



# Biochemical characterization of a novel $\alpha$ -L-fucosidase from *Pedobacter* sp. and its application in synthesis of 3'-fucosyllactose and 2'-fucosyllactose

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## Abstract

Fucosyllactoses have gained much attention owing to their multiple functions, including prebiotic, immune, gut, and cognition benefits. In this study, human milk oligosaccharide (HMO) 2'-fucosyllactose ( $\alpha$ -L-Fuc-(1,2)-D-Gal $\beta$ -1,4-Glu, 2'FL) and its isomer 3'-fucosyllactose ( $\alpha$ -L-Fuc-(1,3)-D-Gal $\beta$ -1,4-Glu, 3'FL) with potential prebiotic effect were synthesized efficiently by a novel recombinant  $\alpha$ -L-fucosidase. An  $\alpha$ -L-fucosidase gene (*PbFuc*) from *Pedobacter* sp. CAU209 was successfully cloned and expressed in *Escherichia coli* (*E. coli*). The deduced amino acid sequence shared the highest identity of 36.8% with the amino sequences of other reported  $\alpha$ -L-fucosidases. The purified  $\alpha$ -L-fucosidase (*PbFuc*) had a molecular mass of 50 kDa. The enzyme exhibited specific activity (26.3 U/mg) towards 4-nitrophenyl- $\alpha$ -L-fucopyranoside (pNP-FUC), 3'FL (8.9 U/mg), and 2'FL (3.4 U/mg). It showed the highest activity at pH 5.0 and 35 °C, respectively. *PbFuc* catalyzed the synthesis of 3'FL and 2'FL through a transglycosylation reaction using pNP-FUC as donor and lactose as acceptor, and total conversion ratio was up to 85% at the optimized reaction conditions. The synthesized mixture of 2'FL and 3'FL promoted the growth of *Lactobacillus delbrueckii* subsp. *bulgaricus* NRRL B-548, *L. casei* subsp. *casei* NRRL B-1922, *L. casei* subsp. *casei* AS 1.2435, and *Bifidobacterium longum* NRRL B-41409. However, the growths of *E. coli* ATCC 11775, *S. enterica* AS 1.1552, *L. monocytogenes* CICC 21635, and *S. aureus* AS 1.1861 were not stimulated by the mixture of 2'FL and 3'FL. Overall, our findings suggest that *PbFuc* possesses a great potential for the specific synthesis of fucosylated compounds.

## Key Points

- A novel  $\alpha$ -L-fucosidase (*PbFuc*) from *Pedobacter* sp. was cloned and expressed.
- *PbFuc* showed the highest hydrolysis activity at pH 5.0 and 35 °C, respectively.
- It was used for synthesis of 3'-fucosyllactose (3'FL) and 2'-fucosyllactose (2'FL).
- The mixture of 3'FL and 2'FL promoted the growth of some *Lactobacillus* sp. and *Bifidobacteria* sp.

**Keywords**  $\alpha$ -L-fucosidase · Transglycosylation · *Pedobacter* sp. · 2'-fucosyllactose · 3'-fucosyllactose

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## Introduction

Fucosyllactoses (FLs), an essential group of human milk oligosaccharides (HMOs), have gained much attention owing to their various beneficial health effects, such as gut maturation (Vandenplas et al. 2018; Yu et al. 2013), pathogens resistance (Yu et al. 2016), immunity improvement (Zehra et al. 2018; Xiao et al. 2018), and nervous system development (Matthies et al. 1996; Vázquez et al. 2015). They usually have a core lactose, and the fucose residues are attached to the terminal galactose residues through  $\alpha(1\rightarrow2)$  glycosidic linkage, or to the glucose units at the reducing end through  $\alpha(1\rightarrow3)$

glycosidic linkage (Saumonneau et al. 2016). 2'FL synthesized by chemical and biotechnological methods has been enlisted in generally regarded as safe (GRAS) (FDA, 2019) and obtained approval from European Union (NFregulation, European Food Safety Authority) as an ingredient in infant formula, dietary supplements, and medical foods (Bych et al. 2019). Thus, much amount of fucosyllactose is required to meet the market.

Enzymatic production of functional oligosaccharides has acquired great interest owing to the specific synthesis of glycoside linkages and mild reaction conditions (Hayes and Pietruszka 2017). Glycosyltransferases (EC 2.4) and glycosidases (EC 3.2.1) can be used for glycoside synthesis. Currently, glycosidases have been much studied since they often possess high availability and activity when compared with glycosyltransferases (Bojarová and Kren 2011; Zeuner et al. 2014).  $\alpha$ -L-Fucosidases (EC 3.2.1.51) are exo-glycoside hydrolases that catalyze the release of L-fucosyl residues from oligosaccharides and glycoconjugates. They are widely distributed in animals, plants, and microorganism (Guzmán-Rodríguez et al. 2019). So far, most of reported  $\alpha$ -L-fucosidases are from bacteria, such as *Bacteroides* sp., *Paenibacillus* sp., *Tannerella* sp., *Xanthomonas* sp., *Thermotoga* sp., and *Bifidobacterium* sp. (Lammerts van Bueren et al. 2010; Benešová et al. 2013; Zeuner et al. 2018; Sela et al. 2012; Rodríguez-Díaz et al. 2011). There are few reports on  $\alpha$ -L-fucosidases from *Pedobacter* sp. According to the amino acid sequence similarities,  $\alpha$ -L-fucosidases are divided into three glycoside hydrolase (GH) families, viz. GH families 29, 95, and 151 (Henrissat 1991; Lombard et al. 2014; Guzmán-Rodríguez et al. 2018a). GH family 29  $\alpha$ -L-fucosidases are typical retaining enzymes which catalyze the reactions through a two-step double-displacement mechanism. GH family 95  $\alpha$ -L-fucosidases are inverting enzymes catalyzing the reactions by a single-displacement mechanism. The reaction mechanisms of GH family 151  $\alpha$ -L-fucosidases are still unknown. Due to these mechanistic differences, only GH family 29  $\alpha$ -L-fucosidases are potential candidates that show transglycosylation activity. However, those  $\alpha$ -L-fucosidases are usually less efficient to synthesize fucosyllactose, such as GH family 29  $\alpha$ -L-fucosidases from *Fusarium graminearum* (Zeuner et al. 2018), *Alcaligenes* sp. (Murata et al. 1999), *Thermotoga maritima* (Guzmán-Rodríguez et al. 2018), and soil-derived metagenome library (Lezyk et al. 2016). Therefore, the discovery of novel  $\alpha$ -L-fucosidases with high transglycosylation activities is very important for fucosyllactose synthesis.

3'-fucosyllactose (3'FL) is an isomer of 2'FL and 3FL in human milk oligosaccharides, which has not been found in nature (Takamura et al. 1981; Murata et al. 1999). However, L-Fuc-(1,3)-D-Gal linkage naturally exists as part of human milk oligosaccharides (non-asaccharide) (Yamashita et al. 1976). 3'FL has been successfully synthesized through

chemical modification of lactose which is unsuitable for large-scale production because of extreme complexity, harmful chemical reagents, and low product yield (Takamura et al. 1981). Enzymatic synthesis of 3'FL has been carried out by  $\alpha$ -L-fucosidase from *Alcaligenes* sp. with a yield of 34%, using p-nitrophenyl  $\alpha$ -L-fucopyranoside and lactose as the substrates (Murata et al. 1999). So far, few studies have been done on the functionalities of 3'FL. Miyauchi et al. have reported that antiserum against 3'-O-a-L-fucosyllactose-p-isothiocyanato-phenethylamine-BSA could preferentially react with adenocarcinoma and embryonal carcinoma cells (Miyauchi et al. 1982). 2'FL and 3FL have been reported to play a critical role in the establishment of a healthy gut microbiome by selectively stimulating the growth of specific *Bifidobacterium* sp. and *Bacteroides* sp. (Ashida et al. 2009; Asakuma et al. 2011; Yun et al. 2019) while also suppressing the growth of pathogens such as *Clostridium perfringens*, *Salmonella enterica* serovar Typhimurium, *Enterobacter cloacae*, and *Escherichia coli* (Yu et al. 2013; Yun et al. 2019). However, the prebiotic effect of 3'FL is unknown.

In this report, a novel  $\alpha$ -L-fucosidase gene (*PbFuc*) from *Pedobacter* sp. CAU209 was successfully cloned and expressed in *E. coli*. *PbFuc* was biochemically characterized and used to synthesize 2'FL and 3'FL. In addition, the prebiotic effect of the mixture of 2'FL and 3'FL towards selected probiotics was investigated.

## Materials and methods

### Materials and chemicals

*E. coli* DH5 $\alpha$  and BL21 (DE3) from Biomed (Beijing, China) were used for plasmid propagation and as hosts for gene cloning and expression, respectively. pET-28 a (+) was obtained from Novagen (Madison, WI, USA). Restriction endonuclease and T4 DNA ligase were purchased from New England Biolabs (Ipswich, MA, USA). Taq DNA polymerase was obtained from Biolabs (Tokyo, Japan).

4-Nitrophenyl- $\alpha$ -L-fucopyranoside (pNP-FUC) was purchased from Megazyme (Chicago, USA). 2'FL and 3FL were obtained from Elicityl (Crolles, France).  $\beta$ -D-Lactose and fucoidan were purchased from Sigma-Aldrich (Steinheim, Germany). Fructooligosaccharide (FOS, Raftilose P95), degree of polymerization (DP) was 2–7, was obtained from Orafit Group (Tienen, Belgium). Ni<sup>2+</sup> Sepharose was obtained from GE Healthcare (Uppsala, Sweden). Bio-Gel P2 was obtained from Bio-Rad Laboratories (Hercules, CA, USA). Selected probiotics used in this study included *L. rhamnosus* AS 1.2466, *L. delbrueckii* subsp. *bulgaricus* NRRL B-548, *L. acidophilus* NRRL B-4495, *L. casei* subsp. *casei* NRRL B-1922, *L. brevis* NRRL B-4527 (ATCC 14869), *L. Rod* NRRL B-4391, *L. casei* subsp. *casei* AS 1.2435, *L.*

*delbrueckii* subsp. *Lactis* AS 1.2132 (JCM 1248), *L. reuteri* CICC-6132, *B. adolescentis* ATCC 15703, *B. infantis* NRRL B-41661, *B. breve* NRRL B-41408, *B. longum* NRRL B-41409, and *B. bifidum* NRRL B-41410. Pathogens including *E. ATCC 11775*, *Samonella enterica* AS 1.1552, *Listeria monocytogenes* CICC 21635, and *Staphylococcus aureus* AS 1.1861 were used in this study. These probiotics were kept at  $-80\text{ }^{\circ}\text{C}$  in MRS broth supplemented with 15% (v/v) glycerol, while pathogens were kept at  $-80\text{ }^{\circ}\text{C}$  in Luria-Bertani (LB) medium supplemented with 15% (v/v) glycerol. All other chemicals used were of analytical grade obtained from commercial sources.

### Cloning of an $\alpha$ -L-fucosidase gene from *Pedobacter* sp. CAU209 and sequence analysis

Two primers, *PbFuc*-F (5'-CCGGAATTCCAGGATTACACACCTACAGCCGC-3') and *PbFuc*-R (5'-ATAAGAATGCGGCCCGCTATCCAATCTCCAAAACAA TCACCTG-3'), were designed to amplify an  $\alpha$ -L-fucosidase gene (*PbFuc*) from the *Pedobacter* sp. CAU209 without the signal sequence. The restriction enzyme sites for *EcoRI* and *NotI* were underlined and located at the 5'-end and 3'-end, respectively. The PCR mixture (50  $\mu\text{L}$ ) contained 10  $\mu\text{L}$  of 5 $\times$  TransStart FastPfu buffer, 4  $\mu\text{L}$  dNTPs mix (2.5 mM), 1.0  $\mu\text{L}$  Aga16A-up/dn (10 pmol/ $\mu\text{L}$ ), 1  $\mu\text{L}$  genomic DNA (300 ng), 1  $\mu\text{L}$  5 $\times$  TransStart FastPfu DNA polymerase (2.5 U/ $\mu\text{L}$ ), and 33  $\mu\text{L}$  H<sub>2</sub>O. PCR conditions were as follows: 5 min at 95  $^{\circ}\text{C}$ , followed by 30 cycles of 20 s at 95  $^{\circ}\text{C}$ , 20 s at 58  $^{\circ}\text{C}$ , and 30 s at 72  $^{\circ}\text{C}$ , with a final extension at 72  $^{\circ}\text{C}$  for 10 min. The PCR product was purified by AxyPrep DNA Gel Extraction Kit (AxyGen, America), and then cloned into pET-28a (+) vector in *EcoRI* and *NotI* sites. Subsequently, the recombinant plasmid was transformed into *E. coli* BL21 (DE3) competent cells.

The potential signal peptide was predicted at the online tool SignalP 4.1 server (Petersen et al. 2011). Nucleotide and predicted amino acid sequences were analyzed with the ExPASy Proteomics tools (Artimo et al. 2012). The multiple sequence alignment analysis of *PbFuc* and other GH family 29  $\alpha$ -L-fucosidases was carried out by Clustal Omega (Sievers et al. 2014).

### Expression and purification of *PbFuc*

A single *E. coli* transformant was cultured in LB medium containing 50  $\mu\text{g}/\text{mL}$  kanamycin at 37  $^{\circ}\text{C}$ , until the optical density (OD) of the broth at 600 nm reached  $A_{600} = 0.6\text{--}0.8$ , then isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG, 1 mM) was added to induce protein expression, followed by incubation at 20  $^{\circ}\text{C}$  for 16 h. *E. coli* cells were harvested by centrifugation (10,000 rpm, 5 min, 4  $^{\circ}\text{C}$ ) and were re-suspended in buffer A (20 mM Tris-HCl buffer pH 8.0 containing 0.5 M NaCl and

20 mM imidazole) and then lysed by sonication. The supernatant as crude enzyme was collected by centrifugation (11,000 rpm, 20 min, 4  $^{\circ}\text{C}$ ), and then loaded on a nickel-iminodiacetic acid column (Ni-IDA: 1  $\times$  5 cm, GE Life Sciences) at 0.5 mL/min. Unbound proteins were washed off with buffer A at 1 mL/min, then the bound proteins were eluted with buffer B (20 mM Tris-HCl buffer pH 8.0, 0.5 M NaCl, 100 mM imidazole). The proteins were collected and dialyzed against 20 mM Tris-HCl buffer pH 7.0 for 12 h. The protein purity was confirmed by SDS-PAGE and the concentrations of protein were quantified by the Lowry method with Bovine serum albumin (BSA) standard (Lowry et al. 1951).

### Enzyme assay

Hydrolysis activity of *PbFuc* was determined using 100  $\mu\text{L}$  of 10 mM pNP-FUC, 100  $\mu\text{L}$  of 50 mM citrate buffer pH 5.0, and 10  $\mu\text{L}$  appropriate diluted enzyme (Paper et al. 2013). The reaction was carried out at 35  $^{\circ}\text{C}$  for 20 min, and stopped by adding 200  $\mu\text{L}$  of 2 M sodium carbonate, then the absorbance was measured at 405 nm. One unit of enzyme activity was defined as the amount of enzyme required to release 1  $\mu\text{mol}$  pNP per minute at the above conditions.

### Characterization of the purified *PbFuc* for hydrolytic activity

The optimal pH of *PbFuc* was measured by assaying enzyme activity at pH values ranging from pH 3.0 to 12.0 in 50 mM different buffers: citrate buffer (■) pH 3.0–6.0; phosphate buffer (○) pH 6.0–8.0; Tris-HCl buffer (▼) pH 6.0–9.0; 2-cyclohexylaminoethanesulfonic acid (CHES) buffer pH (△) 8.0–10.0; 3-cyclohexylaminopropane sulfonic acid (CAPS) buffer (\*) pH 10.0–11.0; and Na<sub>2</sub>HPO<sub>4</sub>-NaOH buffer (●) pH 11.0–12.0. The pH stability of *PbFuc* was evaluated by incubating the enzyme in different buffers at 25  $^{\circ}\text{C}$  for 30 min and assaying residual activity under the standard conditions. The optimal temperature of *PbFuc* was determined by measuring enzyme activity at different temperatures from 25 to 50  $^{\circ}\text{C}$ . Thermostability of *PbFuc* was studied by assaying residual activity after incubation of the enzyme at 35 to 50  $^{\circ}\text{C}$  for 30 min. The thermal denaturing half-life of the enzyme was evaluated at 35, 40, and 45  $^{\circ}\text{C}$  for 4 h, samples were withdrawn at different intervals, and residual activities of treated enzymes were determined by the standard assay.

The impact of metal ions and other chemical reagents on the hydrolysis activity of *PbFuc* was assayed after incubation of the enzyme in the presence of Ag<sup>+</sup>, Ca<sup>2+</sup>, Fe<sup>3+</sup>, Cr<sup>3+</sup>, Co<sup>2+</sup>, Mg<sup>2+</sup>, Mn<sup>2+</sup>, Zn<sup>2+</sup>, Cu<sup>2+</sup>, Ni<sup>2+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Ba<sup>2+</sup>, EDTA,  $\beta$ -mercaptoethanol, and SDS. The enzyme was incubated in 50 mM citrate buffer pH 5.0 with 1 mM metal ions and reagents at 25  $^{\circ}\text{C}$  for 30 min. The residual activities were then

measured according to the standard method; the enzyme without any treatment was used as control.

Substrate specificity of PbFuc was determined by measuring the enzyme activity using 100  $\mu\text{L}$  of 10 mM different substrates (pNP-FUC, 2'FL, 3FL, 3'FL, xyloglucan, and fucoidan), 100  $\mu\text{L}$  of 50 mM citrate buffer pH 5.0, and 10  $\mu\text{L}$  enzyme. The reactions were carried out at 35  $^{\circ}\text{C}$  for 20 min, and stopped by boiling for 10 min. The amount of released fucose was analyzed by HPLC (Agilent HPLC-1260 Infinity) equipped with BP-800 Pb<sup>2+</sup> column at a flow rate of 0.8 mL/min at 80  $^{\circ}\text{C}$ , and was monitored with a refractive index detector (RID) at 35  $^{\circ}\text{C}$ . One unit of enzyme activity was defined as the amount of enzyme required to release 1  $\mu\text{mol}$  fucose per minute at the above conditions. Xyloglucan (XyG) from apple was prepared from NaOH extracts of apple cell walls (Renard et al. 1992).

The kinetic parameters of PbFuc towards pNP-FUC were determined by measuring the enzyme activities with different concentrations of the substrate in 50 mM citrate buffer (pH 5.0) at 35  $^{\circ}\text{C}$  for 5 min. The  $V_{\text{max}}$  and  $K_{\text{m}}$  values were calculated using the Graphpad Prism program 7.04.

### Transglycosylation activity of PbFuc

The transglycosylation activity of PbFuc was explored using 10 mM pNP-FUC as glycosyl donor, 0.5 U/mL purified PbFuc, and 50 mM different saccharides as acceptors (glucose, galactose, fructose, arabinose, fucose, ribose, xylose, rhamnose, glucuronic acid, galacturonic acid, maltose, or lactose). The reactions were carried out in 50 mM citrate buffer (pH 5.0) at 35  $^{\circ}\text{C}$  for 3 h and stopped by boiling for 10 min. The results were analyzed by thin-layer chromatography (TLC), which was performed on Silica gel 60 (Merck, Germany) plates. The samples were applied to the plates and developed twice with 2:1:1 n-butanol-acetic-water aqueous. Samples on the TLC plate were visualized by spraying with 5%  $\text{H}_2\text{SO}_4$  in methanol and heating at 120  $^{\circ}\text{C}$ .

### Isolation, identification, and synthesis optimization of transglycosylation products

To further identify the synthesized saccharides by PbFuc from pNP-FUC and lactose, products were purified by a Bio-gel P2 column (1.2  $\times$  110 cm, MQ water; 0.3 mL/min, Pharmacia, Germany); oligosaccharide fractions were collected after analysis by TLC, then concentrated and freeze-dried. The purity of oligosaccharide was analyzed by HPLC; HPLC analysis was performed using an Agilent-1260 Infinity system equipped with a refractive index detector (RID) and waters XBridge amide column (3.5  $\mu\text{m}$ , 4.6  $\times$  250 mm). The column was maintained at 45  $^{\circ}\text{C}$  and eluted with acetonitrile-water mobile phases (72/28, v/v) at a flow rate of 0.5 mL/min. To determine the molecular weight of purified product, mass spectra (MS)

analysis was performed through Thermo Scientific™ Q Exactive™ equipped with positive-ion mode infusion/offline electrospray ionization (ESI). The structure of purified oligosaccharide was analyzed by NMR. Briefly, the sample was dissolved in  $\text{D}_2\text{O}$  and then transferred into a NMR tube. One-dimensional spectra were recorded on a 500 MHz Varian VNMR SYSTEM™ at 298 K at 500 MHz for  $^1\text{H}$  and 125 MHz for  $^{13}\text{C}$ , respectively. Chemical shifts were expressed in parts per million (ppm) referenced to DSS (4,4-dimethyl-4-silapentane-1-sulfonic acid).

To optimize the synthesis of 3'FL and 2'FL, the reaction conditions including temperature, pH, enzyme dosage, reaction time, and lactose concentration were investigated. The effect of temperature on the synthesis of 3'FL and 2'FL was determined in 50 mM citrate buffer (pH 5.0) for 3 h at 20–50  $^{\circ}\text{C}$ . The effect of pH on the synthesis of 3'FL and 2'FL was evaluated at 35  $^{\circ}\text{C}$  using different buffers (pH 4.0–10.0). The effect of enzyme dosage on the synthesis of 3'FL and 2'FL was studied from 0.05 to 5 U/mL. The influence of reaction time on the synthesis of 3'FL and 2'FL was studied at 0.5 to 24 h, respectively. Finally, the effect of different lactose concentration (0.05–1.0 M) was evaluated for the synthesis of 3'FL and 2'FL.

The reaction was terminated by boiling for 10 min and samples were filtered by 0.22  $\mu\text{m}$  filter, then the products were analyzed by a HPLC system (Agilent-1260 Infinity) equipped with a refractive index detector (RID) and waters XBridge amide column (3.5  $\mu\text{m}$ , 4.6  $\times$  250 mm). The column was maintained at 45  $^{\circ}\text{C}$  and eluted with acetonitrile-water mobile phases (72/28, v/v) at a flow rate of 0.5 mL/min. The conversion ratio (yield) represented the ratio of the amount of the mixture of 3'FL and 2'FL (mM) to the amount of donor (mM, pNP-FUC).

### Growth test of probiotics and pathogens using the mixture of 3'FL and 2'FL as the sole carbon source

To obtain suitable amount of 3'FL and 2'FL, the synthesis reaction (200 mL) was carried out at the optimized conditions. The mixture of 3'FL and 2'FL was collected, concentrated, and freeze-dried.

To investigate the prebiotic effect of the mixture of 3'FL and 2'FL, nine *Lactobacillus* sp. and five *Bifidobacterium* sp. strains were incubated in Man, Rogosa, and Sharpe (MRS) medium without glucose supplemented with 1% (w/v) the mixture of 3'FL and 2'FL, 2'FL, fructooligosaccharide (FOS, DP 2–7, Raftilose P95), or pure water (no sugar) as the sole carbon source. Fermentation broth was prepared in 96 Microwell Plate with a total volume of 250  $\mu\text{L}$  in each well containing 100  $\mu\text{L}$  bacteria in MRS medium without glucose, 100  $\mu\text{L}$  of 2% carbohydrate solution (w/v) or pure water, and 50  $\mu\text{L}$  of mineral oil (Hoeflinger et al. 2015). The anaerobic fermentation was performed at 37  $^{\circ}\text{C}$  for 72 h. To study the



influence of the mixture of 3'FL and 2'FL on the growth of pathogens, *E. coli* ATCC 11775, *S. enterica* AS 1.1552, *L. monocytogenes* CICC 21635, and *S. aureus* AS 1.1861 were incubated in the M9 broth medium at 37 °C for 48 h supplemented with 1% (w/v) of the mixture of 3'FL, 2'FL, FOS, or pure water (Yun et al. 2019). To measure the growth of bacteria, optical density (OD) of each culture media was recorded at 595 nm (OD<sub>595</sub>) with an automated microplate reader (Multiskan FC, Thermo Electron Corporation) at different times during incubation.

### Accession number

The gene sequence (*PbFuc*) is available at the GenBank database under accession number MN902190.

## Results

### Cloning and sequence analysis of *PbFuc*

An  $\alpha$ -L-fucosidase gene (*PbFuc*) was amplified from the genomic DNA of *Pedobacter* sp. CAU209. It is 1326 bp in length and encodes a protein of 441 amino acids. The molecular mass and isoelectric point of deduced protein were predicted to be 50.46 kDa and 8.58, respectively. The amino acid sequence of *PbFuc* was compared with different GH family 29  $\alpha$ -L-fucosidases (Fig. 1). *PbFuc* shared relatively high identity (36.8%) with the  $\alpha$ -L-fucosidase isoenzyme 1 ( $\alpha$ -L-f1wt, PDB entry 6GN6) from *P. thiaminolyticus* (Benešová et al. 2013), followed by the  $\alpha$ -L-fucosidases from *Homo sapiens* (31.6%, GenBank accession number P04066, Ohira et al. 2003), *H. sapiens* (31.3%, GenBank accession number Q9BTY2, Clark et al. 2003), *Macaca fascicularis* (31.1%, GenBank accession number Q60HF8, Fisher and Aronson 1989), and *B. thetaiotaomicron* (*BtFuc*2970, 29.6%, PDB entry 2WVT, Lammerts van Bueren et al. 2010). Two conserved glutamic acid (E260) and aspartic acid (D204) residues were considered the catalytic residues in *PbFuc* (Shaikh et al. 2013). The results indicated that *PbFuc* should be a novel  $\alpha$ -L-fucosidase.

### Expression and purification of *PbFuc*

The  $\alpha$ -L-fucosidase gene (*PbFuc*) was successfully expressed in *E. coli* BL21 (DE3) as a soluble intracellular protein. The enzyme was purified by His-tag Ni-IDA affinity chromatography with a yield of 47.1% (Table 1). SDS-PAGE analysis of purified protein showed that the molecular mass of *PbFuc* was approximately 50 kDa (Fig. 2). The specific activity of purified *PbFuc* was 26.3 U/mg using pNP-FUC as substrate (Table 1).

### Characterization of the purified *PbFuc* for hydrolytic activity

*PbFuc* was optimally active at pH 5.0 in 50 mM citrate buffer (Fig. 3a), and it exhibited broad pH stability from pH 4.0 to 11.0 (Fig. 3b). The optimal temperature of *PbFuc* was 35 °C (Fig. 3c) and the enzyme was stable up to 40 °C (Fig. 3d). The half-lives of *PbFuc* at 35 °C, 40 °C, and 45 °C were determined to be 555 min, 51 min, and 2.8 min, respectively (Fig. 3e). The substrate specificity of *PbFuc* towards different glycosides with fucose residues was examined (Table 2). The enzyme showed high specific activity towards pNP-FUC (26.3 U/mg). It also exhibited hydrolysis activity towards 3'FL (8.3 U/mg) and 2'FL (3.0 U/mg), while *PbFuc* did not show detectable activity towards 3FL, XyG, and fucoidan.

The effect of various metal ions and chemical reagents on the activity of *PbFuc* was studied (Table 3). Ag<sup>+</sup> completely inhibited the enzyme activity, whereas Ca<sup>2+</sup>, Fe<sup>3+</sup>, Cr<sup>3+</sup>, Co<sup>2+</sup>, EDTA,  $\beta$ -mercaptoethanol, and SDS inhibited enzyme activity by 15.9%, 13.6%, 10.9%, 10.7%, 14.1%, 14.9%, and 25.9%, respectively. Mg<sup>2+</sup>, Mn<sup>2+</sup>, Zn<sup>2+</sup>, Cu<sup>2+</sup>, Ni<sup>2+</sup>, Na<sup>+</sup>, K<sup>+</sup>, and Ba<sup>2+</sup> did not influence the enzyme activity of *PbFuc*. The kinetic parameters  $K_m$  and  $V_{max}$  of *PbFuc* towards pNP-FUC were determined to be 0.77 mM and 27.8  $\mu$ mol/min/mg, respectively (data not shown).

### Transglycosylation activity of *PbFuc*

The transglycosylation activity of *PbFuc* was evaluated using pNP-FUC as donor and ten sugars as acceptors. *PbFuc* demonstrated its ability to catalyze the transglycosylation of some acceptors including glucose, galactose, arabinose, ribose, xylose, lactose, and maltose to yield new oligosaccharides detected on TLC (Fig. S1).

### Fucosyllactose synthesis, purification, and identification

Among various fucosyl-oligosaccharides synthesized by *PbFuc*, the oligosaccharides produced using lactose as acceptor were analyzed by HPLC (Fig. 4a), then purified and analyzed by MS and NMR. The positive-ion electrospray ionization (ESI) mass spectrum of purified oligosaccharides showed a peak of [M+Na]<sup>+</sup> ion at  $m/z$  value of 511.1611 (Fig. 4b), which is consistent with the molecular weight of fucosyllactose (488.44). <sup>1</sup>H NMR (Fig. 4c) and <sup>13</sup>C NMR (Fig. S2) spectrometric data for the product suggested that the oligosaccharides were composed of 3'-fucosyllactose ( $\alpha$ -L-Fuc-(1,3)-D-Gal $\beta$ -1,4-Glu, 83%) and 2'-fucosyllactose ( $\alpha$ -L-Fuc-(1,2)-D-Gal $\beta$ -1,4-Glu, 17%).

The optimal temperature, pH, enzyme dosage, reaction time, and lactose concentration on the 3'FL and 2'FL synthesis were investigated (Fig. 5). The results indicated that the



**Table 1** Purification of the  $\alpha$ -L-fucosidase (PbFuc) from *Pedobacter* sp. CAU209 expressed in *E. coli*

Purification step	Total activity (U) <sup>a</sup>	Protein (mg) <sup>b</sup>	Specific activity (U mg <sup>-1</sup> )	Purification factor (-fold)	Recovery (%)
Crude enzyme	1206.7	182.8	6.6	1.0	100.0
Ni-IDA	568.4	21.6	26.3	3.98	47.1

<sup>a</sup> Enzyme activity was determined in 50 mM citrate buffer (pH 5.0) at 35 °C for 20 min using 5 mM pNP-FUC as the substrate

<sup>b</sup> Protein concentration was estimated by the Lowry method

temperature did not show obvious effect on the synthesis of 2'FL and 3'FL (Fig. 5a), whereas pH, enzyme dosage, reaction time, and lactose concentration remarkably affected the synthesis of 2'FL and 3'FL (Fig. 5b–e). The optimized conditions for fucosyllactose synthesis by PbFuc were 25 to 40 °C (Fig. 5a), pH 8.5 (Fig. 5b), 0.5 U/mL (Fig. 5c), 3 h (Fig. 5d), and 700 mM (Fig. 5e), respectively. At the optimized conditions, 85% total conversion ratio of 3'FL (70.5%) and 2'FL (14.5%) from pNP-FUC was achieved, respectively.

### Growth test of probiotics and pathogens using the mixture of 3'FL and 2'FL as carbon source

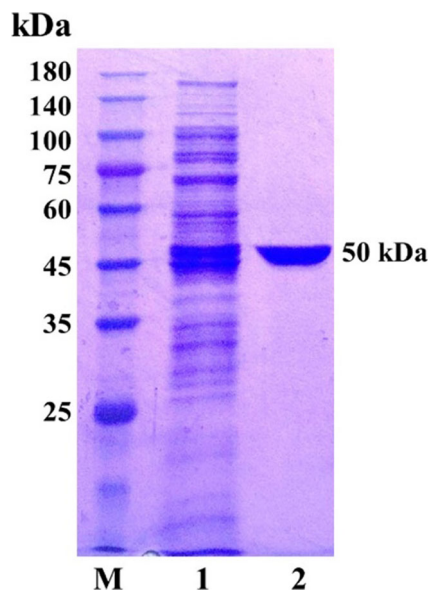
Among the tested probiotics, three *Lactobacillus* and one *Bifidobacterium* strains exhibited higher biomass concentration (OD<sub>595</sub>) in the mixture of 3'FL and 2'FL medium than those of FOS and non-sugar control. The optical densities at 595 nm of *L. casei* subsp. *casei* AS 1.2435 (Fig. 6a), *L. casei* subsp. *casei* NRRL B-1922 (Fig. 6b), *L. delbrueckii* subsp. *bulgaricus* NRRL B-548 (Fig. 6c), and *B. longum* NRRL B-

41409 (Fig. 6d) were 0.362, 0.375, 0.467, and 0.403 at 48 h in the mixture of 3'FL and 2'FL medium, respectively, but were 0.290, 0.320, 0.182, and 0.333 at 48 h in the FOS medium, respectively. Other probiotic strains, including *L. rhamnosus* AS 1.2466, *L. acidophilus* NRRL B-4495, *L. brevis* NRRL B-4527 (ATCC 14869), *L. rod* NRRL B-4391, *L. delbrueckii* subsp. *Lactis* AS 1.2132 (JCM 1248), *L. reuteri* CICC-6132, *B. infantis* NRRL B-41661, *B. adolescentis* ATCC 15703, and *B. breve* NRRL B-41408, grew poorly in the mixture of 3'FL and 2'FL medium, whereas *B. bifidum* NRRL B-41410 did not grow in the mixture of 3'FL and 2'FL medium (data not shown). While supplementation of 2'FL in the MRS medium promoted the growth of *L. casei* subsp. *casei* AS 1.2435 (Fig. S3a), and weakly promoted the growths of *B. longum* NRRL B-41409 (Fig. S3d), *L. rhamnosus* AS 1.2466, *L. reuteri* CICC-6132, and *B. adolescentis* ATCC 15703 (data not shown), other probiotic strains almost did not grow in the 2'FL medium (data not shown).

For pathogenic strains, none of four pathogens could utilize the mixture of 3'FL and 2'FL (Fig. 7) or 2'FL (Fig. S4). The optical densities at 595 nm of *E. coli* ATCC 11775 (Fig. 7a), *S. enterica* AS 1.1552, (Fig. 7b), *L. monocytogenes* CICC 21635 (Fig. 7c), and *S. aureus* AS 1.1861 (Fig. 7d) were 0.091, 0.074, 0.086, and 0.095 after incubation in the mixture of 3'FL and 2'FL medium for 24 h, while were 0.179, 0.186, 0.199, and 0.109 when incubated in the FOS medium for 24 h, and were 0.091, 0.085, 0.085, and 0.089 when incubated with non-sugar MRS broth for 24 h. Also, the optical densities at 595 nm of four pathogens in 2'FL medium were close to non-sugar groups (Fig. S4).

### Discussion

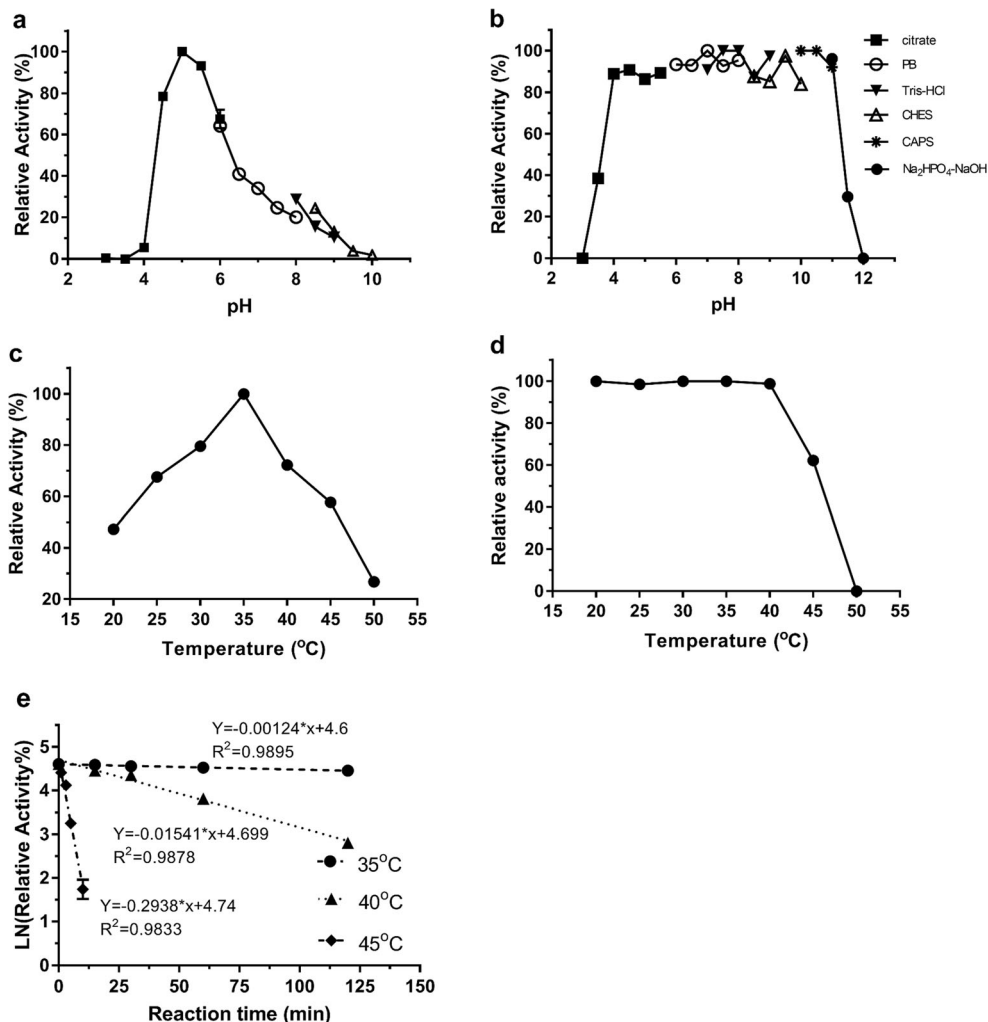
Enzymatic production of fucosyllactoses has acquired much interest owing to the specific synthesis of glycoside linkages and mild reaction conditions (Hayes and Pietruszka 2017). However, the reported  $\alpha$ -L-fucosidases are usually less efficient due to the hydrolysis activity towards synthesized products (Guzmán-Rodríguez et al. 2019). Thus, the discovery of novel  $\alpha$ -L-fucosidases with high transfucosylation activities is very important for fucosyllactose synthesis. In this study, we



**Fig. 2** SDS-PAGE of the purification of PbFuc from *Pedobacter* sp. CAU209. Lane M, molecular weight protein standards; lane 1, crude enzyme; lane 2, purified PbFuc after Ni-IDA column purification



**Fig. 3** Optimal pH (a), pH stability (b), optimal temperature (c), thermostability (d), and thermal denaturing half-lives (e) of the purified  $\alpha$ -L-fucosidase from *Pedobacter* sp. CAU209. Symbols for optimal pH and pH stability: citrate buffer (■) pH 4.0–6.0; phosphate buffer (○) pH 6.0–8.0; Tris-HCl buffer (▼) pH 6.0–9.0; CHES buffer (▲) pH 8.0–10.0; CAPS buffer (\*) pH 10.0–11.0, Na<sub>2</sub>HPO<sub>4</sub>-NaOH buffer (●) pH 11.0–12.0. Symbols for thermal inactivation: 35 °C (●), 40 °C (▲), 45 °C (◻). All experiments were performed with three parallels



described a novel  $\alpha$ -L-fucosidase (PbFuc) from *Pedobacter* sp. CAU209 and the synthesis of 2'FL and 3'FL by an efficient enzymatic method. Also, the prebiotic effect of the mixture of 2'FL and 3'FL was investigated.

A putative  $\alpha$ -L-fucosidase candidate gene (*PbFuc*) was cloned from *Pedobacter* sp. CAU209; the amino acid

**Table 2** Substrate specificity of  $\alpha$ -L-fucosidase (PbFuc) from *Pedobacter* sp. CAU209

Substrate	Specific activity (U mg <sup>-1</sup> ) <sup>a</sup>	Relative activity (%)
pNP- $\alpha$ -L-fucopyranoside	26.3 ± 0.60	100
3'-fucosyllactose	8.26 ± 0.62	37.8 ± 3.9
2'-fucosyllactose	3.04 ± 0.14	13.9 ± 1.0
3-fucosyllactose	ND	ND
XyG	ND	ND
Fucoidan	ND	ND

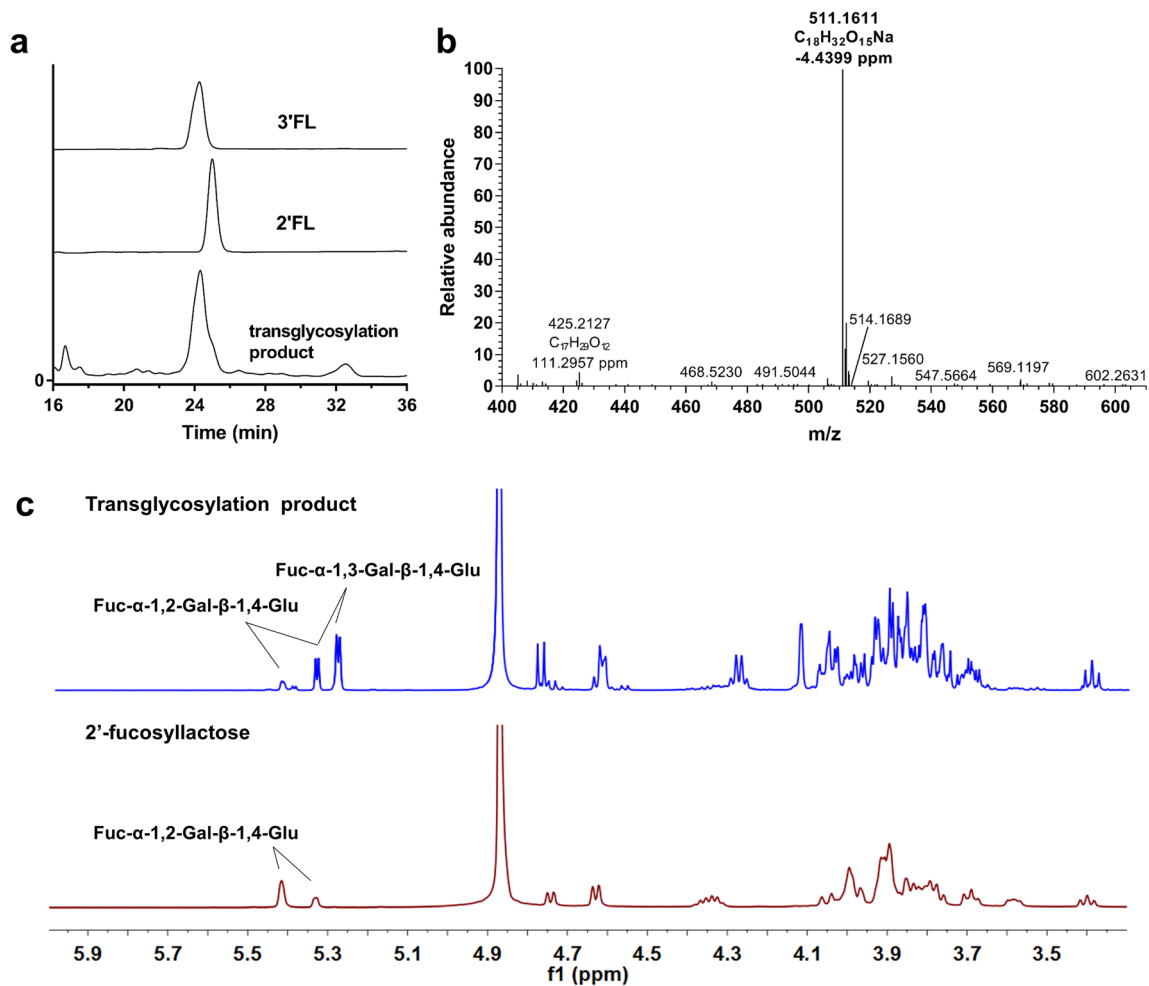
<sup>a</sup> The enzyme activity was measured in 50 mM citrate buffer (pH 5.0) at 35 °C for 20 min using 5 mM different substrates

**Table 3** Effects of metal ions and reagents on the activity of PbFuc

Metal ions (1 mM)	Specific activity (U mg <sup>-1</sup> ) <sup>a</sup>	Relative activity (%)
Control	26.3 ± 0.15	100
Cu <sup>2+</sup>	23.9 ± 0.13	91.0
Ni <sup>2+</sup>	24.8 ± 0.27	94.4
Mg <sup>2+</sup>	27.9 ± 0.01	106.7
Mn <sup>2+</sup>	27.4 ± 0.31	104.0
K <sup>+</sup>	23.7 ± 0.37	90.2
Na <sup>+</sup>	25.9 ± 0.37	98.6
Fe <sup>2+</sup>	27.6 ± 0.19	105.1
Ca <sup>2+</sup>	22.1 ± 1.17	84.1
Ba <sup>2+</sup>	24.1 ± 0.06	91.8
Zn <sup>2+</sup>	28.1 ± 0.88	107.1
Ag <sup>+</sup>	1.2 ± 0.05	0.05
Fe <sup>3+</sup>	22.7 ± 0.19	86.4
Cr <sup>3+</sup>	23.4 ± 1.09	89.1
Co <sup>2+</sup>	23.5 ± 0.01	89.3
SDS	19.5 ± 0.30	74.1
EDTA	22.6 ± 0.83	85.9
$\beta$ -mercaptoethanol	22.3 ± 0.25	85.0

<sup>a</sup> The enzyme activity was measured at 35 °C in 50 mM citrate buffer (pH 5.0) for 20 min using 5 mM pNP-FUC as the substrate after pretreatment at 25 °C for 30 min in the presence of 1 mM various metal ions and reagents





**Fig. 4** HPLC chromatogram of the transglycosylation reaction products with PbFuc (a). MS spectra of the purified oligosaccharides synthesized by PbFuc (b). Mass spectra was recorded on an instrument (Thermo Scientific™ Q Exactive™) equipped with an ESI source in positive-ion mode. One-dimensional <sup>1</sup>H NMR spectra of oligosaccharides synthesized

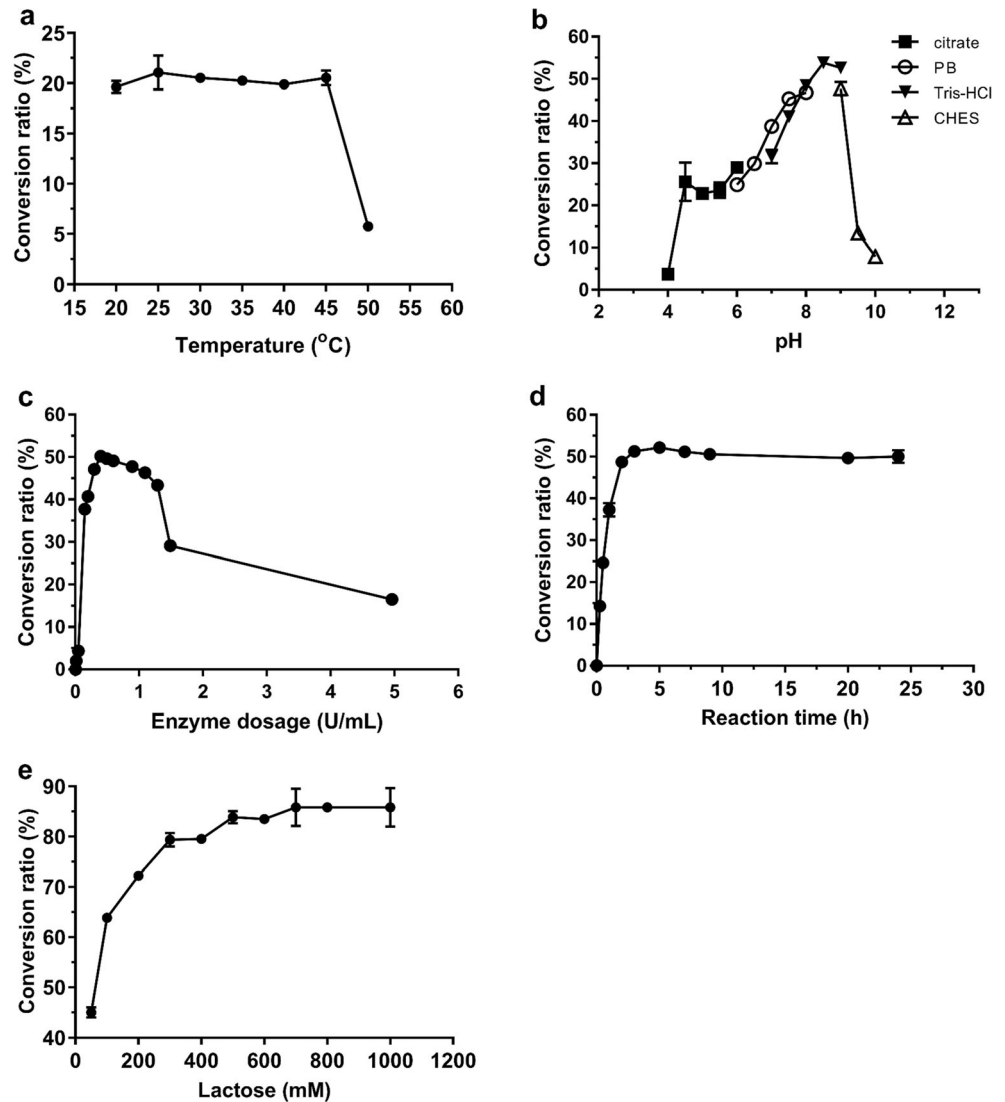
by PbFuc (c). One-dimensional <sup>1</sup>H and NMR spectra were performed on a 500 MHz Varian VNMR SYSTEM™ at 298 K. Chemical shifts are expressed in ppm referenced to DSS (4,4-dimethyl-4-silapentane-1-sulfonic acid)

sequence of PbFuc shared relatively high similarities with several known GH family 29 α-L-fucosidases, especially with the highest identity of 36.8% to that of the α-L-fucosidase (α-L-f1wt) from *P. thiaminolyticus* (Fig. 1), suggesting that it should be a new member of GH family 29 α-L-fucosidases. It has been reported that GH 29 family α-L-fucosidases have two catalytic residues, including a highly conserved nucleophile Asp residue and a structurally conserved general acid/base Glu residue (Sulzenbacher et al. 2004; Lammerts van Bueren et al. 2010; Sakurama et al. 2012). In PbFuc, aspartic acid (D204) and glutamic acid (E260) were predicted to be the putative nucleophilic and acid/base residues, respectively (Fig. 1). Based on the phylogenetic relationship clustering and substrate specificity, the GH family 29 has been further divided into two subgroups (GH 29A and GH 29B). GH family 29A α-L-fucosidases usually have relatively diverse substrate specificities, while

GH 29B α-L-fucosidases display specific activity towards α-1,3/4 linkages (Cao et al. 2014; Lezyk et al. 2016; Zeuner et al. 2018). PbFuc exhibited hydrolysis activity towards pNP-FUC and α-1,2/3 linkages, indicating that it should belong to GH29A, which is similar to several α-L-fucosidases from *Alcaligenes* sp., *X. campestris*, *T. maritima*, and *T. forsythia* (Zeuner et al. 2018).

Generally, most of GH 29A α-L-fucosidases have relatively low molecular masses. PbFuc had a molecular mass of 50 kDa determined by SDS-PAGE (Fig. 2), which is similar to some bacterial α-L-fucosidases, such as TfFuc1 from *T. forsythia* (51 kDa), TmαFuc from *T. maritima* (52 kDa), and iso1 from *P. thiaminolyticus* (51.2 kDa) (Zeuner et al. 2018; Benešová et al. 2013). The optimal pH of PbFuc was 5.0 (Fig. 3a), which is comparable with α-L-fucosidases from *F. oxysporum* strain 0685 (Paper et al. 2013), and *X. campestris* (Dupoirion et al. 2015). PbFuc was stable within

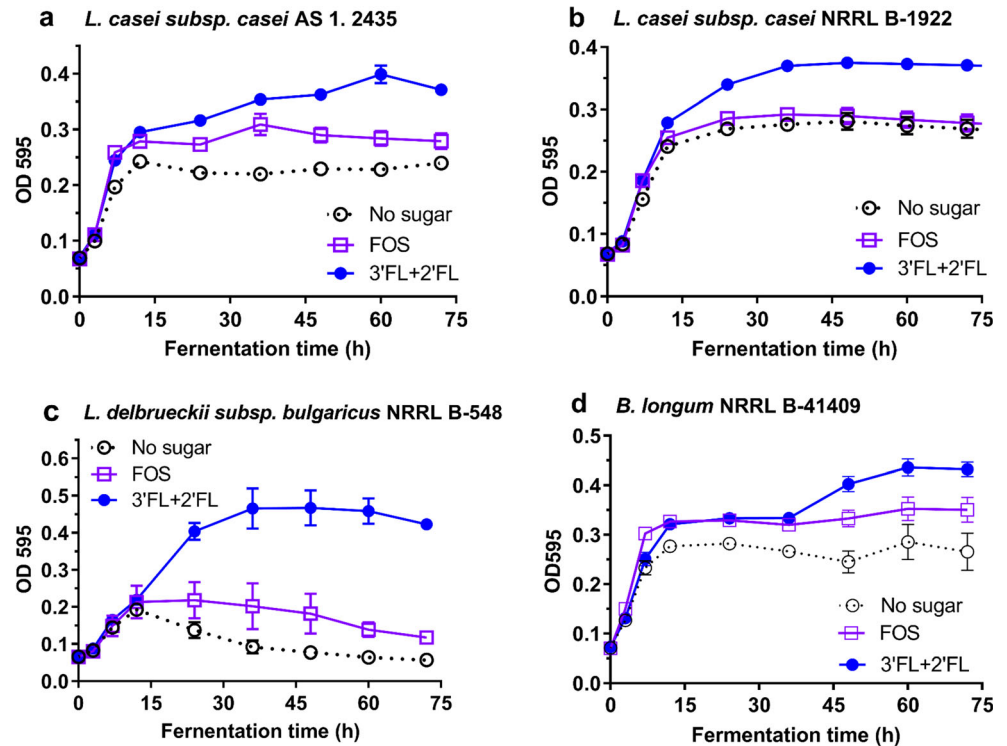
**Fig. 5** Effects of temperature (a), pH (b), enzyme dosage (c), reaction time (d), and lactose concentrations (e) on the synthesis of 3'FL and 2'FL by PbFuc. The effect of temperature on the synthesis of 3'FL and 2'FL was determined at 20–50 °C in 50 mM citrate buffer pH 5.0. The effect of pH on the synthesis of 3'FL and 2'FL was determined at 35 °C using different buffers with pH ranging from 4.0 to 10.0. The effect of enzyme dosage (0.05–5 U/mL) on the synthesis of 3'FL and 2'FL was determined in 50 mM Tris-HCl buffer pH 8.5 at 35 °C. The effect of reaction time on the synthesis of 3'FL and 2'FL was determined in 50 mM Tris-HCl buffer (pH 8.5) with PbFuc (0.5 U/mL); samples were withdrawn at different times within 24 h. The effect of lactose concentrations (0.05–1 M) on the synthesis of 3'FL and 2'FL was determined in 50 mM Tris-HCl buffer (pH 8.5) at 35 °C with 0.5 U/mL PbFuc



a wide pH range 4.0–11.0 (Fig. 3b), which is more stable than most of the reported  $\alpha$ -L-fucosidases from *B. thetaiotaomicron* (pH 4–9, Sakurama et al. 2012), *F. oxysporum* strain 0685 (pH 4–6, Paper et al. 2013), and *Thermus* sp. strain Y5 (pH 4–7.5, Eneyskaya et al. 2001). Typically, bacterial  $\alpha$ -L-fucosidases have optimal temperature of 30–45 °C. PbFuc showed optimal activity at 35 °C (Fig. 3c), which is similar to those of  $\alpha$ -L-fucosidases from *B. thetaiotaomicron* (37 °C, Sakurama, et al. 2012), and *X. campestris* pv. *campestris* (37 °C, Dupoirion et al. 2015). The  $K_m$  value of PbFuc for pNP-FUC was 0.72 mM, which is lower than that of  $\alpha$ -L-fucosidase from *B. thetaiotaomicron* (BT 2970, 1.5 mM, Akurama et al. 2012), whereas is higher than those of the  $\alpha$ -L-fucosidases (iso1 and iso2) from *P. thiaminolyticus* (0.44 and 0.52 mM, Benešová et al. 2013, 2015), Mfuc5 from soil-derived metagenome library (0.13 mM, Lezyk et al. 2016), and Tm $\alpha$ Fuc from *T. maritima* (0.034 mM, Zeuner et al. 2018).

Many GH family 29  $\alpha$ -L-fucosidases with transglycosylation activities can be used to produce fucosylated oligosaccharides (Liu et al. 2016). Recently, several natural fucosyl substrates have been explored as donors in enzymatic synthesis of fucosyllactose, such as fucosylated XyG, fucoidan, and fucosylated chondroitin sulfate (Guzmán-Rodríguez et al. 2019). However, only few GH family 29  $\alpha$ -L-fucosidases possessed the ability to transfer fucose from XyG to lactose, and the product yields were low, such as  $\alpha$ -L-fucosidase FgFCO1 from *F. graminearum* (Zeuner et al. 2018) and Mfuc5 from a soil metagenome (Lezyk et al. 2016). In our study, PbFuc displayed no hydrolytic activity towards XyG or fucoidan (Table 2). Therefore, the transglycosylation activity of PbFuc was only studied using pNP-FUC. PbFuc was capable of catalyzing transglycosylation reaction using pNP-FUC as donor and different sugars as acceptors (Fig. S1). The transglycosylation property is similar to several  $\alpha$ -L-fucosidases from *P. thiaminolyticus* (Benešová et al. 2013, 2015), *Alcaligenes* sp. (Murata et al.

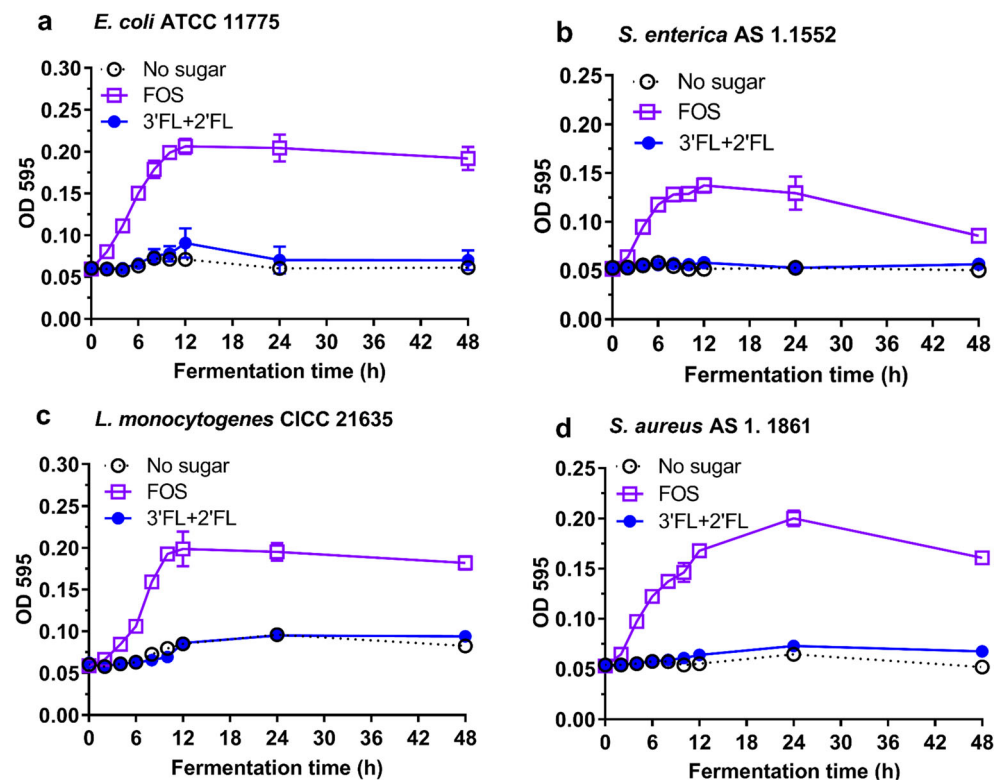
**Fig. 6** Cell growth of *L. casei* subsp. *casei* AS 1.2435 (a), *L. casei* subsp. *casei* NRRL B-1922 (b), *L. delbrueckii* subsp. *bulgaricus* NRRL B-548 (c), and *B. longum* NRRL B-41409 (d) with the mixture of 3'FL and 2'FL (●), or FOS (□), or no sugar (○) as the sole carbon source. Cell density (OD<sub>595</sub>) was monitored. All experiments were performed with three parallels



1999), and *Thermus* sp. strain Y5 (Eneyskaya et al. 2001). Especially, PbFuc was efficient in synthesizing fucosyllactoses when pNP-FUC and lactose were used, 85% of pNP-FUC was converted into 3'FL and 2'FL under the optimized conditions

(Fig. 5). The transglycosylation efficiency for 2'FL synthesis (14.5%) by PbFuc is close to that of  $\alpha$ -L-fucosidase FgFCO1 from *F. graminearum* (14%, Zeuner et al. 2018), higher than that of  $\alpha$ -L-fucosidase from *T. maritima* (6.4%), *T. forsythia* (0.7%),

**Fig. 7** Cell growth of *E. coli* ATCC 11775 (a), *S. enterica* AS 1.1552 (b), *L. monocytogenes* CICC 21635 (c), and *S. aureus* AS 1.1861 (d) with the mixture of 3'FL and 2'FL (●), or FOS (□), or no sugar (○) as the sole carbon source. Cell density (OD<sub>595</sub>) was monitored. All experiments were performed with three parallels





and soil-derived metagenome library (Mfuc2, 0.4%, Lezyk et al. 2016). And also its transglycosylation efficiency for 3'FL synthesis (70.5%) is much higher than that of  $\alpha$ -L-fucosidases from *Alcaligenes* sp. (34%, Murata et al. 1999). In addition, the transglycosylation efficiency (85%) for total fucosyllactose synthesis by PbFuc is higher than that of most reported wild-type  $\alpha$ -L-fucosidases, such as  $\alpha$ -L-fucosidases from *T. maritima* (32.5%, Guzmán-Rodríguez et al. 2018 b), *L. rhamnosus* GG (25% Escamilla-Lozano et al. 2019), soil-derived metagenome library (3.9%, Lezyk et al. 2016), and is comparable with that of  $\alpha$ 1,2-L-fucosynthase (AfcA) mutants, using  $\beta$ -L-fucosyl fluoride and lactose to produce 2'FL with a yield of 88% (Sugiyama et al. 2016). So far, fucosyllactoses including 2'FL, 3FL, and 2',3-difucosyllactose (DFL) have been successfully produced by cell factory approaches (Faijes et al. 2019). However, 3'FL has never been produced by cell factory approach since the lack of suitable glycosyltransferase for the specific synthesis of Fuc- $\alpha$ 1,3-Gal linkage in vivo. Thus, compared with the cell factory methods and other reported  $\alpha$ -L-fucosidases, PbFuc possessed a unique advantage in synthesizing 3'FL due to its efficient transglycosylation activity.

Fucosyl-oligosaccharides such as 2'FL, 3FL, and DFL have promoted the growth of some *Bifidobacterium* spp. and *Lactobacillus* spp. (Yu et al. 2013). Also many in vitro studies suggested that 2'FL promote growth of certain *Bifidobacteria* sp., such as *B. longum* subsp. *Infantis* (LoCascio et al. 2007). However, the prebiotic effect of 3'FL still remains unknown. Supplementation of the mixture of 3'FL and 2'FL in the media significantly promoted the growth of *B. longum* NRRL B-41409, *L. delbrueckii* subsp. *bulgaricus* NRRL B-548, *L. casei* subsp. *casei* NRRL B-1922, and *L. casei* subsp. *casei* AS 1.2435, whereas supplementation of 2'FL in the MRS medium only promoted the growth of *L. casei* subsp. *casei* AS 1.2435 (Fig. S3a), and weakly promoted the growths of *B. longum* NRRL B-41409 (Fig. S3d). The results indicated that the mixture of 3'FL and 2'FL synthesized in this study possessed a broader proliferative effect on the selected probiotics (Fig. 6) when compared with commercial 2'FL (Fig. S3). Recently, several GH families 29 and 95  $\alpha$ -L-fucosidases from *B. longum* sp. and *L. casei* sp. have been demonstrated to be related to the fucosylated HMOs metabolism (LoCascio et al. 2007; Sela et al. 2012; Rodríguez-Díaz et al. 2011). Furthermore, growth curves of four pathogenic bacteria in the mixture of 3'FL and 2'FL medium (Fig. 7) and 2'FL medium (Fig. S4) are close to non-sugar control, suggesting that these pathogens could not utilize 3'FL or 2'FL. This finding is in agreement with previous conclusion that 2'FL, 3FL, and other fucosylated HMOs are not considered ideal energy resource for broad-spectrum pathogenic bacteria (Yu et al. 2013; Hoeflinger et al. 2015). Thus, the mixture of 3'FL and 2'FL synthesized in our study can be used as ideal prebiotic for certain beneficial bacteria and gut maturation.

In conclusion, a novel  $\alpha$ -L-fucosidase gene (*PbFuc*) from the *Pedobacter* sp. CAU209 was cloned and heterologously expressed in *E. coli*. PbFuc efficiently synthesized 3'FL and 2'FL using pNP-FUC and lactose owing to its transglycosylation activity. A high total conversion ratio of 85% was obtained under the optimized reaction conditions. Metabolic characteristics of the mixture of 3'FL and 2'FL in bacterial growth assay illustrated its potential to improve gut microbial environment. Thus, this work furnishes a novel and efficient enzymatic strategy to produce valuable functional fucosylated oligosaccharide.

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## Compliance with ethical standards

**Conflict of interest** The authors declared that they have no conflict of interest.

**Human and animal rights** This article does not contain any studies with human participants or animals.

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