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2-*O*-*α*-D-glucosyl glycerol production by whole-cell biocatalyst of lactobacilli encapsulating sucrose phosphorylase with improved glycerol affinity and conversion rate

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Abstract

Background 2-*O*-*α*-D-glucosyl glycerol (2-*α*GG) is a valuable ingredient in cosmetics, health-care and food fields. Sucrose phosphorylase (SPase) is a favorable choice for biosynthesis of 2-*α*GG, while its glucosyl-acceptor affinity and thermodynamic feature remain largely unknown, limiting 2-*α*GG manufacturing.

Results Here, three SPases were obtained from lactobacilli and bifidobacteria, and the one encoded by *Lb. reuteri* SDMCC050455 (LrSP) had the best transglucosylation ability, with 2-*α*GG accounting for 86.01% in the total product. However, the LrSP exhibited an initial forward reaction rate of 11.83/s and reached equilibrium of 56.90% at 110 h, indicating low glycerol affinity and conversion rate. To improve catalytic efficiency, the LrSP was overexpressed in *Lb. paracasei* BL-SP, of which the intracellular SPase activity increased by 6.67-fold compared with *Lb. reuteri* SDMCC050455. After chemically permeabilized with Triton X-100, the whole-cell biocatalysis of *Lb. paracasei* BL-SP was prepared and showed the highest activity, with the initial forward reaction rate improved to 50.17/s and conversion rate risen to 80.79% within 17 h. Using the whole-cell biocatalyst, the final yield of 2-*α*GG was 203.21 g/L from 1 M sucrose and 1 M glycerol.

Conclusion The food grade strain *Lb. paracasei* was used for the first time as cell factory to recombinantly express the LrSP and construct a whole-cell biocatalyst for the production of 2-*α*GG. After condition optimization and cell permeabilization, the whole-cell biocatalyst exhibited 23.89% higher equilibrium conversion and 9.10-fold of productivity compared with the pure enzyme catalytic system. This work would provide a reference for large-scale bioprocess of 2-*α*GG.

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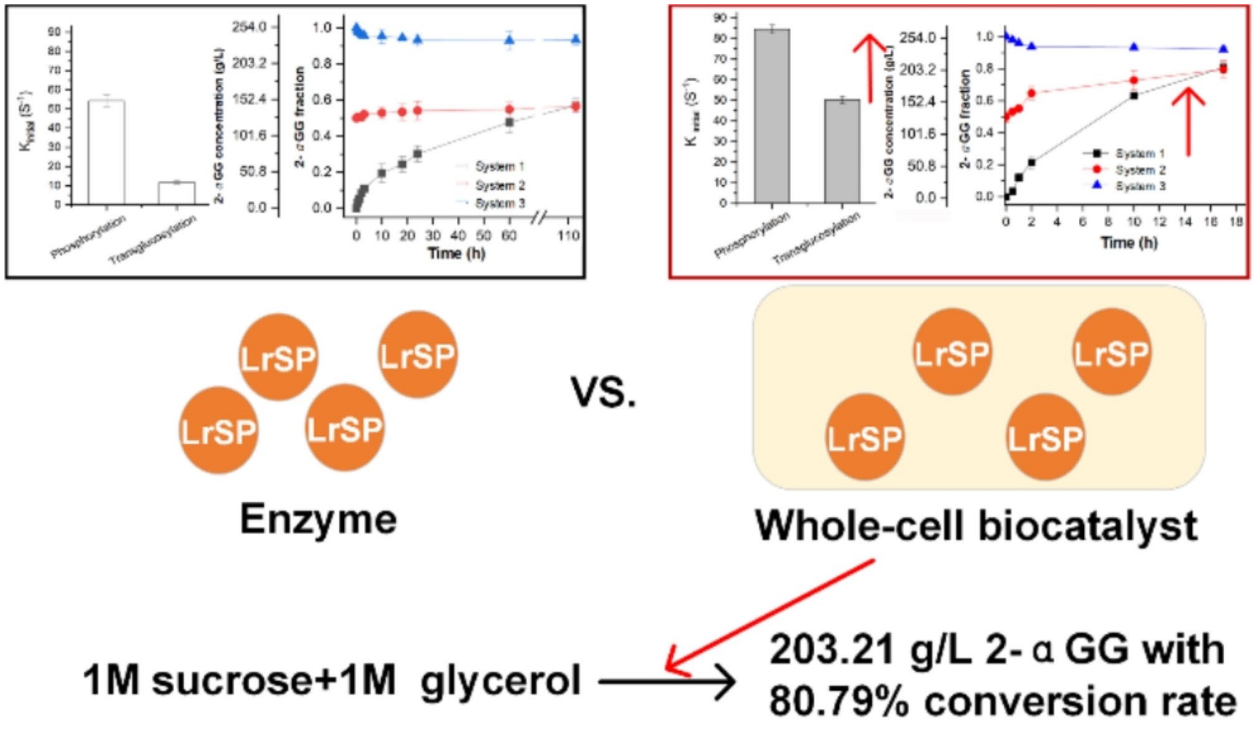
Graphic Abstract



Keywords 2-*α*GG, Sucrose phosphorylase, Glucosyl-acceptor affinity, Equilibrium conversion, Whole-cell biocatalyst

Background

Glucosyl glycerol is a glycoside compound [1]. According to the stereo configuration and location of the glycosidic bond, there are 1-*O*-*α*-D-glucosyl glycerol (1-*α*GG) and 2-*O*-*α*-D-glucosyl glycerol (2-*α*GG). Research found that 2-*α*GG has a better water holding capacity than 1-*α*GG, making it a well-known cosmetics ingredient with moisturizing and antiaging functions [1, 2]. 2-*α*GG can also stabilize protein structure, promote the growth of probiotics, act as a kind of low digestible sweetener, thus showing attractive application potential in health-care and food fields [3]. Therefore, it is valuable to explore efficient approach for 2-*α*GG production.

Several methods have been used for synthesis of 2-*α*GG, including chemical synthesis, microbial fermentation and enzymatic conversion. The product of chemical synthesis is a mixture of different isomers and the 2-*α*GG content is not satisfactory [4], and fermentation with cyanobacteria and other microorganisms produced low yield of 2-*α*GG [5–7]. By comparison, enzymatic synthesis is more suitable for industrialization and gaining more attentions. Many glycoside hydrolases (GHs) including sucrose phosphorylase (EC 2.4.1.7), α-glucosidase (EC. 3.2.1.20), amylosucrase (EC 2.4.1.4), cyclodextrin glucanotransferase (EC 2.4.1.19), GG phosphorylase (EC 2.4.1.332) can catalyze the transfer of glucose moiety from different sugar donors to the C-2 or C-1 position of glycerol via intermolecular transglucosylation, thus generating 2-*α*GG or a mixture of 2-*α*GG and 1-*α*GG [8–12]. Among them, sucrose phosphorylase (SPase) is a favorable enzyme for 2-*α*GG production because of strong regioselectivity [13]. SPases belong to GH13 family, which are commonly encoded by *Leuconostoc mesenteroides*, bifidobacterium and lactobacilli. Besides transglycosylation, SPase catalyzes the reversible conversion of sucrose and phosphate into glucose-1-phosphate and fructose, which is termed phosphorolysis. As the equilibrium constant of phosphorolysis is favorable to the forward reaction, SPase is supposed to serve a catabolic function in vivo [14]. Along with phosphorolysis or transglucosylation, SPase could catalyze the hydrolysis of sucrose to glucose and fructose, but occurs very slowly [15]. Studies of catalytic efficiency of SPase revealed that sucrose is a high-energy glucosyl donor [14–16], while the glucosyl acceptor affinity and thermodynamics in transglucosylation reaction are largely yet unknown.

Suitable approaches could improve 2-*α*GG yield and substrate conversion rate. Routinely, the reaction conditions (temperature, pH, ratio of sucrose to glycerol) have important effects on biosynthesis of 2-*α*GG by SPase. Another effective approach, protein engineering, has been applied to improve the catalytic efficiency of SPases [17, 18]. Besides, whole-cell biocatalysis of SPase has been developed to provide stable reaction conditions and higher 2-*α*GG yield [19, 20]. Recently, *Escherichia coli*, *Bacillus subtilis* and *Corynebacterium glutamicum* have been employed to construct whole-cell biocatalyst of SPase [19–21]. *Lactobacillus paracasei* is an important industrial microorganism for dairy products [22, 23]. As its generally regarded as safe (GRAS) status and efficient protein expression under the NICE (nisin controlled expression) system, *Lb. paracasei* has been adopted as cell factories for the production of bioactive molecules and enzymes of food and health valuable [24]. However, there has been no attempt to develop *Lb. paracasei* whole-cell biocatalysis for 2-*α*GG production.

Here, *Lb. reuteri*, *Lb. acidophilus* and *Bifidobacterium longum* producing SPases were screened using culture method. After cloned and overexpressed in *E. coli*, the SPase from *Lb. reuteri* SDMCC050455 (LrSP) with the best activity was biochemically characterized. To improve catalytic efficiency of the LrSP, a whole-cell biocatalyst was developed based on the *Lb. paracasei* encapsulating LrSP with improved glycerol affinity and equilibrium conversion during 2-*α*GG production. This work would provide a promising approach to achieve industrial production of 2-*α*GG.

Methods

Bacterial strains and growth conditions

Strains and plasmids used in this study are shown in Table 1. *E. coli* DH5α for recombinant DNA manipulation and *E. coli* BL21(DE3) for protein production were grown aerobically in Luria Bertani broth at 37 °C. *Lb. reuteri* SDMCC050455, *Lb. acidophilus* SDMCC050288, *Lb. buchneri* SDMCC050305, *Lb. fermentum* SDMCC050428 and *Lb. paracasei* BL23 [25] were cultivated statically in de Man, Rogosa and Sharpe (MRS) broth at 37 °C. *B. longum* SDMCC050402 was cultivated anaerobically in MRS broth containing 0.05% cysteine at 37 °C. When appropriate, kanamycin (Sangon) was used at 30 μg/mL for *E. coli*, chloramphenicol (Sangon) 2.5 μg/mL for *Lb. paracasei*.

Screening of strains with SPase activity

Lb. reuteri SDMCC050455, *Lb. acidophilus* SDMCC050288, *Lb. buchneri* SDMCC050305, *Lb. fermentum* SDMCC050428 and *B. longum* SDMCC050402 were cultivated in MRS broth or MRS broth with 2% sucrose as carbon source for 14 h. Bacterial cells from 5 mL of the cultures were collected by centrifugation at 6000 g for 3 min, washed twice, and resuspended in PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4) to OD_{600} close to 1.0. Aliquot of cell suspension was supplemented with glass beads (Sigma-Aldrich, St. Louis, MO), and shaken in a bullet blender (Precellys 24, Bertin, France) to crush the cells. By centrifugation of the cell lysate at 12,000 g for 3 min, the intracellular proteins were recovered as crude enzyme for the assay of SPase activity.

Plasmid and recombinant strain construction

Plasmid extraction and bacterial genomic DNA extraction were carried out using Plasmid Mini Kit (Omega) and TIANamp Bacteria DNA kit (TIANGEN, China), respectively. High-fidelity DNA polymerase, restriction enzymes and T4 DNA ligase were used as stated by standard procedures from New England Biolabs (NEB).

Table 1 Bacterial strains and plasmids used in this study

Strains or plasmids	Relevant characteristics	Reference or source
Strains		
E. coli DH5α	Subcloning host	Our lab
E. coli BL21(DE3)	Recombinant protein production	Our lab
Lb. reuteri SDMCC050455[a]	An isolate from probiotic product	Our lab
Lb. acidophilus SDMCC050288	An isolate from probiotic product	Our lab
Lb. buchneri SDMCC050305	An isolate from silage	Our lab
Lb. fermentum SDMCC050428	An isolate from human feces	Our lab
Bifidobacterium longum SDMCC050402	An isolate from human feces	Our lab
Lb. paracasei BL23	A food grade host strain to express the LrSP	[25]
Plasmids		
pET-28a	Kan^r; carries T7 promoter and *lac* operator	Novagen
pNZ8148	Cm^r; lactic acid bacteria and *E. coli* shuttle cloning vector which contains PnisA promoter used for nisin controlled gene expression	[26]
pNSP	Cm^r; Derivative of pNZ8148 with the *LrSP* under the promoter PnisA	This work

[a] "SDMCC" is the abbreviation of "Shandong University Microbiological Culture Collection Center"

Primers used in this work are listed in Table 2. DNA fragments of the SPase from *Lb. reuteri* SDMCC050455, *Lb. acidophilus* SDMCC050288 and *B. longum* SDMCC050402 were PCR amplified using the individual genomic DNA as templates with primers LBF/LBR, LAF/LAR and BLF/BLR. The PCR products were subcloned into the corresponding sites of the pET28a. The resultant plasmids pLrSP, pLaSP and pBlSP were transformed into chemically competent *E. coli* BL21 cells, generating the recombinant strains *E. coli*/pLrSP, *E. coli*/pLaSP and *E. coli*/pBlSP, respectively.

To express the LrSP in *Lb. paracasei* BL23, DNA fragment of the LrSP was PCR amplified from the genomic DNA of *Lb. reuteri* SDMCC050455 with primers LBF2/LBR2, and inserted into the compatible sites of the plasmid pNZ8148 [26], generating the recombinant plasmid pNSP. The pNSP was electroporated into the *Lb. paracasei* BL23 according to the previous method [27], generating recombinant strain *Lb. paracasei* BL-SP.

Sequence analysis

Amino acid sequences of the LrSP, LaSP and BlSP were aligned with those from *B. adolescentis* (accession number AF543301) and *L. mesenteroides* (accession number WP_010279952). Multiple-sequence alignments were performed using Clustal W and ESPript 3.0.

Purification of the SPase protein

The recombinant strains *E. coli*/pLrSP, *E. coli*/pLaSP or *E. coli*/pBlSP were grown in LB broth until OD_{600} reached 0.4. Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.1 mM, and the temperature was lowered to 16 °C for the induction of SPase overexpression. After 16 h of induction, the cells were harvested and the SPase protein was purified by the His-Trap FF column (GE Healthcare) according to the manufacturer's instructions. The buffer solutions for protein purification were binding buffer (20 mM sodium phosphate, 500 mM NaCl, 25 mM imidazole, pH 7.4), wash buffer (20 mM sodium phosphate, 500 mM NaCl, 50 mM imidazole, pH 7.4) and elution buffer (20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4).

Table 2 Primers used in this study

Primers	Sequence (5′-3′)	Restriction site
LBF	CATG*CCATGG*CCCCAATCAAAAACGAAGC	NcoI
LBR	CCG*CTCGAG*TTTTTGTTCCATCACTTTTTCG	XhoI
LAF	CATG*CCATGG*CCAAATTACAAAATAAGGC	NcoI
LAR	CCG*CTCGAG*AAAATTAAAGACCTGATCAT	XhoI
BLF	CATG*CCATGG*CCAAAAACAAAGTGCAACT	NcoI
BLR	CCG*CTCGAG*GTCGATATCGGCAATCGGCG	XhoI
LBF2	TGCA*CTGCAG*ATGCCAATCAAAAATGAAGC	PstI
LBR2	CATG*TCTAGA*CTATTTTTGTTCCATCACTT	XbaI

[a] The restriction sites in the primer sequences are underlined

The SPase protein in elution buffer was dialyzed in 50 mM NaCl and freshly prepared before use. Protein concentration was measured using a BCA protein assay kit (Sangon Biotech, China) with bovine serum albumin as the standard protein.

Enzyme activity assay

Phosphorolysis activity of the LrSP was determined in a 500 μL mixture containing 150 mM sucrose, 50 mM phosphate buffer (pH 6.5) and 25 μg of crude enzyme or 5 μg of the LrSP. After reaction at 50 °C for 10 min, the mixture was supplemented with 500 μL DNS (3,5-dinitrosalicylic acid) and boiled for 10 min, followed by determination of the absorbance at 540 nm. One unit (U) of phosphorolysis activity was defined as the amount of enzyme that released 1 μmol of fructose per minute under reaction conditions. Effects of temperature (from 25 °C to 70 °C) and pH (from 4.0 to 9.0) on the LrSP activity were measured. The stabilities of enzyme were determined at different temperatures and pH values for 1 h.

Transglucosylation activity of the LrSP was determined in a 500 μL mixture containing sucrose, glycerol, buffer and 25 μg of crude enzyme or 5 μg of the LrSP. After reaction at 50 °C for 10 min, 2-*α*GG in the mixture was detected. One unit (U) of transglucosylation activity was defined as the amount of enzyme that produced 1 μmol of 2-αGG per minute under reaction conditions. To determine optimal ratio of donor to acceptor, sucrose and glycerol were used at molar ratios of 3: 1, 2: 1, 1: 1, 1: 2, 1: 3, 1: 4 and 1: 5 in the reaction mixture (pH 6.5, 30 °C). To determine the optimal pH, the reaction mixtures containing 1.0 M sucrose and 1.0 M glycerol were incubated at different pH buffers, including 50 mM of citric acid - sodium citrate buffer (pH 4.0 and pH 5.0), phosphate buffer (pH 5.0–8.0), Tris-HCl buffer (pH 8.0 and 9.0) in 30 °C. To determine the optimal temperature, the reaction mixtures (Tris-HCl buffer pH 8.0) containing 1.0 M sucrose and 1.0 M glycerol were incubated at different temperatures (25 °C to 65 °C).

Preparation of whole-cell biocatalyst

The recombinant *Lb. paracasei* BL-SP was cultured in MRS broth containing chloramphenicol to OD_{600} reaching 0.4, followed by addition of 2 to 12 ng/mL nisin to induce the expression of LrSP. After incubation for 4 h, 8 h, 10 h and 12 h, the cell pellets were collected by centrifugation at 6000 g for 5 min and washed twice with PBS buffer. The cell pellets were supplemented to the reaction mixture to OD_{600} reaching 30 as the whole-cell biocatalyst. The effects of ratio of donor to recipient, temperature and pH on transglucosylation activity of whole-cell biocatalyst were evaluated as described above. To permeabilize the cells, Trition X-100, SDS and Tween-80

at concentrations of 0.5–3.5% were used to incubate the cells at room temperature for 1 h.

Affinities of enzyme and whole-cell biocatalyst to different substrates

The affinities of the LrSP and the whole-cell biocatalyst to different substrates were characterized as the initial reaction rate [28]. For phosphorolysis, the reaction was conducted in a 500 μL mixture containing 200 mM sucrose, 5 μg pure-enzyme (or whole-cell biocatalyst to OD_{600}=30) and 200 mM sodium phosphate buffer (pH 6.0) at 50 ℃. For transglucosylation, the reaction was conducted in a 500 μL mixture containing 200 mM sucrose, 200 mM glycerol, 5 μg pure-enzyme (or whole-cell biocatalyst to OD_{600}=30) and 50 mM Tris-HCl buffer (pH 8.0) at 45 ℃ (or 50 ℃). The reaction was stopped when the sucrose conversion rate did not exceed 10%. Then, fructose in the mixture was measured, and ratio of the amount of fructose to the reaction time represented the initial reaction rate.

Equilibrium conversion

Equilibrium conversions were detected in three 500 μL sucrose-glycerol systems containing 5 μg pure-enzyme (or whole-cell biocatalyst to OD_{600}=30) and 50 mM Tris-HCl buffer (pH8.0) at 45℃ (or 50 ℃). System 1: 1 M sucrose and 1 M glycerol. System 2: 500 mM sucrose, 500 mM glycerol, 500 mM fructose and 500 mM 2-*α*GG. System 3: 1 M fructose and 1 M 2-*α*GG. The reaction was sampled at different time points, and 2-*α*GG was detected in the systems until its content was constant.

HPLC analysis

The concentration of 2-*α*GG was determined on a Shimadzu HPLC system with a RID detector using a Waters Xbridge Amide column (250×4.6 mm, 5 μm) and a mobile phase of 80% acetonitrile at a flow rate of 1.0 mL/min, column temperature at 40 °C.

Statistical analysis

Experiment was carried out in triplicate, and experimental data were described as the mean±standard deviation. Statistical significance between treatment and control conditions was assessed by unpaired 2-tailed Student's t-tests. $P<0.05$ were considered statistically significant.

Results

Screening of strains with SPase activity

SPase catalyzes phosphorolysis of sucrose into fructose and glucose-1-phosphate which subsequently enters the glycolysis pathway to act as carbon and energy sources [14]. Here, four lactobacilli and one bifidobacterium were cultivated in MRS medium supplemented with sucrose as the sole carbon source to detect whether these strains produce SPase. As a result, the five strains could grow in the medium after 14 h cultivation (Fig. 1A), and the biomasses of *Lb. reuteri* SDMCC050455, *Lb. acidophilus* SDMCC050288, *Lb. fermentum* SDMCC050428 were similar as those obtained with glucose as carbon source, while *Lb. buchneri* SDMCC050305 and *B. longum* SDMCC050402 reached about 50%, indicating that all tested strains could catabolize sucrose. The SPase activities could be detected in the crude enzymes of the SDMCC050455, SDMCC050288 and SDMCC050402 cultured in the sucrose medium (14.02, 9.54 and 7.22 U/mg protein), but no SPase activities were detected in the cells cultured in the glucose medium (Fig. 1B), implying sucrose acted as inducer for SPase expression. Unlike these three strains, *Lb. fermentum* SDMCC050428 exhibited SPase activity in both the sucrose and glucose medium.

Using specific primers designed according to the reported sequences, the putative gene encoding SPase was cloned from genomes of the SDMCC050455, as well as SDMCC050288 and SDMCC050402 (*LrSP*, *LaSP* and *BlSP*), but not *Lb. fermentum* SDMCC050428 (data not shown). The *LrSP*, *LaSP* and *BlSP* were 1458, 1443 and 1527 bp in length encoding 485, 480 and 508 amino acid residues, respectively. Sequence alignment showed that amino acid sequences of the LrSP and LaSP had 79.38% and 61.66% identities with that from *L. mesenteroides*, and BlSP had 92.26% identity with that from *B. adolescentis*. The conserved basic residues Asp, Glu and Asp constituting of the catalytic triad of SPase [29] existed in the LrSP, LaSP and BlSP (Fig. 1C), suggesting similar enzymatic functions.

Biochemical characterization of the LrSP

To detect activities of the three putative SPases, the LrSP, BlSP and LaSP were purified after heterologous overexpression in *E. coli* BL21(DE3). Protein bands about 56 kDa, 57 kDa and 56 kDa were observed in the SDS-PAGE (Fig. S1), corresponding to the theoretical molecular mass of each protein plus the C-terminal His_6-tag. The LrSP showed higher phosphorylation and transglucosylation activities than the BlSP and LaSP (Fig. 2A), and the portion of 2-αGG in the total glycosyl glycerols product accounted for 86%, indicating good regioselectivity of the LrSP (Fig. 2B).

Accordingly, the LrSP was further biochemically characterized. As shown in Fig. 3A and B, the optimal phosphorylation activity occurred at 50℃ and pH 6.0, with a specific activity of 223.51 U/mg. The residual enzyme activity of LrSP remained 100% after incubation at 25℃ to 50 ℃ and 80% at 55℃ for 1 h, and above 70% after incubation at pH 6.0–8.0 for 1 h (Fig. 3C and D). The optimal transglucosylation activity was obtained at 45℃

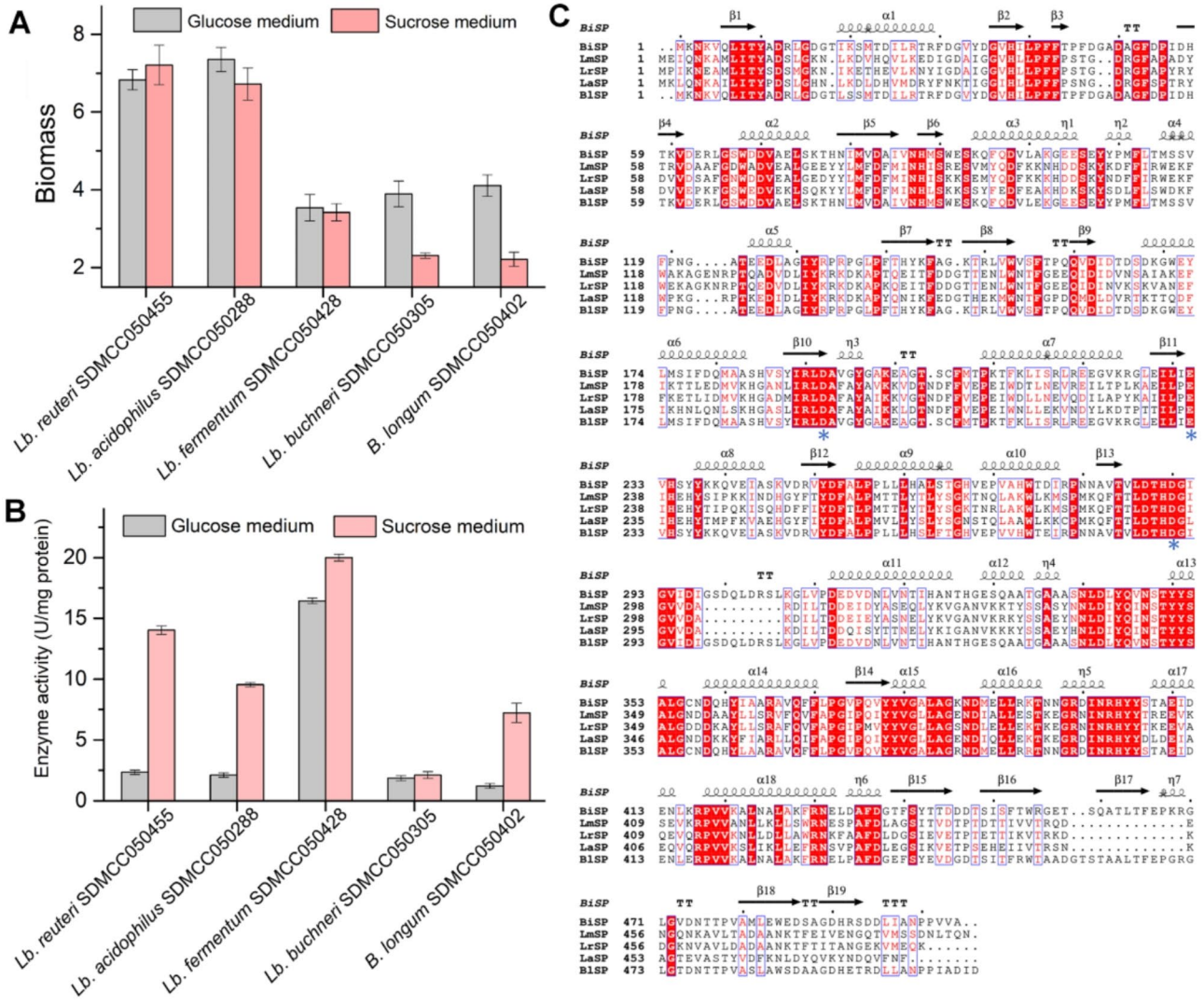


Fig. 1 Screening of strains with SPase activity. **(A)** Biomasses of the five strains cultured in the glucose medium and sucrose medium. **(B)** Intracellular SPase activities of the five strains cultured in the glucose medium and sucrose medium. **(C)** Multiple sequence alignment of the putative SPases from *Lb. reuteri* SDMCC050455 (LrSP), *Lb. acidophilus* SDMCC050288 (LaSP) and *B. longum* SDMCC050402 (BlSP) with those from *Bifidobacterium adolescence* (BiSP, accession number AF543301) and *Leuconostoc mesenteroides* (LmSP, accession number WP_010279952). The alignment was generated using ClustalW, and the figure was prepared using ESPript. Secondary structural elements of BiSP (PDB: 1R7A) are shown as α-helices (coils; α1-α18) and residue numbering across the top refers to the BiSP sequence. The blue stars indicate the conserved basic residues of the catalytic triad, Asp-Glu-Asp

and pH 8.0 at the molar ratio of sucrose and glycerol of 1:1 (Fig. 3E and G).

To compare catalytic efficiency of the LrSP in the phosphorylation and transglucosylation, the initial forward reaction rates ($k_{initial}$) were determined under the optimal conditions. Table 3 showed that the $k_{initial}$ of transglucosylation was 11.83/s, significantly lower than phosphorylation, indicating that glycerol was a low affinity glucosyl-acceptor compared with the phosphage group. Further determination of reaction equilibrium found that the LrSP reached an equilibrium conversion of 56.90% in 110 h, with144.36 g/L of 2-*α*GG yield from 1 M sucrose and 1 M glycerol (Fig. 3H).

Recombinant expression of the LrSP in *Lb. paracasei* BL-SP

To improve catalytic efficiency in transglucosylation, the LrSP was recombinantly expressed in a GRAS host *Lb. paracasei* BL23 under the control of NICE system (Fig. 4A). The recombinant strain *Lb. paracasei* BL-SP was cultivated in MRS medium and induced by nisin of different concentrations. SDS-PAGE analysis and enzymatic detection showed that the LrSP was successfully expressed and the similar expression levels were obtained under the induction of 2 to 12 ng/mL nisin (Fig. S2). Induction duration showed obvious effect on the expression level of LrSP, as the best expression level and enzyme

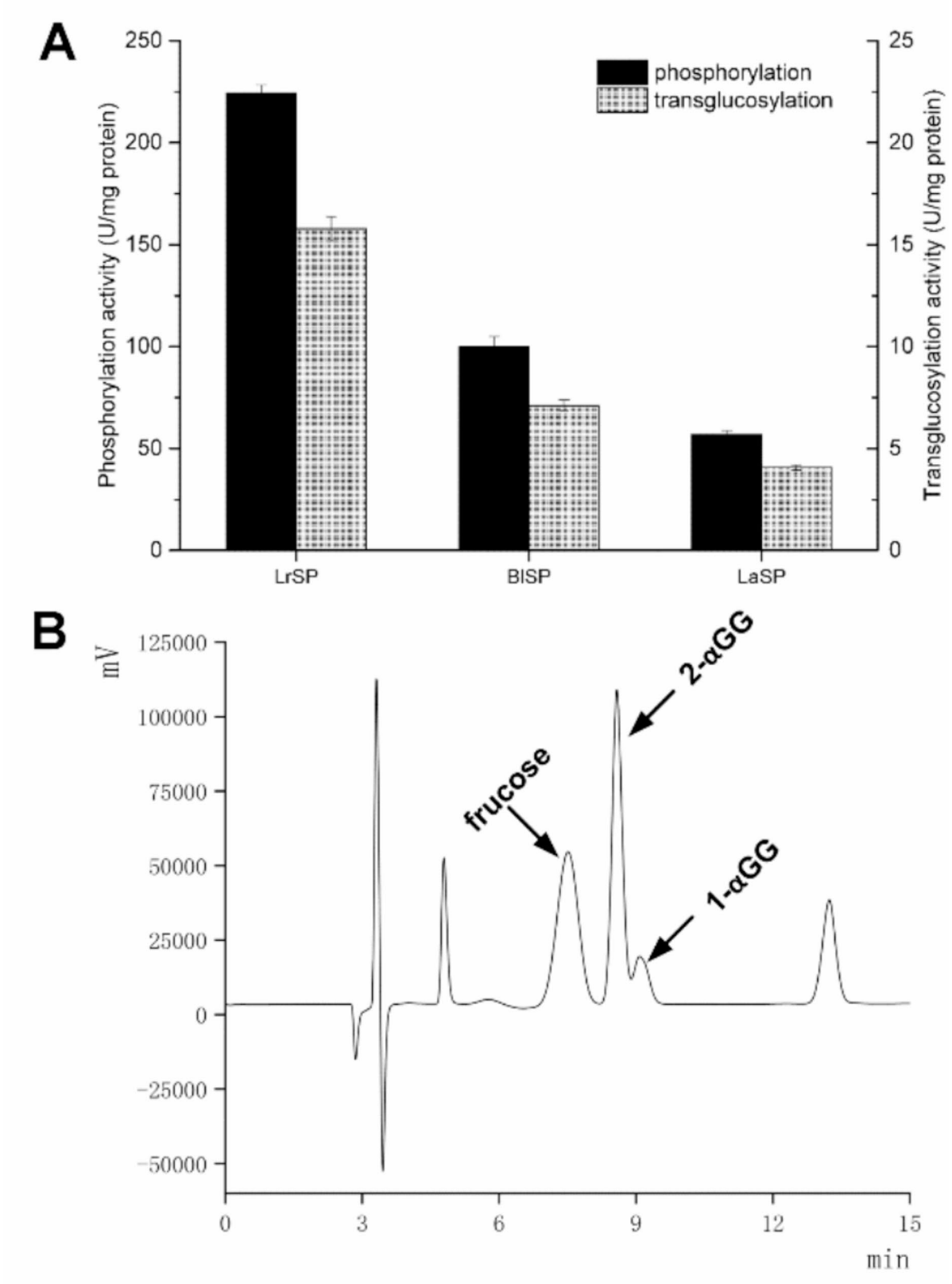


Fig. 2 Activities of the SPases from *Lb. reuteri* SDMCC050455 (LrSP), *B. longum* SDMCC050402 (BlSP) and *Lb. acidophilus* SDMCC050288 (LaSP). **(A)** Phosphorylation and transglucosylation activities of the LrSP, BlSP and LaSP. **(B)** HPLC analysis of the total glycosyl glycerols products of the LrSP

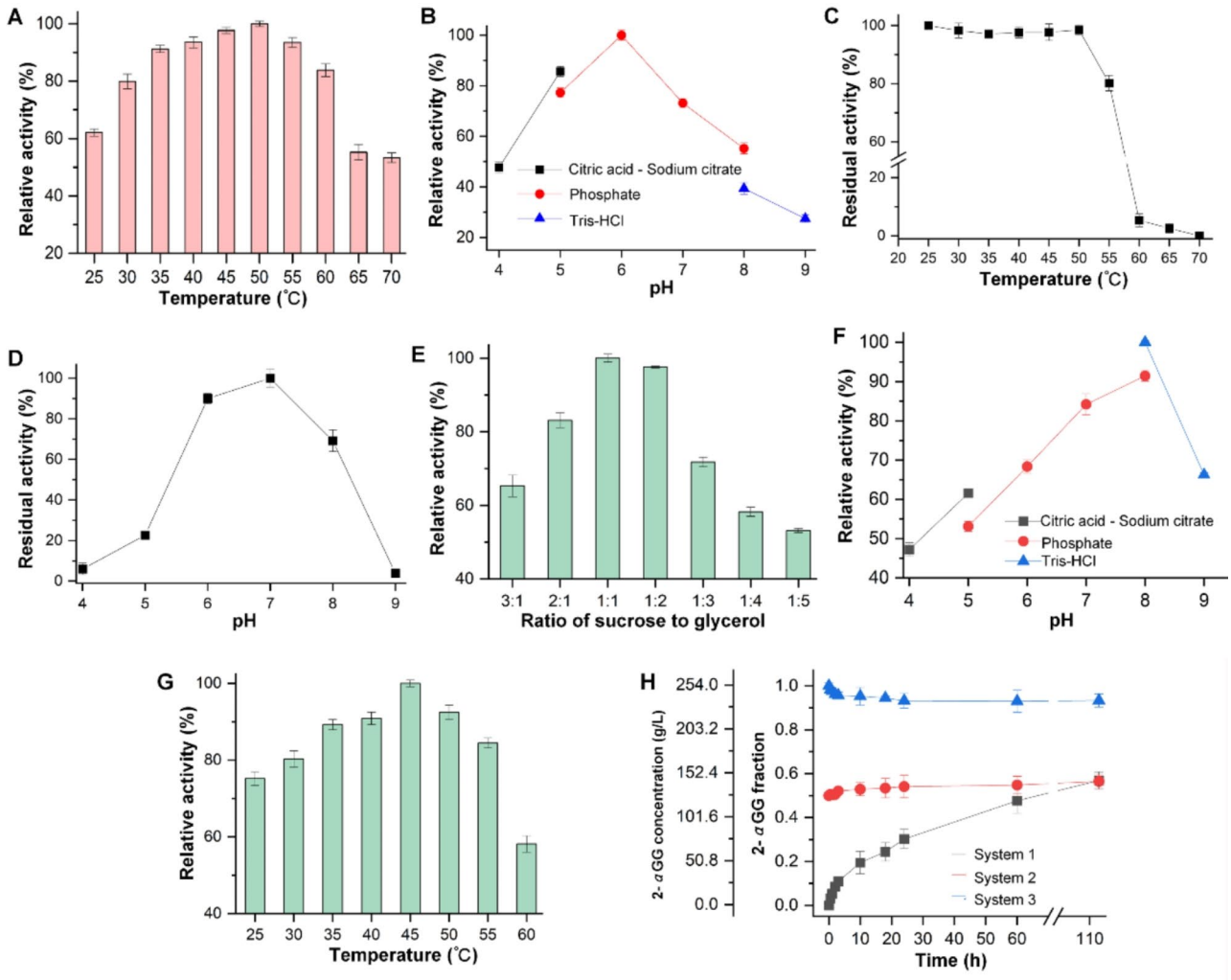


Fig. 3 Biochemical characterization of the LrSP. **(A)** and **(B)** Optimal temperature and pH in the phosphorylation. **(C)** and **(D)** Thermal stability and pH stability in the phosphorylation. The stabilities of enzyme were determined at different temperatures or pH values for 1 h. **(E)**, **(F)** and **(G)** Optimal ratios of sucrose to glycerol, pH and temperature in the transglucosylation. **(H)** Equilibrium conversion of the LrSP starting from three substrate compositions incubated at 45 °C. Additional enzyme was added every 30 h to offset thermal inactivation. System 1, 1 M sucrose and 1 M glycerol; System 2, 500 mM sucrose, 500 mM glycerol, 500 mM fructose and 500 mM 2-*α*GG; System 3, 1 M fructose and 1 M 2-*α*GG

Table 3 Initial forward reaction rate ($k_{initial}$) of the LrSP and the whole cell biocatalyst

Catalytic system	Phosphorylation	Transglucosylation
Recombinant LrSP	54.17 ± 2.97/s	11.83 ± 0.94/s
Whole cell biocatalyst	84.51 ± 2.13/s	50.17 ± 1.77/s

activity were obtained by nisin induction for 10 h (Fig. 4B and C), with SPase activity of 93.50 U/mg protein.

Whole-cell biocatalysis of 2-*α*GG

The optimal conditions for preparing 2-*α*GG by whole-cells of *Lb. paracasei* BL-SP were investigated by varying substrate molar ratio, biotransformation pH, temperature and cell permeabilization. As shown in Fig. 5A and C, the optimal molar ratio of sucrose and glycerol was 1:1, and the optimal pH and temperature were 8.0 and 50 ℃. Then, various permeabilization surfactants were further tested their effects on the enzyme activities of the whole-cells of *Lb. paracasei* BL-SP. The result found that Triton X-100 and SDS had better performances to permeabilize the whole-cells compared with Tween-80 (Fig. 5D). The enzyme activity of the whole-cells was enhanced by 93.96% after permeabilization with 3% Triton X-100 compared with control in PBS buffer.

Under the optimal conditions, the whole-cell biocatalyst of OD_{600}=30 in the reaction system had the equal phosphorylation activity with 5 μg of the LrSP in the reaction system (Fig. S3). Based on this equality relationship, the whole-cell biocatalyst of OD_{600}=30 in the reaction system was subjected to determine the initial forward reaction rates. The result showed that $k_{initial}$ of the whole-cell biocatalyst in transglucosylation was

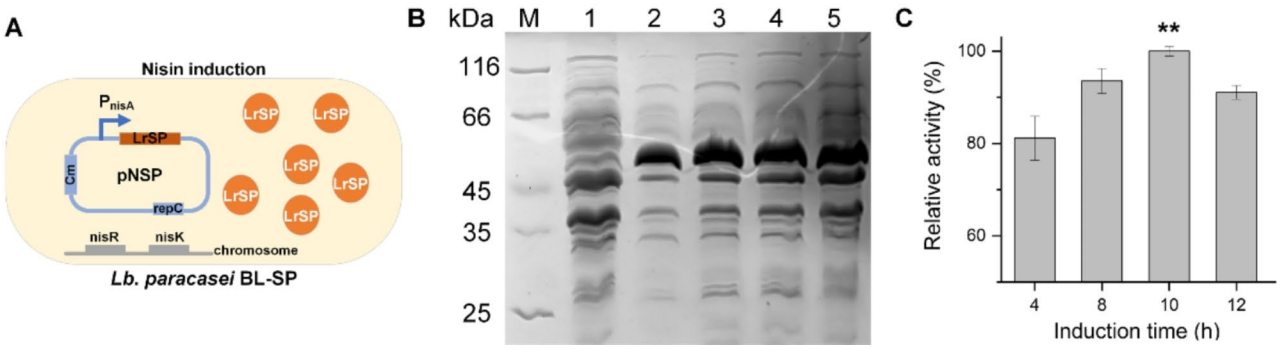


Fig. 4 Recombinant expression of the LrSP in *Lb. paracasei* BL-SP. **(A)** Scheme of recombinant expression of the LrSP under the NICE system. **(B)** SDS-PAGE analysis of the intracellular proteins of *Lb. paracasei* BL23 and *Lb. paracasei* BL-SP after induction by nisin of 6 ng/mL for 4 to 12 h. Lane 1, the intracellular proteins of *Lb. paracasei* BL23 induced for 12 h (control); lane 2, 3, 4 and 5, the intracellular proteins of *Lb. paracasei* BL-SP induced for 4 h, 8 h, 10 h and 12 h, respectively. **(C)** Relative transglucosylation activities of the whole-cells of *Lb. paracasei* BL-SP obtained by nisin induction for 4 h, 8 h, 10 h and 12 h, respectively. $^{**}P < 0.01$

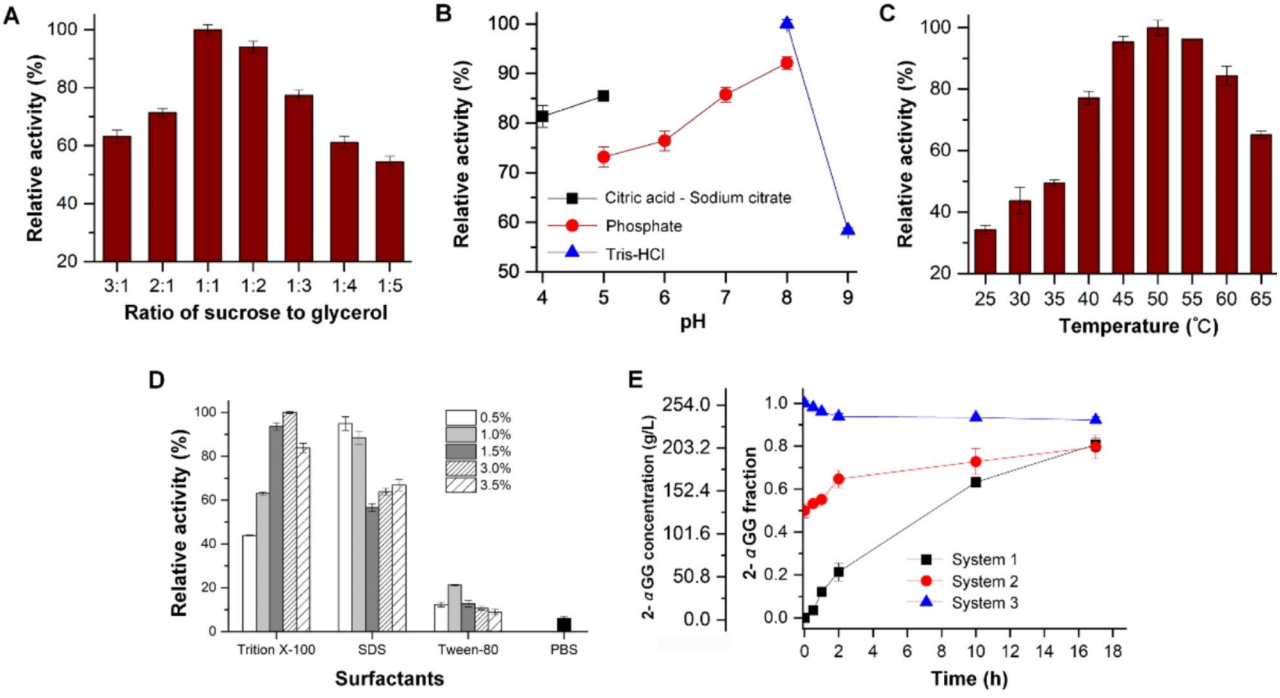


Fig. 5 Optimization of whole-cell biocatalysis for 2-*α*GG production. **(A)**, **(B)** and **(C)** Optimal ratios of sucrose to glycerol, pH and temperature in the transglucosylation catalyzed by the whole-cell biocatalyst of *Lb. paracasei* BL-SP. **(D)** Effects of different surfactants on the activities of the whole-cell biocatalyst. **(E)** Equilibrium conversion of the whole-cell biocatalyst starting from three substrate compositions incubated at 50 °C. System 1, 1 M sucrose and 1 M glycerol; System 2, 500 mM sucrose, 500 mM glycerol, 500 mM fructose and 500 mM 2-*α*GG; System 3, 1 M fructose and 1 M 2-*α*GG

50.17/s, which increased by 4.24-fold compared with the LrSP (Table 3). Determination of reaction equilibrium found that the whole-cells of *Lb. paracasei* BL-SP reached an equilibrium conversion of 80.79% in 17 h (Fig. 5E). Using the whole-cell biocatalysis, the yield of 2-*α*GG was 203.21 g/L and the productivity was 47.06 mM/h.

Discussion

2-*α*GG has broad application potential in cosmetics, food and pharmaceutical fields. At present, the industrial bioprocess for 2-*α*GG is not mature enough, leading to low production efficiency and high production cost. There are few commercial products containing 2-*α*GG on the market, except the Glycoin® of German company BitopAG of which the catalytic enzyme is originating from *L. mesenteroides* [20]. Therefore, efficient enzymes and innovative technologies are both needed to increase 2-*α*GG

production. In this work, three SPases were obtained from two lactobacilli and one bifidobacterium. Among them, the SPase encoded by *Lb. reuteri* SDMCC050455 (LrSP) showed the highest transglucosylation activity and good regioselectivity, but dissatisfactory glycerol affinity and equilibrium conversion. To overcome this limitation, the LrSP was encapsulated in *Lb. paracasei*, leading to improved glycerol affinity and conversion. Using the whole-cell biocatalysis developed here, the yield of 2-*α*GG was 203.21 g/L and productivity was 47.06 mM/h. To our knowledge, this was one of the best productive capabilities of whole-cell biocatalysis of 2-*α*GG.

Previous reports pointed that SPase was mainly carried by bacteria and probably participated in sucrose catabolism [14]. Here, we found that all the tested *Lactobacillus* sp. and *B.* longum could grow in the medium supplemented with sucrose as carbon source, while only *Lb. reuteri* SDMCC050455, *Lb. acidophilus* SDMCC050288 and *B. longum* SDMCC050402 showed inducible SPase activities under the sucrose medium and carried the genes encoding SPases. Other enzymes such as invertase would be responsible for sucrose utilization in *Lb. fermentum* SDMCC050428 and *Lb. buchneri* SDMCC050305, as abundant glucoside hydrolases were presence in lactobacilli [30]. With the development of bioinformatics, genes with interesting function, such as eight SPase genes, could be artificially synthesized to build an enzyme bank for 2-*α*GG production [31]. Here, bacterial strains with SPase activities were obtained by the culture method, which is useful not only for enzyme collection, but also microbial resources with potential to be engineered as cell factories for 2-*α*GG by available genetic tools [32].

Comparison of enzymatic activities of the LrSP, BlSP and LaSP found that the LrSP had the best activity performances as well as good regioselectivity, and the LrSP was thermostable, as its activity could remain 80% after treated at 55℃ for 1 h, agreeing with the previous report [31]. Regioselectivity and thermostability are important feature for industrial application. For example, LmSucP, the most studied and widely applied SPase from *L. mesenteroides*, was more regio-selective for glycerol glycosylation (88%) than BaSucP, SPase from *B. adolescentis* (66%) as well as the LrSP studied here [20], while the BaSucP and LrSP showed higher thermostability than the LmSucP [16, 33]. Further biochemical characterization of the LrSP indicated the optimal conditions for phosphorylation and transglucosylation were different, and there were unfavorable kinetic properties toward glycerol than phosphate group. This limited the efficiency in transglucosylation and resulted in low glycerol conversion rate and long period for equilibrium reaching (Fig. 3H). The relatively unsatisfactory glycerol conversion might be due to the fact that phosphate groups are the natural acceptors of SPase-glucosyl. Therefore, the LrSP has higher affinity to phosphate groups than glycerol, and also higher catalytic efficiency in phosphorylation than in transglucosylation. Similar phenomena have been pointed out for cyclodextrin glycosyltransferase and arabinose isomerase [28, 34]. Here, considering that the hydrolysis of SPase occurs at a low activity (≤50 times of phosphorolysis) [29], the SPase activity of phosphorolysis was determined by the amount of fructose as several previous reports [35–37]. Therefore, the SPase enzyme activity of in phosphorylsis reaction detected here should be slightly higher than its actual activity.

Several strategies have been explored to increase the substrate conversion rates, including novel enzyme exploration, condition optimization, site-directed mutation and whole-cell biocatalysis [18, 28, 34, 38]. Here, the GRAS host *Lb. paracasei* was engineered to overexpress the LrSP, and the resulting SPase activity increased by 6.67-fold compared with the *Lb. reuteri* SDMCC050455. Besides *Lb. paracasei*, another common cell factory *Lactococcus lactis* was also employed to carry the LrSP, whose activity was about 0.93-fold of that in *Lb. paracasei* BL-SP (data not shown). Therefore, the *Lb. paracasei* BL-SP was adopted to develop the whole-cell biocatalyst for 2-*α*GG. Using whole-cell biocatalysis, the bioprocess could be greatly simplified and the production cost is also reduced [19]. What's more, we found that the whole-cell biocatalyst achieved higher glycerol affinity and equilibrium conversion than the purified enzyme (Table 3; Fig. 5E). The possible reason might be that the whole-cell biocatalyst could disproportionately partition substrate and product across their membrane to circumvent the thermodynamic limitation [28, 39]. Cell permeabilization with Triton X-100 or SDS was thought to remove cell surface proteins [28, 40], probably contributing to the exposure of substrate and product channels.

Conclusion

Based on the optimized reaction conditions of whole-cell biocatalyst developed here, the yield of 2-*α*GG was 203.21 g/L with a productivity of 47.06 mM/h from 1 M sucrose and 1 M glycerol. This was one of the best performances reported for whole-cell catalysis of 2-*α*GG using the relative low substrate concentrations. The 2-*α*GG produced by food grade host *Lb. paracasei* would be suitable for cosmetics, food and medicine areas. To further improve 2-*α*GG production, efficient bioprocess would be developed and large-scale reaction still needs to carry out in the future work.

Abbreviations

2-αGG	2-O-α-D-glucosyl glycerol
1-αGG	1-O-α-D-glucosyl glycerol
SPase	sucrose phosphorylase
LrSP	sucrose phosphorylase from Lactobacillus reuteri SDMCC050455

GRAS generally regarded as safe
NICE nisin controlled expression system

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12934-024-02586-9.

Supplementary Material 1

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Author contributions
Yue Cui: Conceptualization, Formal analysis, Investigation, Validation, Writing – original draft. Zhenxiang Xu: Formal analysis, Investigation. Yanying Yue: Formal analysis, Investigation. Wentao Kong: Supervision, Funding acquisition. Jian Kong: Writing – review & editing, Supervision, Conceptualization. Tingting Guo: Writing – original draft, Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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Data availability
The data sets supporting the conclusions of this article are included within the article and its additional files.

Declarations

Ethics approval and consent to participate
This article did not involve any experiment on human participants or animals.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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