Development of A Novel Expression System In Lactic Acid Bacteria Controlled By A Broad-Host-Range Promoter P_{srfA}

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Research

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Abstract

Background:

Lactic acid bacteria (LAB) are exploited for development of gene expression system owing to its health promoting properties and a high degree of safety status. Most of the expression systems were constructed in *Lactobacillus lactis* with inducible promoters. It is necessary to exploit novel promoters to develop LAB host platforms which are indispensable in dairy and health application to satisfy the production demand of increased number of target-genes. Previously, promoter P_{srfA} had been displayed broad host range and used to construct auto-inducible expression system in *B. subtilis* and *E. coli*. In this work, the feasibility of P_{srfA} in lactic acid bacteria (LAB) was estimated.

Results:

Plasmid with the green fluorescent protein (GFP) inserting downstream of P_{srfA} was transformed into *L. casei* 5257, *L. plantarum* 97, *L. fermentum* 087 and *Weissella confusa* 10, respectively. The recombinant strains grew well and displayed different fluorescence which could be detected by spectrophotometer and laser scanning confocal microscope. Moreover, the promoter activity was strain- specifically influenced by particular carbon and nitrogen sources. Heterologous laccase CotA could be expressed by P_{srfA} in *L. casei* 5257-05 and *L. plantarum* 97-06. By adjusting the pH value from 4.5 to 6.5 during incubation, the CotA activity of *L. plantarum* 97-05 and *L. casei* 5257-05 was increased by 137.7 % and 61.5 %, respectively. Finally, the fermentation pH was variably up-regulated along with the production of NADH oxidase controlled by the P_{srfA} and its derivative mutated with core regions.

Conclusions:

These data suggested that P_{srfA} was valid for gene expression in different species of LAB. Moreover, P_{srfA} could be used as an attractive candidate for fine-tuning gene expression in a broad range of procaryotic expression plants.

Introduction

Expression host systems are developed for the expression of heterologous proteins and are becoming more and more attractive along with the development of molecular biology and the emergence of the synthetic biology [1]. So far, the extensively used expression systems were developed in *E. coli*, *Bacillus subtilis* and *Saccharomyces cerevisiae* due to their specific genetic background, mature genetic manipulation and capacity for continuous fermentation [2, 3]. As the developed expression systems are generally have inherent advantages and drawbacks, it is necessary to exploit novel expression host platforms to satisfy the demand of producing the increased number of target-genes for various industrial productions.

Lactic acid bacteria (LAB) are a heterogeneous group of bacteria characterized by their ability to produce lactic acid as a fermentation product, including genera such as *Lactococcus*, *Lactobacillus* and *Streptococcus*. LAB are exploited for development of gene expression system owing to its health promoting properties and a high degree of safety status [4, 5]. Recently, remarkable achievements have been made towards the development of genetic engineering tools and therapeutic delivery systems to produce many different proteins of health interest, such as antigens, cytokines, vitamin, etc [6–8]. The most successfully and widely used expression system is NICE (nisin controlled gene expression) system in *L. lactis* using promoter P_{nisA} which is proved to be a valuable tool for production of heterologous proteins including antimicrobial peptides, membrane proteins and vaccines [9]. Besides, some other inducible expression systems were constructed in LAB with promoters activated by lactose (P_{lacA}), zinc (P_{zn}), xylose (P_{xyIT}), fructo-oligosaccharide (P_{fos}), etc [10]. However, limited constitutive promoters were isolated which were able to be used for the production of food additives or application in the intestinal environment as LAB comprise a probiotic background. For example, promoter P_{11} (a synthetic sequence based on an rRNA promoter from *L. plantarum* WCSF1), promoter P_{tuf33} and P_{tuf34} (the promoter regions upstream of the gene encoding the putative translation elongation factor TU from *L. plantarum* CD033 and from *L. buchneri* CD034) were demonstrated to be feasible for strong constitutive gene expression in *L. plantarum* [11].

The preferred expression system is selected depending upon the required quality and quantity of the target protein. Furthermore, the cost, availability and convenience of an optimal expression system should also be taken into consideration before making an informed choice of a system with which to express a foreign protein [12]. To take an optimal expression system, it is necessary to evaluate the preliminary expression of the interest gene in the possible host cells. As the developed host cells have inherent genetic diversity, construction of cross-species expression system using the same genetic tools is a time- and cost-saving strategy to select the preferred expression cell for the target gene. The promoter, playing the role of expression switch, is commonly strain and context specific and can vary significantly within species. Only a limited number of promoters have been used in a broad range species, such as P_{T7} , P_{xyb} , P_{lac} and P_{BAD} , etc [13–15]. Hence, promoters available for Gram-positive and Gram-negative host strains should be developed to simultaneously examine gene expression in different genetic and physiological backgrounds and thus identify the most suitable expression strain. To date, P_{11} showed cross species activity in *L. plantarum* and *L. sakei* and promoter P_{tuf} was demonstrated highly active in some tested *Lactobacillus* strains which was also recognized in *E. coli* [16].

Hitherto, the development of genetic engineering tools and the molecular characterization have been mainly made in *L. lactis* [5]. Some other LAB are indispensable in dairy and health applications which are potential to be used as production organisms for antimicrobials, exopolysaccharides, flavor-compound and texturing compound. Previously, the promoter of the *srf* operon (P_{srfA}) originated from *B. subtilis*, had been used to construct expression systems in *B. subtilis* and *E. coli* and heterologous proteins were highly produced without inducer [17, 18]. In this work, promoter P_{srfA} was used for construction of expression systems in LAB. The feasibility of promoter P_{srfA} was studied including the range of host strains, the

factors affecting the promoter strength and the applicability of producing heterologous proteins. This work will enrich the efficient genetic toolbox for gene expression in LAB. Meanwhile, it will be helpful for cross-species gene expression or module construction in *E. coli*, *B. subtilis* and LAB.

Materials And Methods

Strains, plasmids and incubated conditions

The bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* JM109 was used as a host for gene cloning. *L. casei* 5257, *L. plantarum* 97, *L. fermentum* 087 and *Weissella confusa* 10 were used for the gene expression. The vector pCold α -Z containing gene *cotA* for enzyme Laccase was kindly given by Zhang Y.Z. (Yancheng Institute of Technology, Jiangsu, China). The media used for *E. coli* and LAB were LB (Luria–Bertani) medium and MRS (de Man, Rogosa and Sharp) medium. When appropriate, growth media for recombinant strains were supplied with ampicillin (10 μ g/mL) and chloramphenicol (10 μ g/mL).

Table 1
Plasmids used in this study

Plasmids	Characteristics	Reference
pMY01	pNE8148 with P _{nisA} replaced by the fragment of P _{srfA} - <i>gfp</i>	[18]
pCold α -Z	pCold α with gene <i>cotA</i>	This work
pMY05	pMY01 with gene <i>gfp</i> replaced by gene <i>cotA</i>	This work
pMY06	pMY01 with gene <i>gfp</i> replaced by gene <i>nox2</i>	This work
pMY07	pMY06 containing promoter P07 (-35 region of P _{srfA} mutated into TTGACA)	This work
pMY08	pMY06 containing promoter P08 (-10 region of P _{srfA} mutated into TATAAT)	This work
pMY09	pMY06 containing promoter P09 (-35 and - 10 regions of P _{srfA} mutated into	This work
pMY10	TTGACA and TATAAT)	This work
pMY11	pMY06 containing promoter P10 (the RBS region of P _{srfA} mutated into	This work
pMY12	TAAGGAGG)	
	pMY06 containing promoter P11 (the CodY site of P _{srfA} deleted with 20bp)	
	pMY06 containing promoter P12 (P _{srfA} deleted the CodY sites)	

Recombinant DNA techniques

Plasmid construction was performed in *E. coli* and DNA extraction was performed by following a standard procedure as previously described [17]. Recombinant plasmids were transformed into LAB as

previously described [16]. The primers used in this study are listed in Table 2. PCR was performed using primerSTAR max DNA polymerase (Takara, Japan). All of the recombinant plasmids constructed in this work were confirmed by DNA sequencing (Shanghai Sangon Biotech Co., Ltd., China).

Table 2
Sequence of primers used in this study

Primers	Sequence
F1	CATTGTCATACCTCCCCTAAT
R1	GGCACTCACCATGGGTACT
F5	GCTTATAAAGATTAGGGGAGGTATGACAATGATGAACCTAGAAAAGTTTG
R5	GCATGCCTGCAGTACCCATGGTGAGTGCCCTAAATGATATCCATCGGCC
F6	AAAGATTAGGGGAGGTATGACAATGATGAAAGTTGCCATTATTGG
R6	TGCCTGCAGTACCCATGGTGAGTGCCCTAATTATTCCGACGATAGC
F7	TGAAACTTTTCACCCATTTTTCGTTGACAAAAACATTTTTTTCATT
R7	CAGTTTAAATGAAAAAATGTTTTTGTCAACGAAAAATGGGTGAAAAG
F8	GGTGATAAAAACATTTTTTTCATTTATAATGAACGGTAGAAAGAT
R8	TATTTTTTATCTTTCTACCGTTCATTATAAATGAAAAAATGTTT
F9	TGAAACTTTTCACCCATTTTTCGTTGACAAAAACATTTTTTTCATT
R9	CATTATAAATGAAAAAATGTTTTTGTCAACGAAAAATGGGTGAAAAG
F10	GCACATGTTCACTGCTTATAAAGTAAGGAGGTATGACAATGATGAAAG
R10	GGCAACTTTTCATCATTGTCATACCTCCTTACTTTATAAGCAGTGAAC
F11	TTCATTTAACTGAACGGTAAACAATGAATAAATAGCCAAAATTGGTT
R11	TTGGCTATTTATTCATTGTTTACCGTTCAGTTTAAATGAAAAAATGT
F12	TTCATTTAACTGAACGGTATCTTATTAGGGTGGGGTCTTGCGGTCT
R12	AAGACCCACCCCTAATAAGATACCGTTCAGTTTAAATGAAAAAATGT

Plasmid pMY01 was linearized using inverse PCR with primers F1 and R1. Gene *cotA* flanked by a 30-bp homology sequence upstream and downstream of the inserted position in pMY01 was amplified from plasmid pCold® using primers F5 and R5. The purified linearized pMY01 and *cotA* fragment were connected according to the manufacturers' protocols of the Clone Express™ II One Step Cloning Kit (Vazyme Biotech Co., Ltd, Nanjing, China). The product was transformed into *E. coli* and the sequenced clone was named as pMY05. To construct plasmid pMY06 was constructed according to the steps described above. Gene *nox* was cloned from *L. plantarum* genome using primers F6 and R6 which was then connected with the purified linear pMY01, yielding plasmid pMY06.

To construct the plasmids containing P_{srfA} with mutation in the core region, plasmid pMY06 was used as the template by means of a megaprimer PCR as previously described [18]. The derivatives of pMY06 (from pMY07 to pMY12 containing the related promoters P07 to P12) with mutation in the core sequence of the promoters were obtained with the corresponding primers.

Cultivation of LAB

A single colony of the appropriate recombinant LAB strain was picked from an MRS agar plates, inoculated in 10 mL of MRS liquid medium and cultured at 37°C for 24 h. Then, 0.3 mL of the culture were inoculated in 10 mL of MRS liquid medium and cultured at 37°C for 12 h to serve as a preculture. Then, 6 mL of the preculture was transferred to 250-mL flasks that contained 200 mL of MRS liquid medium and cultivated at 37°C for 24 h during which the culture medium was sampled with time intervals. After cultivation, the cells and the supernatant were separated by centrifugation. The supernatant was used for pH detection. The harvested cells were washed by PSB buffer (50 mM Tris–HCl, 100 mM NaCl, pH 7.5) for three times, and suspended in an appropriate diluted ratio to be used for detection of fluorescence and enzyme activity.

Detection of cell growth and pH

The cell density was determined by measuring the OD600 with a UV-1800/PC spectrophotometer (MAPADA Instrument Co., Shanghai, Ltd., China). The pH was measured using a laboratory pH meter (Mettler Toledo FE20, Shanghai, China). Each trial was performed in triplicate.

Effect of carbon source, nitrogen source and pH

To analyze the effect of carbon and nitrogen sources, MRS medium with glucose was separately replaced by the equal weight of maltose, fructose and lactose while tryptone replaced by equal weight of soy peptone and polypeptone, respectively. To assay the effect of the pH, sodium hydroxide solution (3 mol/L) was used to control the pH. The strains were cultivated in the MRS medium with the initial pH of 6.5, 7.2, 8.0 and 9.0 to analyze the effect of the initial pH. Besides, to evaluate the effect of the pH during fermentation, the strains were cultivated in the MRS medium and then the pH were separately regulated to 5.5, 6.5 and 7.5 when the pH declined to 4.5 during cultivation. For the above cultivation, assays were done with the usual procedure.

Assay of GFP fluorescence

The fluorescence and the image of the harvested cells were separately detected by spectrophotometer and laser scanning confocal microscope as previously described [18]. All the data were averaged from three independent samples of the same time points.

Assay for NADH oxidase

Three milliliters of the preculture of the suitable strains were incubated in a 250-mL flask loading 100 mL MRS medium and cultivated in a shaker incubator at rotate speed of 120 r/min at 37°C for 15 h. The sample was obtained at 3 h, 9 h and 15 h. After centrifugation, the supernatant and the cells were used for detection of pH and the activity of NADH oxidase, respectively. The harvested cells were disrupted on ice by a cell disruptor (Constant System Ltd., England) and then centrifugated for 10 min (4°C, 10000 r/min). The corresponding supernatant was used for the detection of the protein concentration and the enzyme activity according to the instruction of the NADH oxidase test kit and total protein quantitative assay kit (Jiancheng Bioengineering Institute, Nanjing, China). One unit of activity was defined as the absorbance increase equal to 0.01 in 1 min at 600 nm per milligram protein. The results are the averages of triplicate assays.

Results And Discussion

Feasibility of promoter P_{srfA} in different species of LAB

Genetic engineering tools used for cloning heterologous genes have mainly exploited with *L. lactis* which has been used as the model microorganism in LAB research. Recently, with the increased interest in the probiotic function of the LAB, it is necessary to develop novel expression systems which could work in a broad range of LAB especially in the strains with proven probiotic [19]. Promoter P_{srfA}, originated from *B. subtilis*, had already been used to develop expression system for production of heterologous proteins in *B. subtilis* and *E. coli* in our previous work [17, 18]. In this study, using the GFP as the report protein, the feasibility of P_{srfA} was evaluated in four representative genera of LAB which were widely used in food production.

The recombinant plasmid pMY01 containing the fragment P_{srfA}-*gfp* was separately transformed into *L. casei* 5257, *L. plantarum* 97, *L. fermentum* 087 and *W. confusa* 10, yielding the recombinant strain *L. casei* 5257-01, *L. plantarum* 97 – 01, *L. fermentum* 087 – 01 and *W. confusa* 10 – 01, respectively. After 24 h cultivation, the cell growth and the GFP expression were measured (Fig. 1). The recombinant strains grew well and displayed different fluorescence which were obviously different among the recombinant strains (Fig. 1a). The highest fluorescence was obtained from *L. plantarum* 97 – 01 which was 1.76-, 3.08- and 3.23-times higher than that of *L. casei* 5257-01, *L. fermentum* 087-01 and *W. confusa* 10 – 01, respectively. The fluorescence intensity (defined as fluorescence per biomass of OD₆₀₀) of *L. plantarum* 97 – 01 was ranged from 1.06- to 1.49-times higher than that of the other recombinant strains. Compared with *L. casei* 5257, *L. fermentum* 087 and *W. confusa* 10, *L. plantarum* 97 was demonstrated to be the most feasible host strain to express GFP under the control of P_{srfA}. It was equally shown in different studies that promoter strength was strain and context specific and could vary significantly within LAB [20]. Moreover, the recombinant strains with fluorescence could be obviously detected by laser scanning confocal microscope (Fig. 1b). These data suggested that promoter P_{srfA} had a broad transcriptional compatibility in different species of LAB.

The effect of carbon and nitrogen sources on the activity of promoter P_{srfA}

The usual used carbon and nitrogen components for the cultivation of LAB were employed to estimate their effect on the expression of GFP controlled by P_{srfA} . Firstly, the recombinant strains were cultivated in the medium containing glucose, maltose, fructose and lactose as the solo carbon source, respectively. The strain biomass was ranged from 0.36 (*W. confusa* 10 – 01 with lactose) to 2.09 (*L. plantarum* 97 – 01 with maltose) (Fig. 2a). *L. plantarum* 97 – 01 showed better growth with these carbon sources than the other recombinant strains, especially than that of *W. confusa* 10 – 01. In addition, *L. casei* 5257-01 preferred glucose and fructose while *L. plantarum* 97 – 01, *L. fermentum* 087 – 01 and *W. confusa* 10 – 01 preferred maltose. Meanwhile, the fluorescence of recombinant strains was varied from 1032.95 Au (*W. confusa* 10 – 01 with fructose) to 9102.83 Au (*L. plantarum* 97 – 01 with maltose). Similarly, the recombinant strains were separately cultivated in the medium containing tryptone, soy peptone and polypeptone as the solo nitrogen sources. The strain biomass and fluorescence were ranged from 0.84 (*W. confusa* 10 – 01 with soy peptone) to 1.89 (*L. plantarum* 97 – 01 with polypeptone), 7996.13 Au (*L. plantarum* 97 – 01 with polypeptone) to 696.52 Au (*W. confusa* 10 – 01 with polypeptone), respectively (Fig. 2b).

Moreover, the varied fluorescence intensity was not directly proportional to the changed biomass among the recombinant strains with different carbon and nitrogen sources (Fig. 2). For instance, the biomass, fluorescence and fluorescence intensity of *L. plantarum* 97 – 01 cultivated with maltose were 0.63-, 6.33- and 3.50-times higher than that of *L. casei* 5257-01, respectively. In addition, the biomass, fluorescence and fluorescence intensity of *L. plantarum* 97 – 01 with maltose were 0.10-, 0.45- and 0.31-times than that of glucose. Moreover, to one specific stain, the difference of biomass and promoter activity with nitrogen sources were less than that of with carbon sources. These data suggested that the carbon and nitrogen source not only played an important role to the cell growth but also to the strength of the promoter P_{srfA} .

Application of promoter P_{srfA} in LAB

Expression of heterologous enzyme CotA originated from *B. subtilis*

In spite of feasibility in various host strains, the other crucially important criteria for a mature expression system are the capacity to produce various target proteins [18]. Here, laccase (CotA), originated from *B. subtilis*, was used to estimate the suitability of P_{srfA} for the production of heterologous proteins in LAB. To express CotA, the gene fragment *cotA* was inserted downstream of P_{srfA} to replace *gfp* on plasmid pMY01. The yielded plasmid pMY05 was separately transformed into *L. plantarum* 97 and *L. casei* 5257 obtaining the recombinant strain *L. plantarum* 97 – 05 and *L. casei* 5257-05, respectively. After cultivation, the enzyme activity of CotA was detected. Compared with *L. plantarum* 97 and *L. casei* 5257, enzyme activity of CotA was obviously detected from *L. plantarum* 97 – 05 and *L. casei* 5257-05 (Fig. 3). The CotA activity of strain *L. plantarum* 97 – 06 was 0.40 times higher than that of *L. casei* 5257-05. Laccase represent an interesting group of oxidative enzymes among various enzymes owing to their great

potential for biotechnological and environmental applications in biodegrading fields and food industry, such as wine stabilization, baking, etc [21]. So far, laccase was mainly produced by the wild and recombinant fungus and there were scarce reports about laccase expression in LAB.

Moreover, by adjusting the pH of the medium to 6.5 when the pH reduced below 4.5 during incubation, the enzyme activity of *L. plantarum* 97 – 05 and *L. casei* 5257-05 was increased by 137.7 % and 61.5 %, respectively. Though there was enzyme activity, the corresponding protein band could not be detected on the SDS-PAGE. Considering on CotA and promoter P_{srfA} originated from *B. subtilis*, one explanation for the low expression might due to the different genetic background between Lactobacillus and Bacillus. Similarly, it was reported that nattokinase originating from *B. subtilis* was expressed in *L. lactis* with low yield. Even though active nattokinase was markedly detected by western blotting and fibrinolytic activity analyses, it could not be detected with coomassie brilliant blue [22]. In addition, it was reported that various carbon and nitrogen sources were used to increase the production of laccase [23]. Therefore, to enhance the expression of laccase in LAB, the culture components and the strength of promoter P_{srfA} would be optimized in our subsequent work.

Expression of NADH oxidase to regulate the fermentation pH

LAB produce lactic acid during fermentation and the accumulated acid is a stress for the cell growth of LAB [24]. Normally, lactic acid is produced through glycolysis by converting pyruvate to lactate during which NADH is oxidized to NAD⁺ by NADH oxidase (NOX) to maintain a redox balance. It was reported that when NOX was overexpressed, H⁺ was consumed as well as the reduced pyruvate to lactate flux was almost abolished [25]. Therefore, in this work, the pH of the supernatant was attempted to be regulated by the expression of NOX controlled by the promoter P_{srfA}.

To express NOX, gene fragment *gfp* on the plasmid pMY01 was replaced by gene *nox* obtaining plasmid pMY06. The recombinant strains *L. plantarum* 97 – 06 and *L. casei* 5257-06 was cultured for 15 h as usual procedure. During fermentation, the recombinant strains grew similar to the wild-type strains and the NOX activity was detected from both the wild-type and recombinant strains (Fig. 4b and c). However, the NOX activity and fermentation pH measured from *L. plantarum* 97 – 06 were higher than that of *L. plantarum* 97 during fermentation (Fig. 4b). The NOX activity of *L. plantarum* 97 – 06 detected at 3 h, 9 h and 15 h was 1.03-, 0.18- and 0.24-times higher than that of *L. plantarum* 97, respectively. Meanwhile, the fermentation pH of *L. plantarum* 97 – 06 measured at 3 h, 9 h and 15 h was separately 0.13, 0.19 and 0.15-ΔpH higher than that of *L. plantarum* 97. These data suggested that gene *nox* was successfully expressed in *L. plantarum* 97 under the control of promoter P_{srfA} and the pH of the supernatant could be upregulated along with the expression of NOX. However, there was scarce difference of NOX activity and fermentation pH gap between *L. casei* 5257 and *L. casei* 5257-06 during incubation. It was speculated that gene *nox* expressed by promoter P_{srfA} in *L. casei* 5257 was too low to obviously affect the fermentation pH. The data indicated that the genetic background of *L. plantarum* 97 was more suitable than *L. casei* 5257 for promoter P_{srfA} to trigger the expression of *nox* expression.

Fine-tuning the fermentation pH by promoter mutation

Metabolic engineering and synthetic biology approaches are becoming more and more attractive owing to its capacity to improve industrial application of microbes [1]. Generally, comparing with strong over-expression, strategies for the fine-tuning of gene expression are required to control the aimed metabolic fluxes in metabolic engineering. The most used method for accurate regulation of gene expression is promoter engineering [25, 26]. Here, to evaluate the validity of P_{srfA} for accurate gene expression, the sequence of P_{srfA} was analyzed. Commonly, a full promoter is typically composed of core promoter, spacers and regulatory elements. Specific sequence of these promoter structures is essential for the efficiency of transcription initiation and then for the expression level of a targeted gene. By sequence analysis, besides the usual core regions of -10 box, -35 box and RBS (ribosome recognition site), there was a small sequence downstream of the promoter P_{srfA} which was probably the CodY binding site. CodY was a global regulatory protein which combined to numerous target promoters and repressed transcription of many genes while activated others [27]. Here, the conserved regions of the promoter P_{srfA} mentioned above were mutated using plasmid pMY06 as the template (Fig. 4a). The corresponding plasmids were individually transformed into *L. plantarum* 97 and *L. casei* 5257. The cell growth of the recombinant strains was similar to the wild strains. (Fig. 4b and c). The NOX activity and of the recombinant *L. plantarum* 97 containing the derivatives of P_{srfA} were varied from 9.02 U/mg to 62.55 U/mg which was accordingly 1.04- and 4.99-times higher than that of *L. plantarum* 97 during fermentation. Correspondingly, the pH of the supernatant detected from the recombinant strains was approximately 0.02- to 0.56-ΔpH higher than that of the wide-type strain. The mutant promoters P09 and P10 could obviously enhance the enzyme expression and the fermentation pH was markedly up-regulated. However, the NOX activity and the fermentation pH of the recombinant strains containing promoter P11 and P12 were similar to that of P_{srfA} . In addition, the similar varied trend of enzyme activity and pH was also found in the host train *L. casei* 5257. Likewise, Guo constructed a constitutive promoter library by randomizing the - 35 and - 10 region of the promoter sequence of the *noxE* gene (H₂O-forming NADH oxidase) in *L. lactis* and the fermentation pH was fine-tuned ranging from 5.93 to 6.32 [25]. These data demonstrated that the core regions of P_{srfA} were valid in LAB exhibiting different promoter strength and were able to regulate the fermentation pH by governing the expression of NOX.

The main focus of metabolic engineering has been on *E. coli* and *S. cerevisiae* as microbial workhorse. Recently, LAB has gained increasing attention because of its indispensable in dairy and health applications. Firstly, many engineering efforts were made concentrating on *L. lactis* in which the glycolytic highway and its uncoupling of other catabolic pathways were efficiently rerouted resulting in the high level production of both natural and novel end products, such as vitamin, polyol, extracellular polymeric substances, etc [28]. But there were only a few genetic tools developed in other LAB except *L. lactis* which were used in probiotic products. Secondly, most expression system developed in LAB were using inducible promoters, such as P_{nisA} , P_{zn} , P_{lac} , P_{170} which were induced by nisin, heavy metals, sugar and pH, respectively [29–32]. Inducible expression can be preferable in cases where the aim is to overproduce a desired protein at high levels, e.g. at a specific moment during fermentation. However, as

LAB comprise a food grade background, inducible systems are less suitable in the intestinal environment or when steady-state gene expression is required, instead, constitutive promoters providing expression of a target gene at a suitable level are desirable [33]. In addition, constitutive promoters are preferred to construct synthetic promoter library for the fine-tuning of gene expression which is important for applications as metabolic optimization and metabolic control analysis [34, 35].

In general, promoter P_{srfA} used in this work was able to constitutively trigger gene expression at distinct times during growth in different species of LAB. Moreover, P_{srfA} had been proved to be valid to express various heterologous proteins in *B. subtilis* and *E. coli*. Thus, promoter P_{srfA} was feasible for the construction of circuits functional in multiple species as well as convenient preliminary screening of before deployment in *E. coli*, *Lactobacillus spp.* or *Bacillus spp.*

Conclusion

Developing of genetic engineering tools for gene expression have been mainly made in *L. lactis* and the limited *Lactobacillus spp.* In this work, promoter P_{srfA} originated from *B. subtilis*, was verified to be valid to trigger the expression of GFP and heterologous protein CotA in a broad range of LAB. Moreover, P_{srfA} and its derivatives were able to accurately tune the fermentation pH by managing the production of NOX. Besides, in our previous work, promoter P_{srfA} was available to produce heterologous proteins in different genus of *B. subtilis* and *E. coli*. Hence, promoter P_{srfA} could be used as an attractive candidate in metabolic engineering for tuning gene expression in broad range of prokaryotic expression plant.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All data used this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

CG supervised the experiment and wrote the draft manuscript, YY accomplished the determination of the recombinant expression system and analyzed the data; YM constructed the recombinant vectors; RG and CZ participated in the detection of gene expression; XW polished the draft manuscript; ML and DC conceived of the study, participated in its design, and coordination. All authors read and approved the final manuscript.

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Figures

Figure 1

Expression of GFP controlled by PsrfA in recombinant LAB (a) and fluorescence analyzed by laser scanning confocal microscope (b). Plasmid pMY05 was transformed into *L. casei* 5257, *L. plantarum* 97, *L. fermentum* 087 and *W. confusa* 10, respectively. The fluorescence and cell growth of the recombinant strains were detected.

Figure 2

The effects of carbon (a) and nitrogen (b) sources on the biomass of recombinant strains and the activity of PsrFA. The recombinant strains were individually cultivated in the medium containing various carbon and nitrogen sources. The strain biomass and fluorescence were detected.

Figure 3

Expression of heterologous enzyme CotA controlled by PsrfA. The recombinant strains containing CotA were incubated and the enzyme activity was detected. Further, the expression of CotA was tested by up-regulating the fermentation pH to 6.5 when the pH reduced below 4.5 during incubation.

Figure 4

Regulation of fermentation pH by production of NOX. The sequence of promoter *Psrfa* and its derivatives (a). The recombinant plasmids containing promoter *Psrfa* and its derivatives were transformed into *L. plantarum* 97 (b) and *L. casei* 5257 (c), respectively. The biomass, fermentation pH and NOX activity of the recombinant strains were detected at 3 h, 9 h and 15 h during fermentation.