, *Actinomycetota*, and *Bacteroidota*) commonly found in marine sponges and sediments (Hameş-Kocabaş and Uzel 2012, Thomas et al. 2016) and to 28 genera. Our results are consistent with previous findings that isolation on different media allows the recovery of different taxa, even if the cultivable isolates are not representative of the whole community present in the original sample(s) (Sipkema et al. 2011, Esteves et al. 2016).

CC-agar is the medium commonly used to isolate chitinolytic bacteria (Lau and Furusawa 2024; Saima et al. 2013, MA et al. 2021, Liang et al. 2022). It was on that medium that we recovered the *Motilimonas* isolate. The genus *Motilimonas* has only been recently discovered. (Ling et al. 2017). Three species have been formally described within the genus and all originated from a salty environment (brine spring, coastal sediment, and the gut of a sea cucumber) (Ling et al. 2017, Wang et al. 2019, Kelbrick et al. 2021). This genus has been very little studied, and not in the context of chitin degradation.

The percentage of similarity of the 16S rRNA gene of isolate Spo1_1 with the one of *M. cestriensis* MKS20 (99.79%–99.94%) suggests they belong to the same species, as this value is above the species threshold of 98.7% (Stackebrandt et al. 2021). However, the ANI and dDDH values between

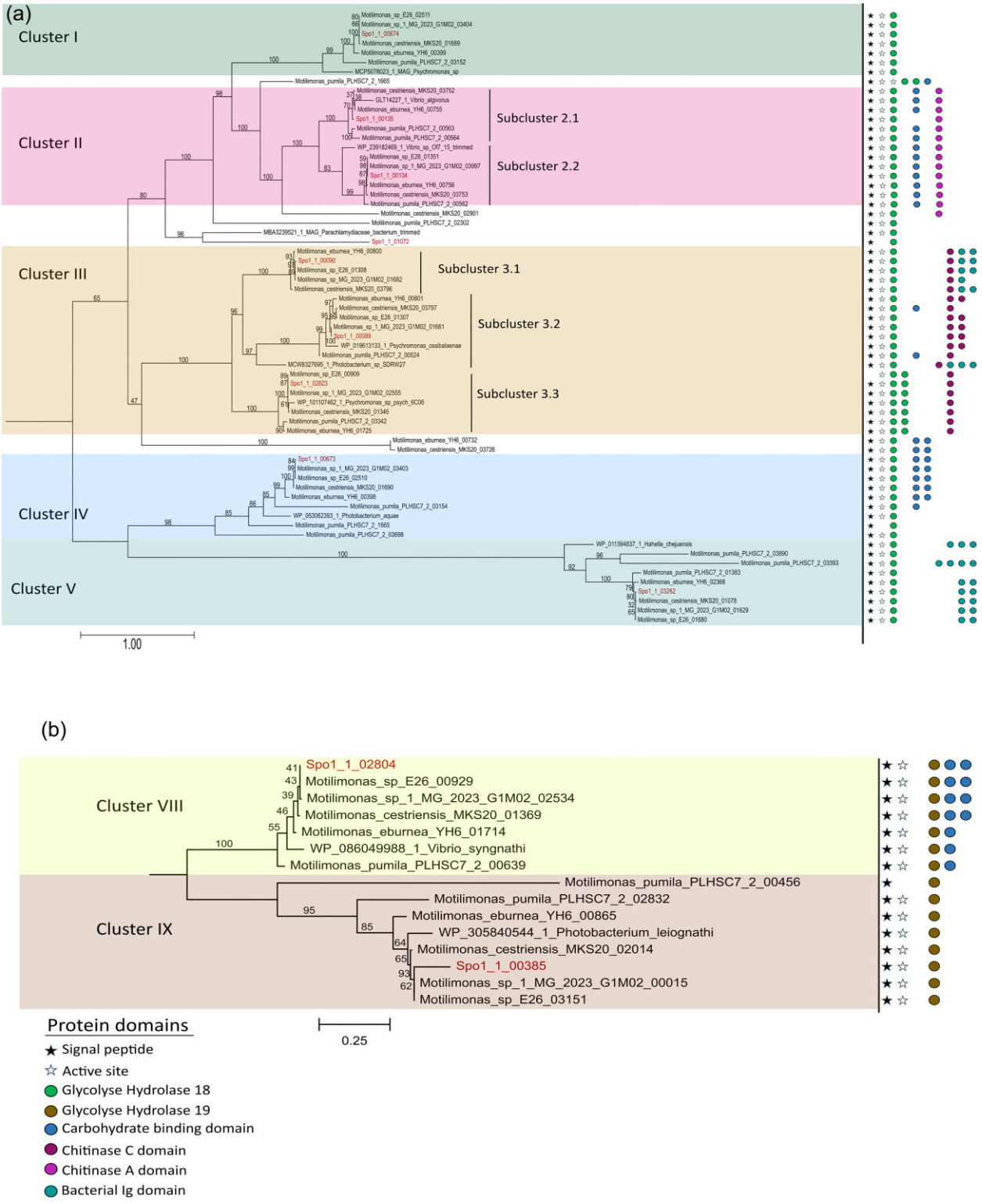

Figure 5. Phylogenetic inference based on all the GH18 (A) and GH19 (B) domains of the chitinase sequences in the *Motilimonas* genomes. The closest amino acid sequences found in NCBI for each of the 11 GH18- or GH19-containing chitinase sequences present in the Spo1_1 genome were added in the trees (following the same procedure). Numbers on branches of the phylogenetic trees are bootstrap support values inferred from 10.000 ultra-fast replicates. The scale bar represents the substitution rate across the tree. Each *Motilimonas* sequence was labeled as follows: name of the strain and number of the annotated protein in the egg-NOG mapper file (e.g. Motilimonas_cestriensis_MKS20_01 078). For the sequences taken from NCBI, the label of the sequence in the phylogenetic tree corresponded to the NCBI label of the sequence. Sequences labeled in red are those of isolate Spo1_1. Protein domains identified in the chitinase sequences are indicated by colored dots as identified in the legend below the tree. Black stars indicate the presence of a signal peptide and white stars the prediction of an active site.

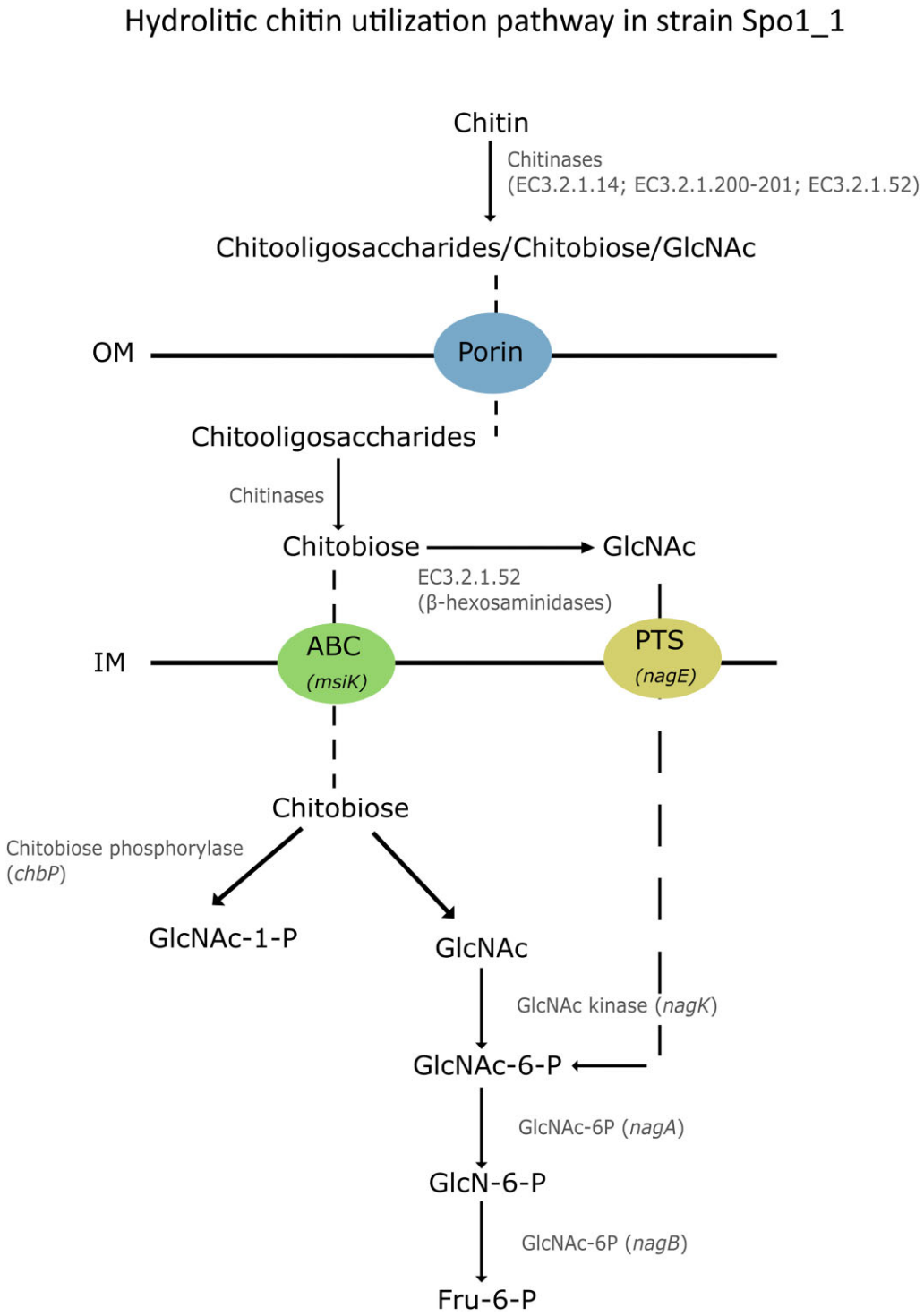

Figure 6. Scheme of the chitin utilisation pathway in strain Spo1_1. Chitinases (EC3.2.1.14, EC3.2.1.200–201 and EC3.2.1.52); GlcNAc, N-acetylglucosamine; ABC, ABC-sugar transporter, PTS, phosphotransferase system; GlcNAc-1-P, N-acetylglucosamine-1-phosphate; GlcNAc-6-P, N-acetylglucosamine-6-phosphate; GlcN-6-P, glucosamine-6-phosphate; fructose-6-P, fructose-6-phosphate. Solid arrows denote enzymatic reactions, and dotted arrows denote transport. Genes identified using BlastKOALA are presented in italics. EC numbers are those mentioned in this paper.

both genomes were 89.6% and 39.4%, i.e. below the thresholds to define a species (95% and 70%, respectively) according to (Stackebrandt et al. 2021). If the 16S rRNA gene similarity between two strains is above 98.7% but the DNA-DNA hybridisation is lower than 70%, they are two separate species within the same genus (Stackebrandt et al. 2021). Therefore, we propose to name isolate Spo1_1 "*Motilimonas chitinivorans*" in reference to its superior ability to degrade chitin.

Interestingly, we also isolated chitinolytic bacteria on a solid medium without chitin, MA (this step was, however, preceded by an enrichment step in a liquid medium containing chitin). Among those was an isolate affiliated to the genus *Pseudophaeobacter*, which was previously unknown for its

ability to degrade colloidal chitin. Since the use of different media compositions permitted the identification of novel (colloidal) chitin degraders, a possibility to increase the diversity of chitinolytic bacteria would be to use commercial or homemade media supplemented with colloidal chitin (Krithika and Chellaram 2016). To the best of our knowledge, the relevance of using an enrichment step in liquid medium prior to isolating chitin degraders has not been debated in the literature, but some studies did use it (Lau and Furusawa 2024; Liang et al. 2022), whereas others did not (Saima et al. 2013, Krithika and Chellaram 2016).

The two most abundant genera among the isolates producing a degradation halo on CC-agar were *Pseudoalteromonas* and *Vibrio*; these genera are well known to include chitin degraders (Techkarnjanaruk and Goodman 1999, Souza et al. 2011, Lin et al. 2018, Paulsen et al. 2019, Tran et al. 2022). However, the extent of colloidal chitin degradation (size of the halo) was highly isolate-dependent within those genera. Our results converge with the experimental and genomic study of Hunt et al. (2008), which showed that not all *Vibrionaceae* could grow on α or β-chitin, and the genomic studies of Paulsen et al. (2019) and Lin et al. (2018), which reported that in the genome of *Pseudoalteromonas* and *Vibrio* species, the number of genes coding for glycosyl hydrolases involved in chitin degradation is variable (Raimundo et al. 2021). Regarding the other taxa, *Bacillus* is a genus known for its chitin degradation capabilities (Alsalman et al. 2022, Tran et al. 2022), and one isolate belonging to the genus *Peribacillus* was reported to produce chitinases (Liang et al. 2024). The latter genus has been proposed in 2020 after a thorough genomic and phylogenetic study to resolve the polyphyly of the genus *Bacillus* (Patel and Gupta 2020). It is therefore possible that multiple "*Bacillus*" isolates studied in the past for chitin degradation are now part of the genus *Peribacillus* or another genus. Finally, the same concern can be raised for the genus *Paeniglutamicibacter* (Busse 2016) previously included in the genus *Arthrobacter*.

When yeast extract was not supplemented in CC-agar, we observed an overall significant decrease of the halos of colloidal chitin degradation. This result is in line with a previous study that showed that yeast extract can increase chitinase activity (Nampoothiri et al. 2004). The four chitinolytic isolates (*Pseudoalteromonas* SpoA_10, *Bacillus* Spo2_2, *Peribacillus* SedA_3, and *Arthrobacter* SpoA_5) that did not grow in the absence of yeast extract might be auxotrophic for compounds provided by the yeast extract powder. Other studies showed that strains isolated from marine sponges can degrade colloidal chitin *in vitro* and/or they reported that sponge metagenomes harbor a wide range of chitinase genes (Raimundo et al. 2021, da Silva et al. 2023). Altogether, these results suggest that the sponge microbiome contributes to nutrient cycling in its host through the process of chitin degradation (Raimundo et al. 2021, da Silva et al. 2023). Finally, among the isolates that did not degrade colloidal chitin on CC-agar, a majority could, however, grow on GlcNAc as sole C and N source. This result was expected as, in natural communities, the release of chitinous hydrolysis products (such as COS and GlcNAc) is not only used by the bacteria able to degrade the large chitin polymers but also by other microorganisms (called "exploiters" or "chitin derivative utilizers") able to use COS and/or GlcNAc, opening the possibilities of cross-feeding (Beier and Bertilsson 2013, Pollak et al. 2021). Moreover, some bacteria in natural communities do not use the hydrolysis products but instead rely on other secreted compounds like fermentation products (e.g. acetate) released by other bacteria of the community. They are called "scavengers" (Pontrelli et al. 2022), and this could explain why some of our isolates did not even grow on GlcNAc.

Next, we wanted to assess the chitinolytic activity of positive isolates on CC-agar when they are challenged with insoluble chitin powder in liquid cultures (for 7 days at 20°C and 85 rpm), It is important to note that those experimental conditions were not optimised for enhanced chitin degradation. For example, the temperature chosen is close to the one observed in coastal water at Audresselles, France, in summer (https://seatemperature.info/fr/audresselles-temperature-de-leau-de-la-mer.html). It is therefore very likely that the percentage of degradation could be higher in optimal conditions of temperature, pH and/or agitation. The ability of environmental isolates to degrade insoluble, powdered chitin in liquid culture has only been investigated in a very limited number of studies; indeed, most studies rely on colloidal chitin instead. Hoang et al. (2011) reported that *Streptomyces* sp. TH-11 (isolated from the sediment of a river) could degrade 32% of insoluble chitin powder after 30 days at 30°C and under vigorous agitation. To calculate the percentage of degradation, the authors measured the dry weight of remaining chitin after incubation. In a more recent study, Lau and Furusawa (2024) isolated a strain belonging to the family *Cellvibrionaceae* from muddy sediments of a mangrove, KSP S5-2, that could degrade 90% of insoluble chitin powder after 10 days at 30°C and under vigorous agitation. They measured the remaining chitin by treating it first with NaOH (2.5 N) and HCl (1.7 N) and then weighing the dry weight. Depending on the study, the method used to measure the weight of the remaining chitin varies, which complicates comparisons across studies. Furthermore, in those two studies, the conditions of incubation were different than in ours (higher temperatures and higher agitation). Other studies investigated the capability of isolates to degrade insoluble chitin powder but measured the chitinase activity and not the % of degradation (Ren et al. 2022). All in all, the isolates of our collection degraded poorly insoluble chitin powder except *Motilimonas* spo1_1 (37% of degradation). Yet, among those, many belonged to genera widely reported in the literature as chitin degraders (*Vibrio*, *Pseudoalteromonas*, and *Bacillus*).

The type of chitin substrate (colloidal, acid swollen, insoluble powdered, chitin flakes) is known to change the number of enzymes produced by bacteria and their chitinase activity (Svitil et al. 1997). Moreover, the efficiency of each enzyme depends on the type of chitin (Svitil et al. 1997, Nampoothiri et al. 2004, Mehmood et al. 2009, Ren et al. 2022, Zhang et al. 2018). Our data illustrate that the efficiency of degradation of a chitinous substrate depends on its nature, as the ability of many isolates to degrade colloidal chitin did not imply an ability to degrade insoluble, powdered chitin.

Due to its superior ability to degrade insoluble powdered and colloidal chitin, we decided to explore the genetic basis for chitin degradation of *Motilimonas* isolate Spo1_1. We sequenced its genome and compared it with that of all other *Motilimonas* genomes available on NCBI. Based on the CAZy database, the different functions necessary to degrade chitin (endo- and exo-chitinase) can be found in multiple glycosyl hydrolase families. The "endochitinase" activity (EC 3.2.1.14) can be found in GH18 and GH19 families and, the "exochitinase" activity (liberating dimeric units

of N-acetyl-glucosamine (EC 3.2.1.200/201) or monomeric ones (EC 3.2.1.52)) can be mostly found in the GH18, GH19, GH20 families (the CAZy database, 2024). Since we did not test the activity of the putative enzymes encoded in the *Motilimonas* genomes, they are all referred to as "chitinase" and not endo- or exo-chitinase. The analysis of the six *Motilimonas* genomes revealed 9–16 sequences coding for putative GH18 and GH19 chitinases. These numbers are in the upper range of those reported in the literature. By way of comparison, three sequences were found in the genome of *Serratia marcescens* (Vaaje-Kolstad et al. 2013); a mean of 5.2 and 2.1 GH18 chitinase genes in the genomes of pigmented and non-pigment strains of *Pseudoalteromonas* spp. (Paulsen et al. 2019); 13 sequences in the genome of the chitinolytic bacterium *Streptomyces coelicolor* (Kawase et al. 2006), and 2–14 sequences in the genomes of five *Aquimarina* strains (Raimundo et al. 2021). Finally, 27 sequences of putative chitinases were identified in the genome of the *Cellvibrionaceae* isolate KSP S5-2 (Lau and Furusawa 2024) and 30 in that of *Chitinolyticbacter meiyuanensis* SYBC-H1 (A. Zhang et al. 2020). It should be noted however that the bioinformatic analysis to identify the sequences coding for chitinases was not identical across the different studies cited above. Based on our analysis of the six *Motilimonas* genomes, chitin degradation is a trait linked to the genus rather than the species or the isolate, but the isolation of more *Motilimonas* strains will be necessary to confirm or invalidate this hypothesis.

Considering the dominance of the GH18 and GH19 domain sequences in the chitinolytic repertoire of *Motilimonas* strains, we then decided to delve deeper into their diversity. All the putative chitinase sequences found within a genome were different, and each *Motilimonas* genome harbored a similar diversity of chitinase sequences. The presence of multiple different chitinase sequences encoded in different operons likely provides several advantages. First, different chitinases (ChiA, ChiB, ChiC, ChiD, …) are known to have a synergistic effect on the degradation of chitin (Orikoshi et al. 2005). Second, it could allow the strains to degrade different forms of chitin and/or under different physical-chemical conditions (Svitil et al. 1997, Orikoshi et al. 2005). All those sequences except one presented a signal peptide, indicating that the chitinases are secreted outside the cytoplasm. Many of them likely interact with chitin, thanks to their CBDs, which have been detected in their sequence and are known to contribute to the degradation of polysaccharides by bringing the enzyme into contact with the substrate (Boraston et al. 2004).

All in all, the hydrolytic chitin utilisation pathway in *Motilimonas* Spo1_1 (*Psychromonadaceae*) closely resembles that of bacteria in the *Vibrionaceae* family described by Jiang et al. (2022). Plus, the presence of a gene coding for a putative enzyme with an LPMO domain in all genomes analysed suggests that chitin degradation is boosted via the oxidative cleavage of glycosidic bonds, as reported by Vaaje-Kolstad et al. (2005), Hemsworth et al. (2015), and Hamre et al. (2015). However, we did not identify any downstream step in that pathway similar to those described for the strains KSP-S5-2 (*Cellvibrionaceae*) (Lau and Furusawa 2024) and *Pseudoalteromonas prydzensis* ACAM 620 (*Pseudoalteromonadaceae*) (Jiang et al. 2022).

This work opens up prospects for the study of the physiology of *Motilimonas* spp. Given that *Motilimonas* Spo1_1 degraded 37% of insoluble powdered chitin in mild conditions in 7 days, it would be worthwhile to optimise the incubation conditions to enhance its chitin degradation ability and to investigate the chitinolytic activity of the other *Motilimonas* strains. Their efficiency toward raw materials (e.g. crushed crustacean shells) should also be tested, with the aim of using them to mineralise chitinous waste, or to produce valuable chito-oligosaccharides from this waste. Finally, characterising and exploiting the chitinases of those strains is another avenue of research. To achieve this goal, chitinase genes could be cloned into *E. coli* for heterologous expression or extracted from the supernatant of *Motilimonas* cultures, and purified by chromatography.

Ethical statement

No ethical approval was needed for this paper (no human or animal materials were used).

Author contributions

Etienne Dechamps (Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft), Arthur Salengros (Methodology, Writing – review & editing), Laurence Meunier (Writing – review & editing), Séverine Chevalier (Methodology), Johan Danguy (Methodology), Sophie-Luise Heidig (Methodology, Software, Writing – review & editing), Jean-François Flot (Software, Writing – review & editing), Tina Keller-Costa (Writing – review & editing), Rodrigo Costa (Writing – review & editing), and Isabelle F. George (Conceptualization, Resources, Supervision, Validation, Writing – review & editing).

Supplementary data

Supplementary data is available at *JAMBIO Journal* online.

Conflict of interest: None declared.

Funding

This work was supported by a PhD grant to E. Dechamps from the Fonds de la Recherche dans l'Industrie et l'Agriculture (FRIA) at the Fonds de la Recherche Scientifique (F.R.S-FNRS).

Data Availability Statement

The 16S rRNA fragments are deposited in the NCBI database at accession numbers PQ351673-PQ351743. The genome sequence of isolate Spo1_1 can be accessed at BioSample SAMN43845440 in the NCBI database.

References

Alldredge AL, Gotschalk CC. the relative contribution of marine snow of different origins to biological processes in coastal waters. *Cont Shelf Res* 1990;**10**:41–58. https://doi.org/10.1016/0278-4343(90)90034-J

Alsalman A J, Farid A, Al Mohaini M. *et al*., Chitinase activity by chitin degrading strain (*Bacillus salmalaya*) in shrimp waste. *IJCRR* 2022;**14**:11–17. https://doi.org/10.31782/IJCRR.2022.141107.

Beier S, Bertilsson S. Bacterial chitin degradation-mechanisms and ecophysiological strategies. *Front Microbiol* 2013;**4**: 149https://doi.org/10.3389/fmicb.2013.00149

Beygmoradi A, Homaei A, Hemmati R. *et al*. Marine chitinolytic enzymes, a biotechnological treasure hidden in the ocean? *Appl Microbiol Biotechnol* 2018;**102**:9937–48. https://doi.org/10.1007/s00253-018-9385-7.

Bonal M, Goetghebuer L, Joseph C. *et al*. Deciphering interactions within a 4-strain riverine bacterial community. *Curr Microbiol* 2023;**80**: 238https://doi.org/10.1007/s00284-023-03342-9

Boraston AB, Bolam DN, Gilbert HJ. *et al*. Carbohydrate-binding modules: fine-tuning polysaccharide recognition. *Biochem J* 2004;**382**:769–81. https://doi.org/10.1042/BJ20040892

Busse HJ. Review of the taxonomy of the genus Arthrobacter, emendation of the genus *arthrobacter* sensu lato, proposal to reclassify selected species of the genus Arthrobacter in the novel genera *Glutamicibacter* gen. Nov., *Paeniglutamicibacter* gen. nov., *Pseudoglutamicibacter* gen. nov., *Paenarthrobacter* gen. nov. and *pseudarthrobacter* gen. nov., and emended description of *Arthrobacter* roseus. *Int J Syst Evol Microbiol* 2016;**66**:9–37.

Cantalapiedra CP, Hernąndez-Plaza A, Letunic I. *et al*. eggNOG-mapper v2: functional annotation, orthology assignments, and domain prediction at the metagenomic scale. *Mol Biol Evol* 2021;**38**:5825–9. https://doi.org/10.1093/molbev/msab293.

Crauwels C, Heidig S-L, Díaz A. *et al*. Large-scale structure-informed multiple sequence alignment of proteins with SIMSApiper. *Bioinformatics* 2024;**40**(5):btae276https://doi.org/10.1093/bioinformatics/btae276

da Silva DMG, Pedrosa FR, Ângela Taipa M. *et al*. Widespread occurrence of chitinase-encoding genes suggests the *Endozoicomonadaceae* family as a key player in chitin processing in the marine benthos. *ISME Commun* 2023;**3**. https://doi.org/10.1038/s43705-023-00316-7

Dhole NP, Dar MA, Pandit RS. Recent advances in the bioprospection and applications of chitinolytic bacteria for valorization of waste chitin. *Arch Microbiol* 2021;**203**:1953–69. https://doi.org/10.1007/s00203-021-02234-5.

Elieh-Ali-Komi D, Hamblin MR, Daniel E-A-K. Chitin and Chitosan: production and application of versatile biomedical nanomaterials HHS public access. *Int J Adv Res (Indore)* 2016;**4**:411–27.

Esteves AIS, Amer N, Nguyen M. *et al*. Sample processing impacts the viability and cultivability of the sponge microbiome. *Front Microbiol* 2016;**7**:499https://doi.org/10.3389/fmicb.2016.00499

Gohel V, Singh A, Vimal M. *et al*. Bioprospecting and antifungal potential of chitinolytic microorganisms. *Afr J Biotechnol* 2006;**5**:54–72. http://www.academicjournals.org/AJB

Gooday GW. The ecology of chitin degradation. In: *Advances in Microbial Ecology*. 1990;**10**:387–430.https://link.springer.com/chapter/10.1007/978-1-4684-7612-5_10

Gortari MC, Hours RA. Biotechnological processes for chitin recovery out of crustacean waste: a mini-review. *Electron J Biotechnol* 2013;**16**: 1–14https://doi.org/10.2225/vol16-issue3-fulltext-10

Hameş-Kocabaş EE, Uzel A. Isolation strategies of marine-derived actinomycetes from sponge and sediment samples. *J Microbiol Methods* 2012;**88**:342–7. https://doi.org/10.1016/j.mimet.2012.01.010.

Hamre AG, Eide KB, Wold HH. *et al*. Activation of enzymatic chitin degradation by a lytic polysaccharide monooxygenase. *Carbohydr Res* 2015;**407**:166–9. https://doi.org/10.1016/j.carres.2015.02.010.

Hemsworth GR, Johnston EM, Davies GJ. *et al*. Lytic polysaccharide monooxygenases in biomass conversion. *Trends Biotechnol* 2015;**33**:747–61. https://doi.org/10.1016/j.tibtech.2015.09.006.

Hoang KC, Lai TH, Lin CS. *et al*. The chitinolytic activities of *Streptomyces* sp. TH-11. *Int J Mol Sci* 2011;**12**:56–65. https://doi.org/10.3390/ijms12010056.

Hou F, Gong Z, Jia F. *et al*. Insights into the relationships of modifying methods, structure, functional properties and applications of chitin: a review. *Food Chem* 2023;**409**: 135336https://doi.org/10.1016/j.foodchem.2022.135336

Hsu SC, Lockwood JL. Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. *Appl Microbiol* 1975;**29**:422–6. https://doi.org/10.1128/am.29.3.422-426.1975

Hunt DE, Gevers D, Vahora NM. *et al*. Conservation of the chitin utilization pathway in the Vibrionaceae. *Appl Environ Microb* 2008;**74**:44–51. https://doi.org/10.1128/AEM.01412-07.

Jiang, WX., Li, PY., Chen, XL. *et al*.. A pathway for chitin oxidation in marine bacteria. *Nat Commun* 2022; **13**: 5899.https://doi.org/10.1038/s41467-022-33566-5

Kalyaanamoorthy S, Minh BQ, Wong TKF. *et al*. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat Methods* 2017;**14**:587–9. https://doi.org/10.1038/nmeth.4285.

Kanehisa M, Sato Y, Morishima K. BlastKOALA and GhostKOALA: KEGG tools for functional characterization of genome and metagenome sequences. *J Mol Biol* 2016;**428**:726–31. https://doi.org/10.1016/j.jmb.2015.11.006.

Kawase T, Yokokawa S, Saito A. *et al*. Comparison of enzymatic and antifungal properties between family 18 and 19 chitinases from *S. coelicolor* A3(2). *Biosci Biotechnol Biochem* 2006;**70**:988–98. https://doi.org/10.1271/bbb.70.988

Kaya M, Mujtaba M, Ehrlich H. *et al*. On chemistry of γ-chitin. *Carbohydr Polym* 2017;**176**:177–86. https://doi.org/10.1016/j.carbpol.2017.08.076.

Kelbrick M, Abed RMM, Antunes A. *Motilimonas cestriensis* sp. nov., isolated from an inland brine spring in Northern England. *Int J Syst Evol Microbiol* 2021;**71**(3): 004763https://doi.org/10.1099/ijsem.0.004763

Keyhani NO, Roseman S. Physiological aspects of chitin catabolism in marine bacteria 1. *Biochim Biophys Acta* 1999;**1473**:108–22. https://doi.org/10.1016/S0304-4165(99)00172-5

Kielak AM, Cretoiu MS, Semenov AV. *et al*. Bacterial chitinolytic communities respond to chitin and pH alteration in soil. *Appl Environ Microb* 2013;**79**:263–72. https://doi.org/10.1128/AEM.02546-12.

Kolmogorov M, Yuan J, Lin Y. *et al*. Assembly of long, error-prone reads using repeat graphs. *Nat Biotechnol* 2019;**37**:540–6. https://doi.org/10.1038/s41587-019-0072-8.

Krithika S, Chellaram C. Isolation, screening, and characterization of chitinase producing bacteria from marine wastes, *Int J of Pharmacy and Applied Pharmaceutical Sciences* 2016.**8**(**5**) 34–36

Kumari R, Kumar M, Vivekanand V. *et al*. Chitin biorefinery: a narrative and prophecy of crustacean shell waste sustainable transformation into bioactives and renewable energy. *Renew Sust Energy Rev* 2023;**184**: 113595https://doi.org/10.1016/j.rser.2023.113595

Lau N-S, Furusawa G. Polysaccharide degradation in *Cellvibrionaceae*: genomic insights of the novel chitin-degrading marine bacterium, strain KSP-S5–2, and its chitinolytic activity. *Sci Total Environ* 2024;**912**:169134. https://doi.org/10.1016/j.scitotenv.2023.169134.

Lavy A, Keren R, Yahel G. *et al*. Intermittent hypoxia and prolonged suboxia measured in situ in a marine sponge. *Front Mar Sci* 2016;**3**:263https://doi.org/10.3389/fmars.2016.00263

Liang C, Yang D, Dong F. *et al*. Biocontrol potential of bacteria isolated from vermicompost against *Meloidogyne incognita* on tomato and cucumber crops. *Horticulturae* 2024;**10**: 407https://doi.org/10.3390/horticulturae10040407

Liang YY, Yan LQ, Tan MH. *et al*. Isolation, characterization, and genome sequencing of a novel chitin deacetylase producing *Bacillus aryabhattai* TCI-16. *Front Microbiol* 2022;**13**: 999639https://doi.org/10.3389/fmicb.2022.999639

Lin H, Yu M, Wang X. *et al*. Comparative genomic analysis reveals the evolution and environmental adaptation strategies of vibrios. *BMC Genom [Electronic Resource]* 2018;**19**: 135https://doi.org/10.1186/s12864-018-4531-2

Lin Z, Akin H, Rao R. *et al*. Evolutionary-scale prediction of atomic-level protein structure with a language model. *Science* 2023;**379**:1123–30. https://doi.org/10.1126/science.ade2574

Ling SK, Guo LY, Chen GJ. *et al*. *Motilimonas eburnea* gen. Nov., sp. nov., isolated from coastal sediment. *Int J Syst Evol Microbiol* 2017;**67**:306–10. https://doi.org/10.1099/ijsem.0.001621.

Ma Z, Tong J, Wang Y. *et al*. Isolation and characterization of a thermostable alkaline chitinase-producing Aeromonas strain and its potential in biodegradation of shrimp shell wastes. *Rom Biotechnol*

Lett 2021;**26**:2511–22. https://doi.org/10.25083/rbl/26.2/2511.2522.
Madeira F, Pearce M, Tivey ARN. *et al*. Search and sequence analysis tools services from EMBL-EBI in 2022. *Nucleic Acids Res* 2022;**50**:W276–9. https://doi.org/10.1093/nar/gkac240.
Manni M, Berkeley MR, Seppey M. *et al*. BUSCO update: novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. *Mol Biol Evol* 2021;**38**:4647–54. https://doi.org/10.1093/molbev/msab199.
Mapleson D, Accinelli GG, Kettleborough G. *et al*. KAT: a K-mer analysis toolkit to quality control NGS datasets and genome assemblies. *Bioinformatics* 2017;**33**:574–6. https://doi.org/10.1093/bioinformatics/btw663.
Mathew GM, Sukumaran RK, Sindhu R. *et al*. Green remediation of the potential hazardous shellfish wastes generated from the processing industries and their bioprospecting. *Environ Technol Innov* 2021;**24**: 101979https://doi.org/10.1016/j.eti.2021.101979
Mehmood MA, Xiao X, Hafeez FY. *et al*. Purification and characterization of a chitinase from *Serratia proteamaculans*. *World J Microbiol Biotechnol* 2009;**25**:1955–61. https://doi.org/10.1007/s11274-009-0094-3.
Meier-Kolthoff JP, Auch AF, Klenk H-P. *et al*. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinf* 2013;**14**:60. https://doi.org/10.1186/1471-2105-14-60
Meunier L, Costa R, Keller-Costa T *et al*.. Selection of marine bacterial consortia efficient at degrading chitin leads to the discovery of new potential chitin degraders. *Microbiol Spectr* 2024;**12**: e00886–24https://doi.org/10.1128/spectrum.00886-24
Minh BQ, Schmidt HA, Chernomor O. *et al*. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol Biol Evol* 2020;**37**:1530–4. https://doi.org/10.1093/molbev/msaa015.
Nampoothiri KM, Baiju TV, Sandhya C. *et al*. Process optimization for antifungal chitinase production by *Trichoderma harzianum*. *Process Biochem* 2004;**39**:1583–90. https://doi.org/10.1016/S0032-9592(03)00282-6.
Oliva G, Fontesit MR, Garratt RC. *et al*. Structure and catalytic mechanism of glucosamine 6-phosphate deaminase from *Escherichia coli* at 2.1 A resolution. *Structure* 1995;**3**:1323–32. https://doi.org/10.1016/S0969-2126(01)00270-2
Orikoshi H, Nakayama S, Miyamoto K. *et al*. Roles of four chitinases (ChiA, ChiB, ChiC, and ChiD) in the chitin degradation system of marine bacterium Alteromonas sp. strain O-7. *Appl Environ Microb* 2005;**71**:1811–5. https://doi.org/10.1128/AEM.71.4.1811-1815.2005.
Patel S, Gupta RS. A phylogenomic and comparative genomic framework for resolving the polyphyly of the genus Bacillus: proposal for six new genera of Bacillus species, Peribacillus gen. nov., Cytobacillus gen. nov., Mesobacillus gen. nov., Neobacillus gen. nov., Metabacillus gen. nov. and Alkalihalobacillus gen. nov. *Int J Syst Evol Microbiol* 2020;**70**:406–38.
Paulsen SS, Strube ML, Bech PK. *et al*. Marine chitinolytic pseudoalteromonas represents an untapped reservoir of bioactive potential. *Appl Environ Sci* 2019;**4**: e00060–19
Pollak S, Gralka M, Sato Y. *et al*. Public good exploitation in natural bacterioplankton communities. *Sci Adv* 2021;**7**:4717–45. https://doi.org/10.1126/sciadv.abi4717
Pontrelli S, Szabo R, Pollak S. *et al*. Metabolic cross-feeding structures the assembly of polysaccharide degrading communities. *Sci Adv* 2022;**8**:3076. https://doi.org/10.1126/sciadv.abk3076
Quast C, Pruesse E, Yilmaz P. *et al*. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2013;**41**: D590–D596 https://doi.org/10.1093/nar/gks1219
Raimundo I, Silva R, Meunier L. *et al*. Functional metagenomics reveals differential chitin degradation and utilization features across free-living and host-associated marine microbiomes. *Microbiome* 2021;**9**: 43https://doi.org/10.1186/s40168-020-00970-2
Rathore AS, Gupta RD.. Chitinases from bacteria to human: properties, applications, and future perspectives. *Enzyme Res* 2015;**2015**: 791907https://doi.org/10.1155/2015/791907
Ren XB, Dang YR, Liu SS. *et al*. Identification and characterization of three chitinases with potential in direct conversion of crystalline chitin into N,N′-diacetylchitobiose. *Mar Drugs* 2022;**20**: 165https://doi.org/10.3390/md20030165
Rinaudo M. Chitin and chitosan: properties and applications. *Prog Polym Sci* 2006;**31**:603–32. https://doi.org/10.1016/j.progpolymsci.2006.06.001.
Roy JC, Salaün F, Giraud S. *et al*. Solubility of chitin: solvents, solution behaviors and their related mechanisms. In: *Solubility of Polysaccharides*. Xu Zhenbo , London:InTech, 2017. https://doi.org/10.5772/66033
Saima K, M. R, Ahmad IZ. Isolation of novel chitinolytic bacteria and production optimization of extracellular chitinase. *J Genetic Eng Biotechnol* 2013;**11**:39–46. https://doi.org/10.1016/j.jgeb.2013.03.001.
Sakai K, Yokota A, Kurokawa H. *et al*. Purification and characterization of three thermostable endochitinases of a noble *Bacillus* strain, MH-1, isolated from chitin-containing compost. *Appl Environ Microb* 1998;**64**:3397–402. https://doi.org/10.1128/AEM.64.9.3397-3402.1998
Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 2014;**30**:2068–9. https://doi.org/10.1093/bioinformatics/btu153.
Sipkema D, Schippers K, Maalcke WJ. *et al*. Multiple approaches to enhance the cultivability of bacteria associated with the marine sponge *Haliclona (gellius)* sp. *Appl Environ Microb* 2011;**77**:2130–40. https://doi.org/10.1128/AEM.01203-10.
Souza CP, Almeida BC, Colwell RR. *et al*. The importance of chitin in the marine environment. *Mar Biotechnol* 2011;**13**:823–30. https://doi.org/10.1007/s10126-011-9388-1.
Stackebrandt E, Mondotte JA, Fazio LL. *et al*. Authors need to be prudent when assigning names to microbial isolates. *Curr Microbiol* 2021;**78**:4005–8. https://doi.org/10.1007/s00284-021-02678-4.
Svitil AL, Ad S, Ní chadhain M. *et al*. Chitin degradation proteins produced by the marine bacterium *Vibrio harveyi* growing on different forms of chitin. *Appl Environ Microb* 1997;**63**:408–13. https://doi.org/10.1128/aem.63.2.408-413.1997
Taboada B, Estrada K, Ciria R. *et al*. Operon-mapper: a web server for precise operon identification in bacterial and archaeal genomes. *Bioinformatics* 2018;**34**:4118–20. https://doi.org/10.1093/bioinformatics/bty496.
Techkarnjanaruk S, Goodman AE. Multiple genes involved in chitin degradation from the marine bacterium *Pseudoalteromonas* sp. strain S91. *Microbiology* 1999;**145**:925–34. https://doi.org/10.1099/13500872-145-4-925
Thomas T, Moitinho-Silva L, Lurgi M. *et al*. Diversity, structure and convergent evolution of the global sponge microbiome. *Nat Commun* 2016;**7**: 11870https://doi.org/10.1038/ncomms11870
Tran DM, Huynh TU, Nguyen TH. *et al*. Molecular analysis of genes involved in chitin degradation from the chitinolytic bacterium Bacillus velezensis. *Antonie Van Leeuwenhoek* 2022;**115**:215–31. https://doi.org/10.1007/s10482-021-01697-2.
Vaaje-Kolstad G, Horn SJ, Sørlie M. *et al*. The chitinolytic machinery of *Serratia marcescens*—a model system for enzymatic degradation of recalcitrant polysaccharides. *FEBS J* 2013;**280**:3028–49.https://doi.org/10.1111/febs.12181.
Vaaje-Kolstad G, Horn SJ, Van Aalten DMF. *et al*. The non-catalytic chitin-binding protein CBP21 from *Serratia marcescens* is essential for chitin degradation. *J Biol Chem* 2005;**280**:28492–7.https://doi.org/10.1074/jbc.M504468200.
Wang FQ, Ren LH, Lin YW. *et al*. *Motilimonas pumila* sp. Nov., isolated from the gut of sea cucumber *Apostichopus japonicus*. *Int J Syst Evol Microbiol* 2019;**69**:811–5. https://doi.org/10.1099/ijsem.0.003242.

Weisburg WG, Barns SM, Pelletier DA. *et al*. 6S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 1991;**173**:697–703. https://doi.org/10.1128/jb.173.2.697-703.1991

Wick RR, Schultz MB, Zobel J. *et al*. Bandage: interactive visualization of de novo genome assemblies. *Bioinformatics* 2015;**31**:3350–2. https://doi.org/10.1093/bioinformatics/btv383.

Xu Y, Bajaj M, Schneider R. *et al*. Transformation of the matrix structure of shrimp shells during bacterial deproteination and demineralization. *Microb Cell Fact* 2013;**12**: 90https://doi.org/10.1186/1475-2859-12-90

Yurgel SN, Nadeem M, Cheema M. Microbial consortium associated with crustacean shells composting. *Microorganisms* 2022;**10**:1033https://doi.org/10.3390/microorganisms10051033

Zhang A, Mo X, Zhou N. *et al*. Identification of chitinolytic enzymes in *Chitinolyticbacter meiyuanensis* and mechanism of efficiently hydrolyzing chitin to N-acetyl glucosamine. *Front Microbiol* 2020;**11**: 572053https://doi.org/10.3389/fmicb.2020.572053

Zhang A, Wei G, Mo X. *et al*. Enzymatic hydrolysis of chitin pretreated by bacterial fermentation to obtain pure: n-acetyl-d-glucosamine. *Green Chem* 2018;**20**:2320–7. https://doi.org/10.1039/C8GC00265G.

Zhang M, Haga A, Sekiguchi H. *et al*. Structure of insect chitin isolated from beetle larva cuticle and silkworm *(Bombyx mori) pupa exuvia*. *Int J Biol Macromol* 2000;**27**:101–5. https://doi.org/10.1016/S0141-8130(99)00123-3

Zobell CE, Rittenberg SC. The occurrence and characteristics of chitinoclastic bacteria in the sea [Contributions from the Scripps Institution of Oceanography New Series]. *J Bacteriol* 1937;**35**:275–87. https://doi.org/10.1128/jb.35.3.275-287.1938

Received 30 October 2024; **revised** 26 June 2025; **accepted** 22 July 2025