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Xylooligosaccharides production using multi-substrate specific xylanases secreted by a psychrotolerant *Paenibacillus* sp. PCH8

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ABSTRACT

Xylanases are industrial enzymes with multiple applications in the food, pharmaceuticals, bio-bleaching, and textiles industries. The present study explores a putative novel bacterium *Paenibacillus* sp. PCH8 shows xylanolytic activity from Himalayan glacial soil. Genome sequencing and analysis revealed multiple genes encoding xylanases, cellulases, and other lignocellulolytic enzymes. The bacterium utilized oat spelt xylan substrate and showed xylanolytic activity in wide pH (4.0 – 12.0) and temperature (4 to 90 °C). Proteomic analysis revealed 1,4-β-xylanase, arabinan endo-1,5-α-L-arabinosidase, and 11 hypothetical proteins in partially purified protein fraction. Multi-substrate enzymatic activity (IU/mg) was observed for beechwood (21.42), oat spelt xylan (19.8), CMC (5.17), avicel (7.7), and starch (1.62) in protein fraction. The hydrolysis of xylan led to the formation of xylose, xylobiose, xylotriose, and xylotetraose upon analysis by LC-MS. The xylooligosaccharides (XOS) containing hydrolysate enhanced the growth of probiotic microbes, suggesting prebiotic potential. Thus, the study provides a new source of xylanases from *Paenibacillus* sp. PCH8, with potential applications in lignocellulosic biomass hydrolysis and XOS production.

1. Introduction

Hemicellulose is a heterogeneous matrix of polysaccharides consisting of xylan, arabinan, mannan, and galactan (Saha, 2003; Kumar, Binod, Sindhu, Gnansounou, & Ahluwalia, 2018). Xylan is the predominant component of hemicelluloses with β,1–4 linkage backbone between xylose residues or other substituted residues like arabinose, acetyl, glucuronic acid, and 4-O-methylglucuronic acid (Scheller & Ulvskov, 2010; Rennie & Scheller, 2014). The polymerization and diversity of substitution are major hurdles in depolymerizing the xylan. Additionally, hydrogen bonding of xylans with a hydrophilic surface on the cellulose microfibrils interferes with the action of hydrolytic enzymes (Simmons, 2016).

Xylanases, an enzyme group comprised of endo-1,4-β-D-xylanases (EC 3.2.1.8), α−1-arabinofuranosidases (EC 3.2. 1.99), β-D-xylosidases (EC 3.2.1.37), and α-glucuronidases (EC 3.2.1.139), perform endohydrolysis of xylan (Bhardwaj, Kumar, & Verma, 2019). The hydrolytic action of xylanases on hemicelluloses produces xylooligosaccharides (XOS) of 2–10 units (Cano et al., 2020; Nieto-Domínguez, 2017; Peralta, Venkatachalam, Stone, & Pattathil, 2017). The emerging applications of XOS as prebiotics in food and pharmaceuticals have attracted researchers' worldwide (Mhetras, Mapre, & Gokhale, 2019; Rashid & Sohail, 2021). Prebiotics are indigestible carbohydrate oligomers that selectively enhance the growth of beneficial gut bacteria of the host (Nieto-Domínguez, 2017) and offer several health benefits (Aachary, Gobinath, Srinivasan, & Prapulla, 2015; Singh, Banerjee, & Arora, 2015; Yang, 2015).

The production of XOS can be carried out through physical (autohydrolysis), chemical (acid/alkali), and biological (enzymes) methods (Brienzo, Siqueira, & Milagres, 2009; Chapla, Pandit, & Shah, 2012; Palaniappan, Antony, & Emmambux, 2021; Tan et al., 2008). The physical and chemical-based bioprocesses generate undesirable side products and require specialized equipment. On the contrary, the xylanolytic enzyme-based approaches are product-specific (Chapla et al., 2012). Besides XOS production, xylanases play a critical role in biorefinery, textiles, paper, pulp, and food industry (Kuhad and Singh, 1993; Woldesenbet, Virk, Gupta & Sharma, 2012; Singh et al., 2020; Wang, Liang, Li, Tian, & Wei, 2021). Considering the broad applications of microbial xylanases across several industries, novel producers of robust and multi-substrate-specific xylanases are the need of the hour.

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Several bacterial genera like *Bacillus, Streptomyces, Arthrobacter, Microbacterium, Pseudomonas, Anoxybacillus,* and *Paenibacillus* have been reported for the production of xylanolytic enzymes (Rättö et al., 1992; Kim, Park, Park, Kim, & Lee, 2005; Xu, Bai, Xu, Shi, & Tao, 2005; Khandeparkar and Bhosle, 2007; Dheeran, Sachin, Jaiswal, & Adhikari, 2012; Meryandini, 2007; Yadav et al., 2018; Dutta et al., 2020). The members of the genus *Paenibacillus* were isolated from various environments like Alaskan tundra, Kafni glacier, selenium mines, and rhizosphere (Khan, 2020; Kim, 2009; Kishore, Begum, Pathan, & Shivaji, 2010; Lee, 2013; Nelson, Glawe, Labeda, Cann, & Mackie, 2009; Yao et al., 2014). *Paenibacilli* are well characterized for the production of hydrolytic enzymes, XOS, plant growth-promoting attributes, biofertilizers, biopesticides, exopolysaccharide, and antimicrobials (Grady, MacDonald, Liu, Richman, & Yuan, 2016; Liu et al., 2018; Liyaskina et al., 2021; Lorentz, Ártico, Da Silveira, Einsfeld, & Corção, 2006; Priest, 2015; Raza, Yang, & Shen, 2008; Xie et al., 2016). With the help of advanced biotechnological techniques such as genome sequencing, several attempts have been made to identify potential lignocellulose depolymerizing enzymes, including xylanases. The understanding of the genomic structure of the microbe and the genes further assists in rapid characterizing the mode of action of the xylanases. Realizing the potential, a putative novel Himalayan *Paenibacillus* sp. PCH8 showing xylanase activity was explored. The study performed genome sequencing and mined genes encoding lignocellulolytic enzymes including xylanases. Proteomic and enzymatic characterization was carried out for secreted proteins showing xylanase activity. Further, concentrated and partially purified protein fraction was employed for xylan hydrolysis and XOS production.

1.1. Hypothesis

The Himalayan niches provide a plethora of bioresources and biomolecules such as lignocellulolytic enzymes with unique properties and diverse applications.

2. Materials and methods

2.1. Materials

The whole-genome sequencing kits were procured from Pacific Biosciences, California, Inc., USA. The media, buffer components, and standards were purchased from Himedia Laboratories Limited, Mumbai (India).

2.2. Microorganism

Bacterial strain PCH8 was isolated from a glacial soil sample of the Pangi-Chamba Himalayan (PCH) region and identified as *Paenibacillus* sp. PCH8 by 16S rDNA sequence (NCBI GenBank accession no. KY628835) analysis (Thakur, Kumar, Kumar, & Singh, 2018). The strain was submitted to Microbial Type Culture Collection (MTCC), CSIR-IMTECH, Chandigarh (India). The probiotic strains *Lactobacillus paracasie* PCH265, *Saccharomyces cerevisiae* PCH359, and *Kluyveromyces marxianus* PCH397 were previously isolated in our laboratory (Nag, Kumar, Kumar, & Kumar, 2021).

2.3. Morphological, physiological, and biochemical characterization of bacterial isolate PCH8

The cell morphology was studied using Transmission Electron Microscopy (JEOL JEM-1010). The physiological parameters affecting growth viz. temperature (4–37 ℃), pH (4.0 – 12.0), and salt tolerance (0 – 5%) were observed in the Antarctic bacterial medium (g/L: peptone 5.0, yeast extract 2.0). Biochemical characteristics (sugar utilization, gram staining, catalase test, and antibiotic sensitivity) were examined by various HiMedia Kits (HiMedia, Mumbai, India). Pure culture of the bacterium was maintained on a nutrient agar medium (g/L: meat extract 1.0, peptone 5.0, sodium chloride 5.0, yeast extract 2.0, agar 15.0) at 20 ℃ and stored in glycerol stock at -80 ℃.

2.4. Genome sequencing and phylogenomic positioning of Paenibacillus sp. PCH8

The genomic DNA isolation, genome sequencing using PacBio RSII, assembly, and annotation were carried out as described earlier (Kumar et al., 2020a; Thakur, Kumar, Kumar, & Singh, 2021). The draft genome assembly was deposited to NCBI WGS database under Bioproject PRJNA427453 and RefSeq NZ_PKQK00000000.1. Phylogeny of the bacterium was studied by analysing the 16S rRNA gene sequence in the EzBioCloud server (https://www.ezbiocloud.net/?bannerId=6). Average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) were performed with available genomes of 10 closest matches for studying genomic relatedness. A phylogenetic tree was constructed using the 16S rDNA sequence (EzTaxon) and with the whole-genome sequence in Type Strain Genome Server (TYGS) (Meier-kolthoff & Göker, 2019). As described earlier, functional genome annotation was performed through different web servers (Thakur et al., 2021). The presence of signal peptides in the protein sequences was predicted through SignalP 5.0 server (https://services.healthtech.dtu.dk/service.php?SignalP-5.0).

2.5. Qualitative and quantitative estimation of xylanase activity of Paenibacillus sp. PCH8

The bacterium was screened for xylanase production through qualitative plate assay on the medium containing (g/L) yeast extract 5.0, $MgSO_4{\cdot}7H_2O$ 10.0, KCl 2.0, $NaNO_3$ 5.0, oat spelts xylan 5.0, and agar 20.0. The bacterial culture was spotted on the solid medium and grown for 48 h at 28 ℃. The plate was flooded with 0.1% Congo red solution and washed with 1 M NaCl after 30 min (Samanta, Kolte, Senani, Sridhar, & Jayapal, 2011). The zone of clearance around the colony represents the xylanase activity. The strain PCH8 was grown in the above medium, excluding agar (xylanase production medium) for 84 h at 28 ℃ and 160 rpm. After that, culture was harvested every 12 h and centrifuged at 10,000 *g* for 15 min. The supernatant was used as a crude enzyme for the xylanase activity assay. The xylanase assay was performed using 1% oat spelts xylan as substrate in 25 mM potassium phosphate buffer (pH 7.0) at 50 ℃ for 40 min. Quantitative estimation of reducing sugars was carried out using the DNSA method (Miller, 1959). In control, the supernatant was added to the reaction mixture after incubation. All the reactions were performed in triple biological repeats. One unit of enzyme activity is defined as μmoles of reducing sugar produced per minute by one mg of the enzyme under standard assay conditions.

2.6. Proteomic analysis of protein fraction showing xylanolytic activity produced by Paenibacillus sp. PCH8

Paenibacillus sp. PCH8 was grown in a 500 mL xylanase production medium at 28 ℃ and 160 rpm for 60 h. The supernatant was dialyzed with 25 mM potassium phosphate buffer (pH 7.0) and concentrated to 200 mL in AKTA flux (GE Healthcare, Chicago, Illinois, United States) using a 3 kDa hollow fiber membrane at a feed rate of 1.5 L/min and 9 psi pressure. The concentrated fraction was lyophilized and resuspended in a 20 mL phosphate buffer. The partially purified protein fraction was resolved on 10% SDS-PAGE, and clear bands were visualized after Coomassie blue staining. The xylanase activity was reconfirmed by zymogram analysis of native protein fraction as described elsewhere (Jiang et al., 2004). Briefly, the protein fraction was resolved on a polyacrylamide gel copolymerized with 1% oat spelts xylan under native conditions. The gel was then incubated in 25 mM potassium phosphate buffer (pH 7.0) at 50 ℃ for 1 h. After incubation,

Table 1
The in silico average nucleotide identity (ANI) and digital DNA hybridization (dDDH) analysis of whole-genome sequence of *Paenibacillus* sp. PCH8 with the closest neighbours.

S. No.	Organism	NCBI RefSeq	Genome size (Mb)	GC (%)	16S sequence similarity (%)	ANI (%)	DDH (%)
1.	PCH8	NZ_PKQK00000000.1	6.75	46.0	–	–	–
2.	*Paenibacillus amylolyticus* NBRC 15957(T)	NZ_BIMJ00000000.1	7.11	45.6	97.05	87.24	33.30
3.	*Paenibacillus xylanexedens* DSM 21292	NZ_JAGIKV000000000.1	6.99	46.0	96.76	87.38	33.30
4.	*Paenibacillus tundra* A10b(T)	NA	–	–	96.76	–	–
5.	*Paenibacillus xylanivorans* A59(T)	NZ_LITU00000000.1	7.08	46.1	95.73	81.62	25.20
6.	*Paenibacillus cucumis* AP-115(T)	NA	–	–	95.72	–	–
7.	*Paenibacillus polysaccharolyticus* BL9(T)	NZ_FMVM00000000.1	6.49	45.6	95.58	78.70	22.60
8.	*Paenibacillus pabuli* NBRC 13638(T)	NZ_BCNM00000000.1	7.32	46.5	95.50	81.40	24.90
9.	*Paenibacillus intestini* LAH16(T)	NA	–	–	95.50	–	–
10.	*Paenibacillus taichungensis* BCRC 17757(T)	NZ_JABMCC000000000.1	7.23	45.7	95.43	81.52	25.10
11.	*Paenibacillus silvae* DB13031(T)	NZ_BMFU00000000.1	6.85	46.9	95.05	78.61	22.90

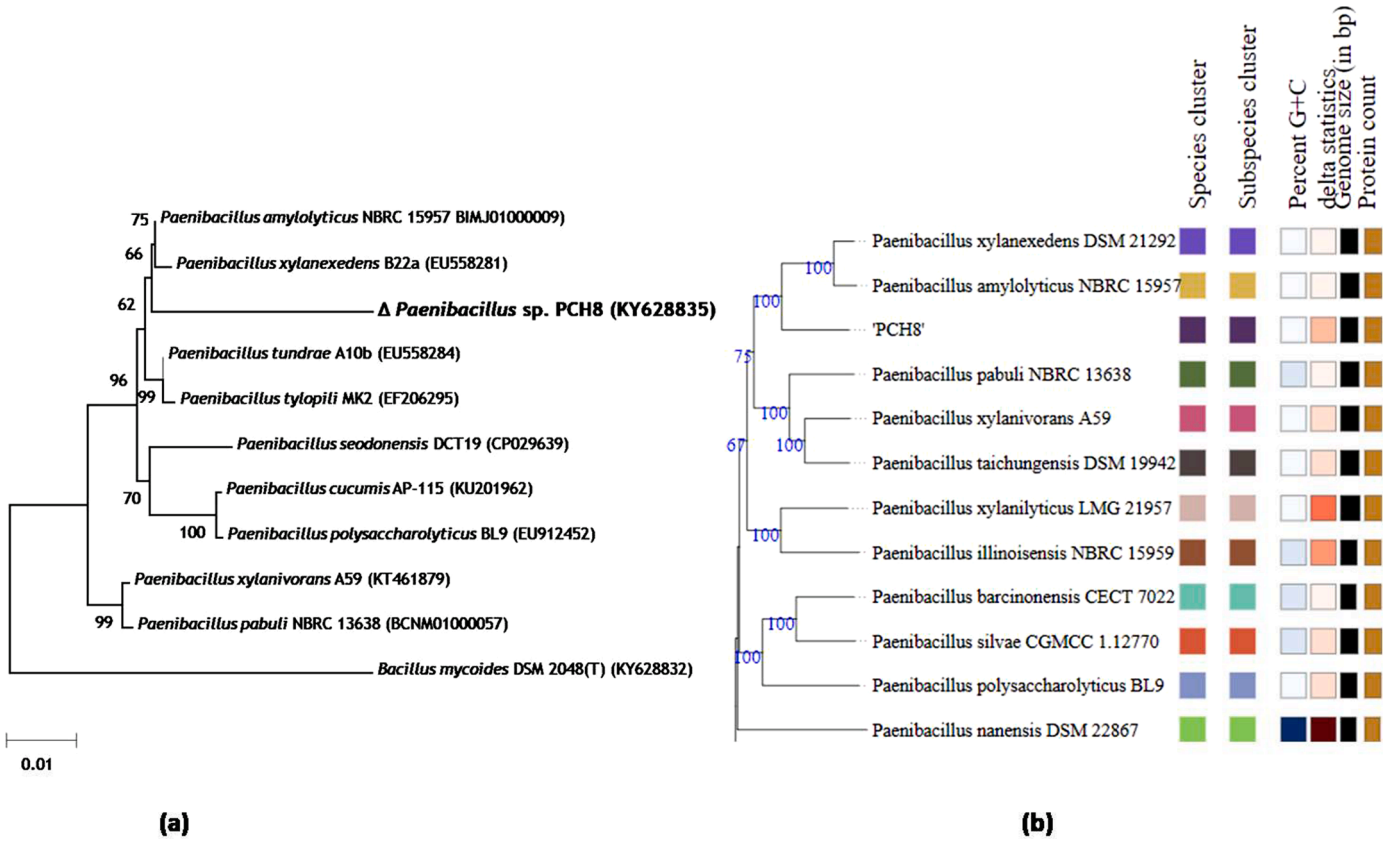

Fig. 1. Taxonomical representation of a novel *Paenibacillus* sp. PCH8, (a) 16S rRNA gene based phylogenetic tree, (b) Type (strain) genome server (TYGS) based phylogenetic tree.

the gel is washed three times with distilled water and stained using 0.1% Congo red for 0.5 h, followed by de-staining with 1 M NaCl (Geib, Tien, & Hoover, 2010). The complete proteome analysis of the enzymatic fraction showing xylanase activity was performed in 6550 Q-TOF IMS-LC-MS instruments as described elsewhere (Kumar et al., 2020b). The detection was performed with three replicates. The spectrum mill software was used for peptide identification using SwissProt and UniProt databases as search parameters.

2.7. Biochemical characterization of xylanolytic activity

The xylanolytic activity was assayed with 0.5% oat spelts xylan as substrate at 50 °C for 10 min. The effect of pH and temperature on the xylanase activity of the enzymatic fraction was assessed using a one-factor at time (OFAT) approach. Five buffer systems (25 mM with different pH) namely citrate (4.0 – 6.0), potassium phosphate (6.0 – 8.0), Tris–HCl (7.0 – 9.0), carbonate-bicarbonate (9.0 - 10.0) and disodium hydrogen phosphate-sodium hydroxide (11.0 – 12.0) were tested. The effect of temperature was studied from 4 to 90 °C.

2.8. Substrate specificity of protein fraction with xylanolytic activity

Substrate specificity of the partially purified fraction with xylanolytic activity was assessed using 0.5% of different substrates i.e., oat spelts xylan, beechwood xylan, carboxymethyl cellulose (CMC), avicel, and starch. The enzymatic reactions were carried out at 50 °C in 25 mM Tris–HCl buffer (pH 7.0) for 10 min. Individual reaction controls and three biological replicates were taken for each reaction.

Table 2
Glycosyl hydrolase gene families present in the genome of *Paenibacillus* sp. PCH8 annotated through dbCAN2 meta server.

S. No.	Ligno-cellulolytic enzymes	Glycosyl hydrolases (GH) families
1.	Xylanase (EC 3.2. 1.8)	GH1, GH2, GH3, GH5, GH8, GH10, GH11, GH16, GH30, GH31, GH43,GH51, GH52, GH67, GH74
2.	Arabinosidase (EC 3.2.1.185)	GH2, GH5, GH42, GH43, GH51, GH146
3.	Mannosidase (EC 3.2.1.25)	GH1, GH2, GH5, GH31, GH38, GH130, GH125
4.	Cellulase (EC 3.2.1.4)	GH1, GH3, GH4, GH5, GH6, GH8, GH10, GH13, GH16, GH30, GH48, GH51, GH74
5.	Amylase (EC 3.2.1.1)	GH13, GH126
6.	β-Galactosidase (EC 3.2.1.23)	GH1, GH2, GH16, GH35, GH42
7.	α-Galactosidase (EC 3.2.1.22)	GH27, GH31, GH36
8.	Laccase (EC 1.10.3.2)	AA1
9.	Chitinase (EC 3.2.1.14)	GH5, GH8, GH16, GH18, GH48

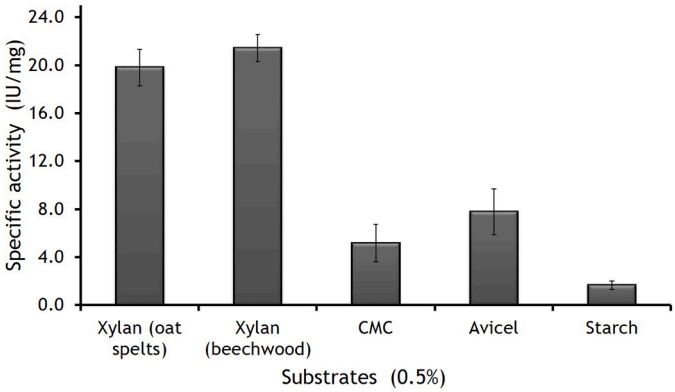

Fig. 4. The multi-substrate activity of partially purified enzyme fraction on diverse polysaccharides.

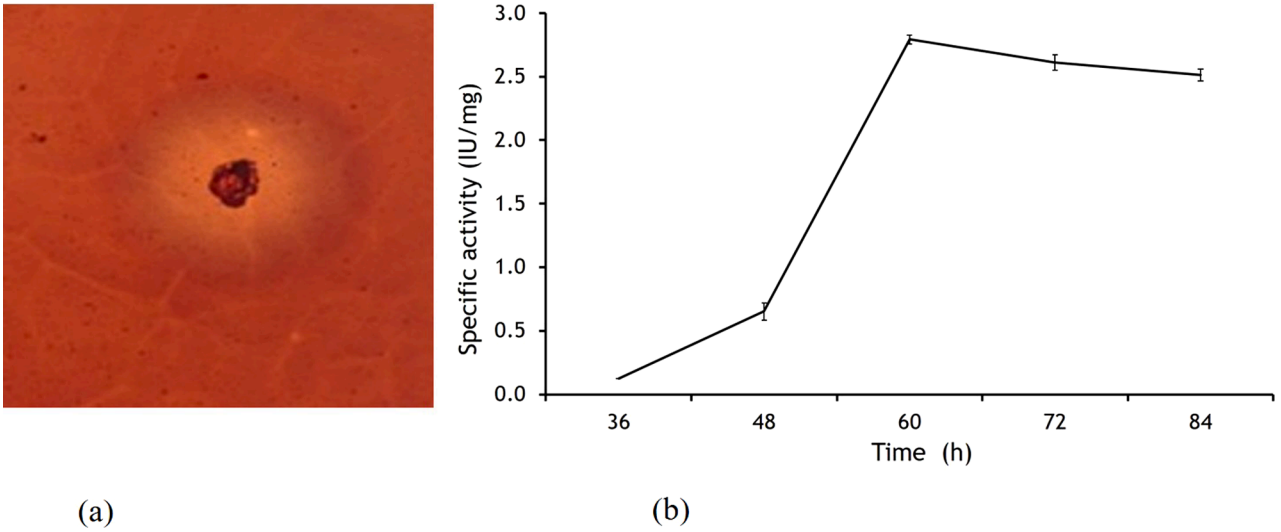

Fig. 2. The estimation of the xylanolytic potential of *Paenibacillus* sp. PCH8, (a) Qualitative estimation of xylanase activity, (b) Quantitative estimation of xylanase activity.

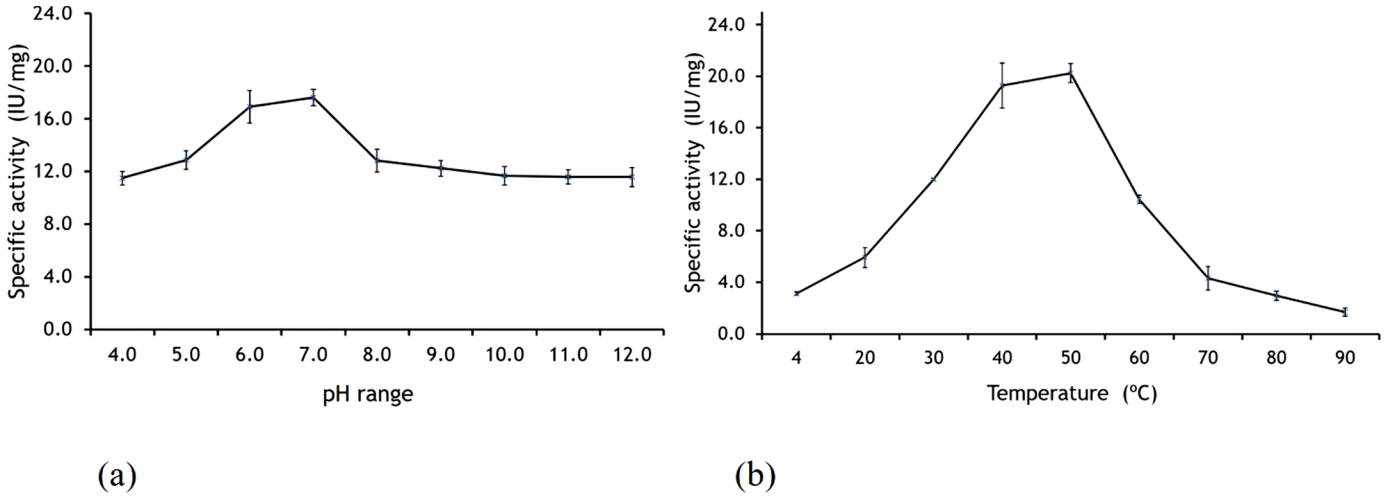

Fig. 3. Optimization of reaction parameters for partially purified xylanolytic enzyme fraction, (a) Buffer pH, (b) Temperature range.

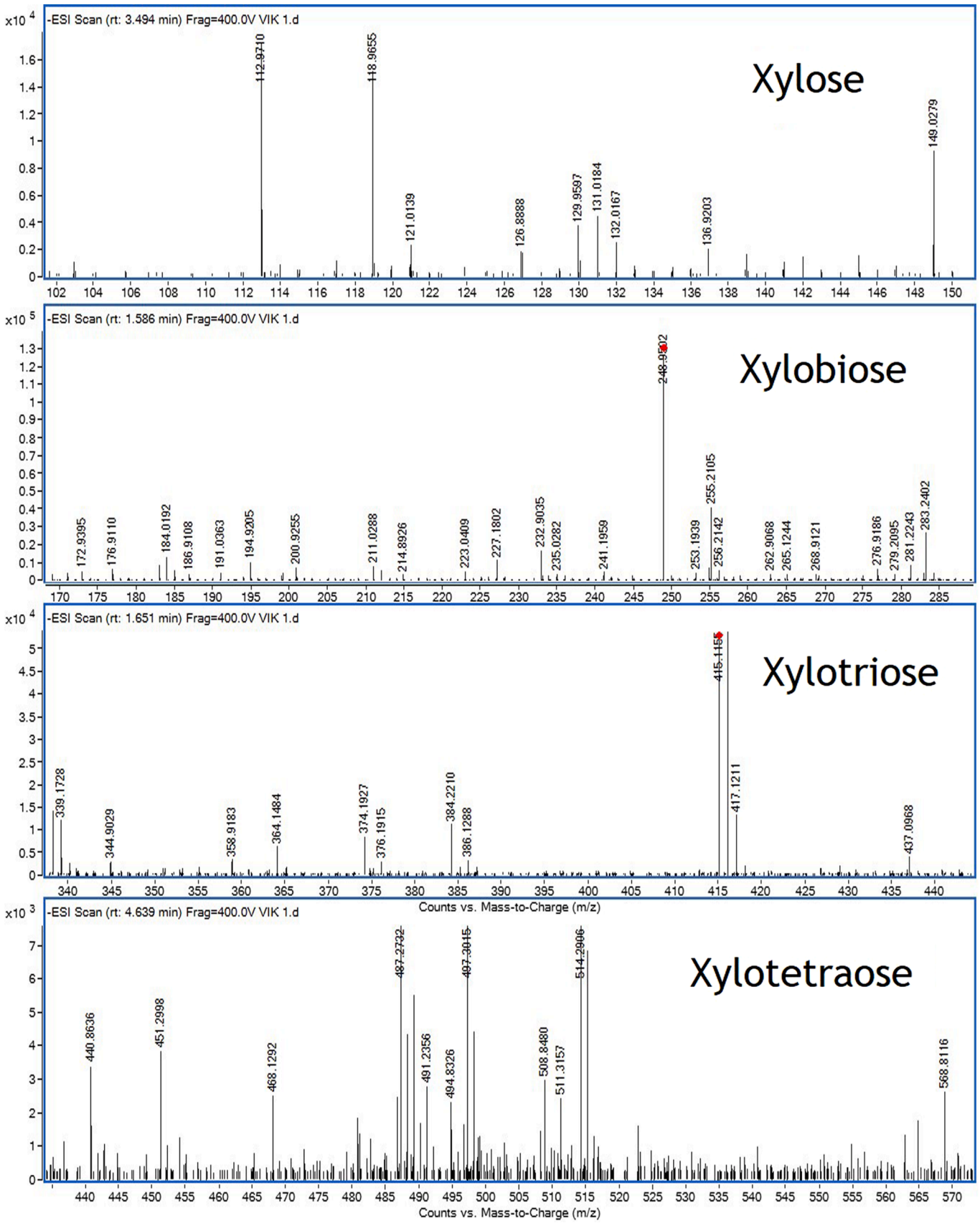

Fig. 5. The hydrolyzed products of beechwood xylan identified as xylose and xylooligosaccharides (XOS) by LC-MS analysis.

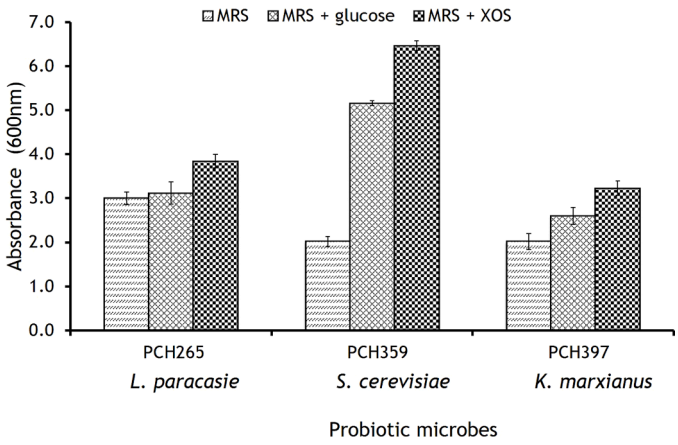

Fig. 6. The investigation of prebiotic potential of xylooligosaccharides (XOS) in the growth of probiotic microbes such as *Lactobacillus paracasie* (*L. paracasie*), *Saccharomyces cerevisiae* (*S. cerevisiae*), and *Kluyveromyces marxianus* (*K. marxianus*).

2.9. Xylan hydrolysis and xylooligosaccharides production and validation of their prebiotic potential

The protein fraction having xylanase activity was used for hydrolysis of 1% beechwood xylan. The enzyme-substrate mixture was incubated for 1 h at 50 ℃. The amount of reducing sugars formed was estimated by using DNSA method (Miller, 1959). The samples were subjected to LC-MS analysis to investigate the xylan hydrolysis and formation of XOS. The prebiotic potential of XOS was studied using probiotic strains *Lactobacillus paracasie* PCH265, *Saccharomyces cerevisiae* PCH359, and *Kluyveromyces marxianus* PCH397 isolated in our laboratory (Nag, Kumar, Kumar, & Kumar, 2021). The MRS media with following composition was prepared: (g/L) peptone-10.0, yeast extract-5.0, sodium acetate-10.0, K_2HPO_4–2.0, $MgSO_4{\cdot}7H_2O$-0.2, and tween 80–0.1%. Probiotic strains were grown in MRS medium, MRS medium supplemented with 0.25% glucose, and MRS medium supplemented with 0.25% XOS.

2.10. Statistical analysis

All the experiments were performed in triplicates. The results were expressed as mean of three replications with standard deviation. The standard deviation (SD) was applied to all the calculated enzyme activities and SD of ±2 was accepted as significant.

3. Results and discussions

3.1. Isolation, identification, and biochemical characterization of strain PCH8

The strain PCH8 was isolated on Antarctic bacterial medium at 10 °C from a glacial soil sample of the PCH region (Thakur et al., 2018). The bacterium grows in a temperature range of 4 – 37 °C and pH of 5.0 – 9.0. The optimum growth temperature and pH were 28 °C and pH 7.0, respectively. The bacterial culture tolerated 3% NaCl and utilized lactose, xylose, fructose, dextrose, galactose, trehalose, melibiose, sucrose, and L-arabinose as carbon sources (Table S1). As visualized by TEM, the bacterial cells were rod-shaped, 0.8–1.2 μm wide, and 3–4 μm long (Fig. S1). The biochemical analysis showed the strain as Gram-positive, catalase-positive, sensitive to erythromycin, ciprofloxacin, gentamycin, azithromycin, vancomycin, penicillin G, kanamycin, tetracycline, rifampicin, and resistant to streptomycin. The 16S rDNA phylogeny of the bacterium showed 97.5% sequence similarity with closest match type strain *Paenibacillus amylolyticus* NRRL NRS-290(T). The sequence similarity is well below the threshold mark (≤98.6%) for the demarcation of a novel species (Kim, Oh, Park, & Chun, 2014). This insinuated *Paenibacillus* sp. PCH8 is a putative novel species in the genus *Paenibacillus*

3.2. Whole-genome sequencing and phylogenomic analysis of Paenibacillus sp. PCH8

The genome assembly of *Paenibacillus* sp. PCH8 consists of 12 contigs summing up to 6,751,460 bases with average reference coverage of 180.7X. *In-silico* ANI and digital DDH score of the genome with available ten closest genomes were in the range of 78.61 – 87.38% and 22.60 – 33.30%, respectively (Table 1). The phylogenetic analysis of 16S rDNA sequence of *Paenibacillus* sp. PCH8 showed a separate clade with *P. amylolyticus* and *P. xylanexedens* (Fig. 1a). The genome-based phylogenetic tree constructed through the TYGS server further validated the strain PCH8 as a novel bacterial species in the genus *Paenibacillus* (Fig. 1b). The bacterial strain having 16S rDNA sequence identity <98.6%, ANI <94%, and DDH score <70% is considered to be a novel species (Kim et al., 2014). The *Paenibacilli* are well documented for their potential for hydrolytic enzymes production. Hence, a novel *Paenibacillus* species from the Himalayas opens new horizons for unearthing potentially industrial important enzymes.

The functional genome analysis using Rapid Annotation and Subsystem Technology (RAST) server indicated 46% GC content, a total of 6114 coding sequences, 147 RNA's, 6 CRISPR arrays under 442 subsystems (Fig. S2a). Interestingly, the highest subsystem feature count of 724 genes was observed for the carbohydrate metabolism category, including 11 genes for polysaccharides, 211 genes for oligosaccharides, 216 genes for monosaccharide utilization, and 43 genes for fermentation. The presence of genes encoding for carbohydrate-active proteins further reconfirmed by Prokaryotic Genome Annotation Pipeline (PGAP) and dbCAN meta server (Fig. S2b). The dbCAN server revealed 199 genes encoding for CAZymes, which includes various lignocellulolytic enzymes like xylanase, arabinosidase, mannosidase, cellulase, amylase, β-galactosidase, α-galactosidase, laccase, and chitinase activities (Table 2). The xylanase showed the highest number of glycosyl hydrolase families, which intrigued further investigation. A total of 9 genes encoding for xylanolytic enzymes were identified through PGAP annotation. Among these, 5 xylanolytic genes had signal peptides responsible for extracellular expression (Table S2).

3.3. Production of extracellular enzyme fraction and its proteomic analysis

Paenibacillus sp. PCH8 showed xylanase activity with a zone of clearance (zone ratio = 4.5) after Congo red staining (Fig. 2a). The bacterium showed the highest xylanase activity of 2.79 ± 0.18 IU/mg after 60 h of growth in the xylanase production medium (Fig. 2b). The partially purified enzyme fraction after 60 h grown culture showed 16.4 ± 0.5 IU/mg activity and 5.87-fold purification. It is comparatively higher than the specific activity reported previously for *Paenibacilli* (0.06 IU/mg, Park and Cho, 2010; 4.25 IU/mg, KURRATAA & Meryandini, 2015). Earlier, crude xylanases from *Anoxybacillus kamchatkensis* (Yadav et al., 2018) and *Thermomyces dupontii* (Seemakram, Boonrung, Aimi, & Ekprasert, 2020) showed xylanase activity of 3.01 and 2.01 IU/mg, respectively, which is lesser as compared to the present study.

The partially purified enzyme fraction showed clear bands from 30 to 75 kDa on 10% SDS-PAGE stained with Coomassie blue (Fig. S3a). The zymogram of the native protein fraction showed a clear band against a red background. This re-confirmed the xylanase activity in-gel assay (Fig. S3b). The partially purified fraction revealed 66 protein hits in LC-MS-based total proteome analysis (Fig. S4). Amongst, two proteins showed similarity with 1,4-β-xylanase and arabinan endo-1,5-α-L-arabinosidase in the BLAST analysis. Further, 11 hypothetical proteins hits

were also found in this analysis (Table S3). Previously, the extracellular proteome of *Aspergillus oryzae* showed 73 protein hits, including endo-1,4-β-xylanase (Bhardwaj, Verma, Chaturvedi, & Verma, 2018). The extracellular proteome studies of bacterial xylanolytic cocktails are limited in literature to a few microorganisms (Ghio et al., 2018; Osiro, 2017). Thus, the present results validated the genome-based identification of carbohydrate-active proteins for extracellular expression, including xylanases in *Paenibacillus* sp. PCH8.

3.4. Biochemical characterization of xylanolytic activity

The partially purified enzyme fraction from *Paenibacillus* sp. PCH8 showed xylanase activity across a broad pH (4.0 – 12.0) and temperature (4 – 90 °C). The highest xylanase activity (20.25 ± 0.45 IU/mg) was observed at 50 °C and pH 7.0 in 25 mM Tris–HCl buffer (Fig. 3a, 3b). Surprisingly, the pH and temperature optima of the xylanolytic activity were higher than optimum growth conditions of the bacterium (at 28 °C). The optimum buffer system, pH, and temperature provide an ambient environment for the enzyme to work efficiently. Earlier, partially purified xylanases from *Bacillus altitudinis* (Adhyaru, Bhatt, & Modi, 2014) and *Cladosporium oxysporum* (Guan et al., 2016) showed wide pH (3.0 – 10.0) and temperature activity (25 – 75 °C). Purified xylanase of *Anoxybacillus kamchatkensis* was active at wide pH (4.0 – 11.0) and temperature (30 – 80 °C) (Yadav et al., 2018). *Paenibacillus montaniterrae* produced xylanase with a pH range of 4.0 – 11.0 and a temperature range of 20 – 100 °C (Arora, Krishna, Malik, & Reddy, 2014). Though several strains reported broad pH active xylanases, the activity drops significantly for them at pHs other than pH optima. However, the xylanolytic enzyme fraction from PCH8 exhibited a stable activity profile at pH extremes. The enzyme fraction showed 65% activity on acidic (pH 4.0) and alkaline (pH 12.0) pH in comparison to optimum pH of 7.0 (Fig. 3a). All mentioned strains are either mesophilic or thermophilic, whiles PCH8 is a psychrotolerant bacterium with thermophilic enzyme properties.

The broad pH and temperature-active xylanases have applications in varied industries. Acidic xylanases are utilized in the food industry, whereas alkaline xylanases have application in bio-bleaching (Beg, Kapoor, Mahajan, & Hoondal, 2001; Goluguri et al., 2012; Luo et al., 2009). Low-temperature active xylanases are favoured in the food industry, whereas biobleaching and bioconversion-based industries require high-temperature active xylanases (Dornez, Verjans, Arnaut, Delcour, & Courtin, 2011; Wang et al., 2014). The enzyme fraction of *Paenibacillus* sp. PCH8 showed xylanolytic activity with desirable attributes i.e. broad pH and temperature activity. This makes PCH8 a potential bioresource for diverse industrial processes.

3.5. Hydrolysis of hemicellulosic and cellulosic substrates

The partially purified enzyme fraction showed hydrolytic activity for polysaccharides like oat spelt xylan, beechwood xylan, CMC, avicel, and starch (Fig. 4). The enzyme activities on oat spelt (19.8 ± 0.28 IU/mg) and beechwood xylan (21.42 ± 0.46 IU/mg) confirm hemicellulolytic activities. Additionally, the enzyme fraction hydrolyses CMC (5.17 ± 0.80 IU/mg), avicel (7.7 ± 0.60 IU/mg), and starch (1.65 ± 0.40 IU/mg), thus, validating the cellulolytic and amylolytic activity. The capability of partially purified enzyme fraction in the hydrolysis of diverse polysaccharides is intriguing. This revelation makes strain PCH8 a suitable bioresource for the lignocellulose depolymerization and bioethanol industry.

3.6. Xylooligosaccharides production and validation of their prebiotic potential

The enzymatic fraction having xylanase activity hydrolysed beechwood xylan to form XOS. The hydrolysis carried out under optimized conditions resulted in the generation of 5.4 g/L reducing sugars. The LC-MS analysis of hydrolysed products revealed that XOS contains xylobiose, xylotriose, and xylotetraose (Fig. 5). The reaction showed ~25.5% conversion of beechwood xylan into products including xylose and XOS in 1 h. Earlier studies also reported the production of XOS from various xylan substrates. For example, xylanase from fungi i.e., *Aspergillus foetidus, Talaromyces amestolkiae*, and *Aureobasidium pullulans* yielded 6.75 g/L, 5.95 g/L, and 7.7 g/L XOS from corncob xylan (Chapla et al., 2012), birchwood xylan (Nieto-Domínguez, 2017), and beechwood xylan (Gautério et al., 2021). Although the enzyme fraction is partially purified in the present study, still, it resulted in 25% conversion efficiency, which shows its potential hydrolysis capacity.

The XOS is the favourable substrate for the growth of probiotic strains. In our experimental conditions, the xylan hydrolysed product, including XOS, enhanced the growth of probiotic strains when supplemented in MRS medium (Fig. 6). The *Saccharomyces cerevisiae* PCH359 showed the highest increase in growth (~80%) in XOS enriched MRS medium compared to MRS medium containing glucose only. Prebiotics are well known for stimulating the growth of probiotic microflora in the human gut. Several studies have demonstrated the positive effects of xylose enriched XOS on glucose regulation and total cholesterol reduction (Kim et al., 2016; Lim, 2016; Sheu, Lee, Chen, & Chan, 2008). The findings insinuate that the xylanolytic enzyme fraction of *Paenibacillus* sp. PCH8 can be employed for the production of prebiotic XOS with applications in the food industry.

4. Conclusions

The harsh and hostile environmental conditions in the Himalayas challenge all life forms, including microbes. Microbes adapt to these conditions through various unique enzymes that perform specialized biological functions and are of commercial applications. In this study, a putative novel Himalayan bacterium *Paenibacillus* sp. PCH8 possessing many lignocellulolytic enzymes, including xylanases, has been identified and characterized. Enzymatic characterization confirmed the extracellular xylanase activity at a broad pH and temperature. Interestingly, it showed activity for various xylans and cellulose substrates, suggesting its applicability in hemicellulosic and cellulosic hydrolysis. The enzyme fraction also depolymerizes beechwood xylan to produce XOS, which enhances the growth of probiotic bacteria and yeast. The current findings have supported our hypothesis that the Himalayan niches are a goldmine of bioresources that produces biomolecules with unique properties for diverse applications. Further, the findings also implied that *Paenibacillus* sp. PCH8 has xylanolytic activity with potential applications for cellulose/hemicellulose hydrolysis and XOS-based food and pharmaceutical industries.

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Author contribution

Vikas Thakur: Designed, performed experiments, data analysis, and manuscript writing. **Virender Kumar:** Enzyme purification and XOS production. **Vijay Kumar:** Genome annotation and data analysis. **Dharam Singh:** Conceived the study, research methodology and design, data interpretation, manuscript writing and research supervision.

Data availability

The assembled whole genome sequence of *Paenibacillus* sp. strain PCH8 was submitted to NCBI database with BioProject ID

PRJNA427453, BioSample ID SUB3395370.

Compliance with ethical standards

Animal Research The authors declare that this research did not involve human participants and/or animals. The authors declare that this work involved no unusual hazard, animal or human subjects.

Informed consent

All authors have read and approved the submission of the manuscript.

Declaration of Competing Interest

The authors declared no potential conflict of interest with respect to research, authorship, and/or publication of this article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.carpta.2022.100215.

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