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MINI-REVIEW

Natural and engineered promoters for gene expression in *Lactobacillus* species

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Abstract

Lactobacillus species are attractive hosts for the expression of heterologous proteins, antigens, vaccines, and drugs due to their GRAS (generally recognized as safe) status. The bioengineering techniques open new possibilities of improving *Lactobacillus* strains. In this regard, the control of the gene expression in *Lactobacillus* strains through the adequate native or engineered promoters acquires a key role in the development of biotechnological applications and for their function as probiotic bacteria. Depending on the objective sought, the protein produced and the strain used, inducible or constitutive promoters can be chosen. Whereas, when a fine-tuning of gene expression is required, the development of synthetic promoter libraries could be the best approach. In this work, we revise the main constitutive and inducible natural promoters from *Lactobacillus* strains or from other genus that have been applied in *Lactobacillus*, as well as the few engineered promoters developed for these bacteria.

Keywords *Lactobacillus* · Promoter · Constitutive · Inducible

Introduction

Lactobacillus is a genus of Gram-positive bacteria, facultative anaerobic, bacilliform, non-spore-producing bacteria. *Lactobacillus* species are part of lactic acid bacteria (LAB) due to their ability to produce lactic acid from sugars. Lactobacilli are used as starters to manufacture cheeses, yoghurt, sourdough breads, silage, table olives, sauerkraut, fermented fish, and sausages, and have been proposed as natural biopreservatives in non-fermented vegetables (Leroy and Vuyst 2004; Wiernasz et al. 2017). Moreover, lactobacilli are natural inhabitants of the human intestinal tract and some strains have probiotic functions (Saarela et al. 2000). The wide utilization of many *Lactobacillus* species has granted them the GRAS (generally recognized as safe) status. Moreover, lactobacilli are good candidates to be used as microbial cell factories producing recombinant proteins, chemicals, or biofuels (Heiss et al. 2016; Bosma et al. 2017).

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Lactobacilli are therefore an important target for the genetic modification in order to widen and improve their multiple applications. Therefore, there has been an effort in exploring the optimization of the expression systems for lactobacilli. A key element of the expression vectors is the promoter, which regulates the timing and levels of expression of the introduced gene. The election of a suitable promoter is determined by several factors, including the compatibility with the host *Lactobacillus* strain, the desired pattern of expression, and the nature of the transcriptional product of the gene (McCracken et al. 2000; Jensen and Hammer 1998a). Given the importance of host compatibility, most of the promoters used in *Lactobacillus* strains have been obtained from lactobacilli (Table 1 and Table 2), because the expression can drop dramatically when a promoter from another microorganism is used (Scheirlinck et al. 1989; Jensen and Hammer 1998a). Nevertheless, there are some examples of heterologous genes expressed in lactobacilli under the control of promoters from bacteria belonging to different genus such as the constitutive promoters P_{32} and P_{lac} and the inducible promoter P_{nisA} from *Lactococcus lactis* (Table 3).

Regarding how they regulate the genetic expression, the promoters present in the genome of organisms (natural promoters) can be differentiated into constitutive promoters and inducible promoters. The techniques for the determination of the gene expression such as microarray, RNAseq, and

Table 1 Examples of applications of constitutive promoters from *Lactobacillus* strains

Constitutive promoter (gene and organism of origin)	Heterologous gene or protein	Host species/strain	Function/characteristic	Reference
P_{tu} (elongation factor Tu; *L. reuteri* CECT925)	*evoglow*-Pp1 of *Pseudomonas putida*	*Lactobacillus* strains	Molecular markers for monitoring LAB in food and fecal environments	Landete et al. (2015), Landete et al. (2017)
P_{tu} (elongation factor Tu; *L. reuteri* CECT925)	β-Glucosidase of *L. mucosae* INIA P508	*L. fermentum* INIA P584, *L. plantarum* WCFS1	Hydrolysis of glucose from flavonoids and lignans	Unpublished data
P_{ldh} (lactate dehydrogenase gene; *L. plantarum* WCFS1)	Glucosamine-6-phosphate synthase gene (*glm*S1) of *L. plantarum* WCFS1	*L. plantarum* WCFS1	Food-grade vector	Chen et al. (2018)
P_{ldh} (lactate dehydrogenase gene; *L. plantarum* NCIMB8826)	Oxalate decarboxylase gene (*oxd*C) of *Bacillus subtilis*	*L. plantarum* NC8	Degraded oxalate efficiently under in vitro conditions.	Anbazhagan et al. (2013)
P_{ldh} (lactate dehydrogenase gene; *L. plantarum* 80	Single-chain antibody against SA I/II adhesin of *Streptococcus mutans*	*L. paracasei* ATCC 393	Passive immunization	Krüger et al. (2005)
P_{ldh} (lactate dehydrogenase gene; *L. casei* ATCC393)	Carboxymethyl cellulose of *B. subtilis* BSE616	*B. subtilis* RM125	Development of an efficient expression and secretion system	Baek et al. (1997)
P_{pgm} (phosphoglycerate mutase gene; *L. acidophilus* NCFM)	Oxalate degradation pathway of *L. acidophilus* NCFM	*L. gasseri* ATCC 33323, *L. acidophilus*	Overexpression of oxalate degradation pathway. Complementation of oxalate-deficient mutant	Duong et al. (2010)
P_{pgm} (phosphoglycerate mutase gene (*pgm*); *L. acidophilus* NCFM) and P_{slpA} (S-layer protein A gene; *L. acidophilus* ATCC 4356)	Mannanase gene (*man*B) of *Bacillus licheniformis* DSM13	*L. plantarum* WCFS1	Constitutive expression and cell-surface display of a bacterial β-mannanase	Nguyen et al. (2019)
P_{slpA} (*slp*A; *L. brevis*)	Cytokine IL-10	*L. casei* IGM393	Immunization	Kajikawa et al. (2010)
P_{slpA} (*slp*A; *L. brevis*)	β-Glucuronidase (*gus*A), luciferase (*luc*) and aminopeptidase N (*pep*N) as reporter genes	*L. plantarum, L. gasseri, Lc. lactis*	Develop of an efficient expression system	Kahala and Palva (1999)
P_{cbh} (conjugated bile acid hydrolase gene; *L. plantarum* Lp8O)	α-Amylase gene (*amy*L) of *B. licheniformis* and levanase gene (*sac*) from *B. subtilis*	*L. plantarum* NCIB8826	Integration for α-amylase and levanase expression	Hols et al. (1994)
P_{apf} (aggregation-promoting factor gene; *L. crispatus*)	Prolyl endopeptidase gene (*pep*) of *Myxococcus xanthus*	*L. casei* BL23	Prevention of celiac disease. Food-grade vector	Alvarez-Sieiro et al. (2014)
P_{acc} (acetyl coenzyme A carboxylase gene; *L. plantarum* L137)	Cholesterol oxidase gene (*cho*A) of *Streptomyces*	*L. plantarum* NCL21	Expression of heterologous cholesterol oxidase	Kiatpapan et al. (2001)

proteomic approach allow the functional study of the promoters. As an example, microarray analysis of the genome of *Lactobacillus acidophilus* found operons that were differentially expressed in response to the available carbohydrate source, and operons constitutively expressed regardless of carbohydrate source (Duong et al. 2010). The correspondent inducible and constitutive promoters were used by these authors to construct a series of expression vectors for use in lactobacilli. Moreover, promoters influence the yield of protein expressed, being usually classified into strong or weak promoters. Strength of the promoters is an important trait when choosing recombinant vectors and is a target of the promoter engineering, which pursues the optimization of promoter activity and is an emerging strategy for genetic modification of lactobacilli.

Constitutive promoters of *Lactobacillus*

A constitutive promoter is an unregulated promoter that allows the continual transcription of its associated gene. The most common strategies to identify constitutive promoters consist on screening random chromosomal DNA fragments by cloning them in vectors harboring reporter genes or genes that complement auxotroph phenotypes (Bron et al. 2004). Moreover, constitutive promoters can be identified easier from housekeeping

Table 2 Examples of applications of inducible promoters from *Lactobacillus* strains

Inducible promoter (gene and organism of origin)	Heterologous gene or protein	Host species/strain	Function/characteristic	Reference
P_{sppQ} (sakacin P cluster; *L. sakei*)	β-Galactosidase of *L. reuteri*	*L. plantarum* WCFS1	Inducible pSIP expression system by the inducing pheromone (IP-673)	Nguyen et al. (2015)
P_{sppA} and P_{sppQ} (sakacin P cluster; *L. sakei*)	β-Galactosidase of *L. reuteri* L103 and *L. plantarum* WCFS1	*L. plantarum* WCFS1	Inducible pSIP expression system by SppIP pheromone. Food-grade gene expression systems for lactic acid bacteria are useful for applications in the food industry	Nguyen et al. (2011)
P_{orfX} (sakacin P cluster; *L. sakei* LTH673)	Chloramphenicol acetyltransferase of *Bacillus pumilus*, aminopeptidase N of *Lc. lactis*, and chitinase B of *Serratia marcescens*	*L. plantarum* C11	Expression induced by peptide pheromone (IP-C11).	Mathiesen et al. (2004)
P_{orfX} (sakacin P cluster; *L. sakei* LTH673)	Heterologous oxalate decarboxylase gene (*oxdc*) from *B. subtilis*	*L. plantarum* NC8	Expression of the ability to degrade intestinal dietary oxalate	Kolandaswamy et al. (2009)
$P_{\alpha\text{-}amylase}$ (α-amylase gene of *L. amylovorus*)	Single-chain antibody (scFv) against the SA I/II adhesin of *St. mutans*	*L. paracasei* ATCC393	Inducible by mannitol	Krüger et al. (2005)
P_{lac} (*lac* operon, *L. casei*)	*ilv*BN genes of *Lc. lactis*	*L. casei* BL23	Repressed by glucose and induced by lactose. Integrative expression vector for the obtaining of stable food-grade integrants	Gosalbes et al. (2000)
$P_{\alpha\text{-}amylase}$ (α-amylase gene of *L. amylovorus*)	Phytase gene (*phy*C) from *B. subtilis* VTT E-68013	*L. plantarum* strain 755	Inducible by cellobiose	Kerovuo and Tynkkynen (2000)
P_{xyl} (xylose operon of *L. casei*)	Porcine parvovirus (PPV) major structural polypeptide VP2.	*L. casei* ATCC393	Inducible by xylose mucosal vaccine against PPV infection	Yigang and Yijing (2008)
P_{lp_0775} (argininosuccinate synthase of *L. plantarum* WCFS1)	Cytokine IL-10	*L. gasseri* ATTC33323	Stress-inducible promoter	Allain et al. (2016)

genes, which, since required for the maintenance of basal cellular functions, are expressed irrespective of the developmental stage, cell cycle state, or environmental factors. The rRNA promoter of any strain of *Lactobacillus* is a good candidate for constitutive promoter (Rud et al. 2006). Other constitutive promoters are the promoters of factors of initiation or elongation such as the promoters of elongation factor Tu from *Lactobacillus plantarum* CD033 (P_{tuf33}), *Lactobacillus buchneri* CD034 (P_{tuf34}) (Tauer et al. 2014), and *Lactobacillus reuteri* CECT925 (P_{tufR}). The last one was used in place of P_{nisA} in pNZ8048 vector, with good results in the expression of the reporter green fluorescent protein for the traceability of *Lactobacillus* and other LAB strains (Landete et al. 2015). Other constitutive promoter are the promoter of elongation factor P from *L. buchneri* CD034 (P_{efp}) (Tauer et al. 2014) and the promoter of elongation factor G and P and the promoter of initiation factor IF-2 from *Lactobacillus casei* BL23 (Landete et al. 2017).

Other constitutive promoters of *Lactobacillus* strains are the promoters of the phosphoglycerate mutase (Duong et al. 2010) and the L-lactate dehydrogenase (Anbazhagan et al. 2013), which has been used in a lot of applications, even as promoter for the expression of the chimeric single guide RNA of a CRISPR-Cas9 system developed for *L. casei* (Song et al. 2017).

Inducible promoters of *Lactobacillus*

Gene expression is regulated by different cellular mechanisms, starting with the control of transcription at the promoter level. Hence, many genes and operons are not constitutively expressed but rather their expression is regulated in response to activator agents; and thus, the correspondent promoters can be used for controlling the time of the expression in *Lactobacillus*. Inducible promoters are often regulated by a two-component regulatory system (Sørvig et al. 2005; Pfeiler et al. 2007), whose encoding genes must be present in the bacteria in order to exert their regulatory function.

Table 3 Examples of promoters from other organisms used in *Lactobacillus* strains

Promoter (gene and organism of origin)	Heterologous gene or protein	Host species/strain	Function/characteristic	Reference
P_{spa} (protein A gen of *Streptococcus aureus*)	Gene fusion SEZZ-VD4 (*St. aureus-Chlamydia psittaci*)	*Lactobacillus* strains	Live vaccine vectors	Rush et al. (1997)
P_{25} (*Streptococcus thermophilus*)	M6-gp41E of *Streptococcus pyogenes*	*L. plantarum* NCIMB 8826	Heterologous secretion of antigens	Hols et al. (1997)
P_{nisA} (nisinA of *Lc. lactis)*	Betaine uptake system (*Bet*L) of *Listeria*	*L. salivarius* UCC118	Enhancement of stress tolerance	Sheehan et al. (2006)
P_{lacA} (lactose operon of *Lc. lactis*)	Rumen microbial fibrolytic enzyme genes of *Neocallimastix patriciarum*, *Fibrobacter succinogenes* and *Piromyces rhizinflata*	*L. reuteri* Pg4	Acquired ability to secrete fibrolytic enzymes, adherence to mucin and tolerance of acid and bile salts	Liu et al. (2005)
P_{nisA} (nisinA of *Lc. lactis)*	Amylase *(B. licheniformis)*	*L. reuteri* DSM20016	Expression of amylase under nisin induction	Wu et al. (2006)
P_{32} (*Lc. lactis*)	Alcohol dehydrogenase (*Zymomonas mobilis*)	*L. casei* 686	Production of ethanol	Gold et al. (1996)
P_{32} (*Lc. lactis*)	Exendin-4 (artificial sequence, originally isolated from *Heloderma suspectum*)	*L. paracasei* L14	Expression of the therapeutic peptide drug for type 2 diabetes	Zeng et al. (2016)
P_{32} (*Lc. lactis*)	*ctsR* of *Oenococcus oeni*	*L. plantarum* WCFS1	Study of the acid-ethanol stress response	Zhao et al. (2019)
P_{32} (*Lc. lactis*)	Bile salt hydrolase (*bsh*) of *L. plantarum AR113*	*L. casei* LC2W	Improve of the BSH activity	Xiong et al. (2017)

Bacteriocin promoters for gene expression

One of the most used bacteriocin-inducible promoters, the nisin-controlled gene expression system (NICE) has been successfully adapted to several LAB; nevertheless, it was found to be less appropriate for some *Lactobacillus* species (Wu et al. 2006). In lactobacilli, promoters from operons of the bacteriocins sakacin A and P, found in *Lactobacillus sakei*, have been used together with the correspondent regulatory system to construct vector for inducible gene expression in *L. sakei* and *L. plantarum* (Sørvig et al. 2005). Other promoters from well-known bacteriocin genes have been those of plantaricin NC8 (Maldonado et al. 2003), the class IIb bacteriocins (salivaricinT, salivaricin P, and ABP-118), and bactofencin A (Guinane et al. 2015).

Similar to NICE, the activity of those promoters is controlled via a three-component signal transduction system, which responds to an externally added peptide pheromone (Maldonado et al. 2004). Once the required inducing peptide level is reached, the signal is processed by the regulatory system, which interacts with the promoter of the bacteriocin genes to allow bacteriocin production (Maldonado et al. 2003, 2004). These promoters and peptide pheromone can be used to express genes of interest using expression vectors or even after insertion in the bacterial genome.

Carbon catabolism pathways–controlled expression systems

In lactobacilli, the regulation of gene expression has been studied mainly for carbon catabolism pathways, such as those of fructooligosaccharides, lactose, trehalose, xylose, ribose, maltose, malic acid, sorbitol, myo-inositol, and arginine (Duong et al. 2010; Zúñiga et al. 1998; Yebra et al. 2007; Alcántara et al. 2008; Monedero et al. 2008; Landete et al. 2010). Moreover, promoters from *L. plantarum* WCFS1 lactose/galactose-inducible have been identified recently (Zhao et al. 2019). Genes involved in transport and catabolism of carbohydrates are usually organized into strongly expressed operons, which are controlled by the catabolite control protein A (CcpA) (Muscariello et al. 2001). The promoters present in these operons have two regulatory mechanisms. On one hand, the catabolite repression element (cre) sequence produces the repression of the system in the presence of an easily assimilable carbon source such as glucose. On the other hand, the absence of glucose and the presence of the activator produce the induction of expression. Two of those cre sites have been identified in the operon of the arginine deiminase, which is induced by arginine and repressed by glucose through the PTS-CcpA signal transduction pathway (Zúñiga et al. 1998). Thus, consensus sequences have been suggested for the identification of these cre sites in the genome of some bacteria (Miwa et al. 2000). These regulatory systems rely on the

carbohydrates available in the media and have a more feasible application in industrial fermentations compared with promoters induced by peptides.

These promoters can be used for the regulated heterologous expression of genes of interest in vectors or in integrative food-grade expression systems. An interesting strategy has been described for the integration of foreign genes into the lactose operon of *L. casei*, putting the heterologous gene under the same glucose repression and substrate induction than that of the lactose operon (Gosalbes et al. 2000).

Stress-inducible promoters

The addition of a compound as activator of gene expression is not always desirable, economical, or even feasible. In those scenarios, the use of environmental stimuli-based expression systems may be of interest. Therefore, gene expression induced by environmental stresses (SICE for stress-induced controllable expression) such as low pH, temperature, bile salts, or NaCl are a good option (Derzelle et al. 2002; Martínez-Fernández et al. 2019).

The dnaK operon of *L. sakei* encodes several heat shock proteins and is heat induced. Its promoter region has been probed to respond with a similar heat shocking transcription induction when included in an expression plasmid (Schmidt et al. 1999). Many promoters from *Lactobacillus* strains are also regulated in response to oxidative stress (Serrano et al. 2007). Hertel et al. (1998) showed that the promoter of the *Kat*A, which encodes the true catalase of *L. sakei* LTH677, is regulated by the addition of H_2O_2 to anaerobic cultures, as well as by a switch to aerobic conditions, resulting in a strong increase in the induction of the gene.

Temperature conditions also can influence the gene expression. P_{cspL} and P_{cspP} from *L. plantarum* are induced in response to cold shock (Mayo et al. 1997; Derzelle et al. 2002). Binishofer et al. (2002) isolated a thermoinducible promoter-repressor cassette from the temperate *L. casei* phage φFSW-TI, which is repressed at 28 °C and expressed at 42 °C.

Regarding engineering of probiotic lactobacilli, the control of the gene expression under gastrointestinal conditions could allow obtaining their improved effects once the probiotic is in the intestine. The promoter P16090 from *L. casei* BL23 was selected and its bile induction confirmed by means of a gene reporter in *L. casei* BL23, *L. plantarum* WCFS1, *Lactobacillus rhamnosus* INIA P232, *L. rhamnosus* INIA P426, and *L. reuteri* INIA P572. The developed vector, pNZ:16090-aFP, constitutes a promising tool suitable for the expression of genes of interest under intestinal conditions in probiotic *Lactobacillus* (Martínez-Fernández et al. 2019).

Finally, a novel system is based on the manganese starvation-inducible promoter from a specific manganese transporter of *L. plantarum* NC8. The induction of expression was achieved by cultivating *L. plantarum* NC8 at low manganese concentrations (Böhmer et al. 2013). This expression system does not need the addition of an external inducing agent.

Strong and weak promoters of *Lactobacillus*

Constitutive and inducible promoters are classified as strong or weak according to its affinity for the RNA polymerase, which is one of the most influencing factors defining the amount of protein finally produced. That affinity is related to the sequence architecture of the promoter. In *Lactobacillus*, consensus hexamers appear at − 35 (TTGACA) and − 10 (TATAAT) with respect to the transcription initiation site (Fig. 1), similarly to other prokaryotes (Pouwels and Leer 1993), and are the location where the bacterial RNA polymerase binds. How closely the promoter sequence resembles the ideal consensus sequence of the − 35 and − 10 hexamers, alongside with the sequence and length of the spacer region connecting the two hexamers, influences greatly the strength of the promoter (Matern et al. 1994). The presence of the TG motif appears to be of considerable importance in Gram-positive organisms, where introduction or deletion of the motif can influence promoter activity substantially (Voskuil and Chambliss 1998; McCracken and Timms 1999). Additionally, the UP element, an AT-rich sequence upstream of the − 35 hexamer which is contacted by the C-terminal domain of the RNA polymerase α-subunit has been described to influence the transcription as well (Ross et al. 1993). Other elements adjacent to the promoter have also influence in the regulation of the transcription, such as the sequence of the ribosome binding site (RBS) (Salis et al. 2009) and length of the space between it and the start codon (Tauer et al. 2014).

A strategy for the determination of strong or weak promoters is usually the utilization of reporter genes. So, we demonstrated that the elongation factor Tu promoter from *L. reuteri* CECT925 and the elongation factor P promoter from *L. casei* BL23 are strong and constitutive promoters and they could be expressed in different Gram-positive bacteria (Landete et al. 2015, 2017). Likewise, other constitutive promoters corresponding to housekeeping genes are also strong promoters, such as the rRNA promoters and elongation factors already mentioned in the constitutive promoters section.

Engineered promoters for *Lactobacillus*

Natural promoters do not encompass all the possibilities of transcription regulation, and thus, several strategies have been developed to obtain new synthetic promoters, which would allow the fine-tuning of gene regulation, of special interest in metabolic engineering in order to optimize production (Blazeck and Alper 2013; Jensen and Hammer 1998a). Promoter engineering is an evolving field that has developed diverse technologies for the manipulation of the promoter

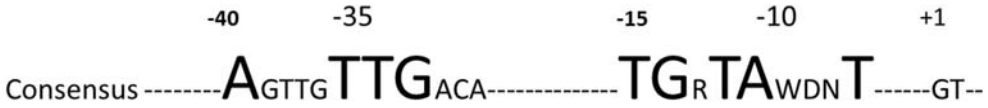

Fig. 1 Consensus sequence of 16S rRNA promoters from *L. plantarum* WCFS1 (Rud et al. 2006). Semi-conserved bases: R = A or G; W = A or T; D = A, G, or T; N = A, G, T, or C

DNA sequence, aimed towards generating a wide range of gene transcription levels. In this regard, new promoters can be obtained by constructing hybrid promoters or by altering the sequence of a natural promoter (Blazeck and Alper 2013).

Promoter engineering for *Lactobacillus* should take into account the knowledge about promoters and their structure. So far, there are just a few examples of engineered promoters for lactobacilli. Rud et al. (2006) constructed a synthetic promoter library for *Lactobacillus* strains by randomizing the non-consensus spacer sequence of the rRNA constitutive promoters of *L. plantarum* WCFS1. The resulting promoter library was tested in *L. plantarum* and *L. sakei* obtaining a wide range of promoter activities, evidencing the influence of the spacer sequence in the promoter strength. Within the spacer, the TG motif located upstream of the − 10 hexamer has shown to influence the transcription in *Lactobacillus*. Hence, the introduction of consensus sequences − 35 and − 10 and a TG motif into the *L. acidophilus* ATCC 4356 *ribosomA* promoter resulted in a increment in transcriptional activity in *L. fermentum* BR11, although not in *L. rhamnosus* GG, showing that both strain and context-dependent effects are critical factors influencing transcription in *Lactobacillus* (McCracken and Timms 1999).

A different approach was used for optimizing two weak lactose/galactose-inducible promoters of *L. plantarum* WCFS1 (Zhang et al. 2019). The sequences on − 35, − 10 regions, and RBSs were replace with consensus sequences, in different combinations, obtaining strength increases in almost all the cases compared with their original promoters. Similarly, the mutagenesis of P15, a promoter-like sequence from *L. acidophilus* ATCC 4359, resulted in the generation of hexamers in − 35 and − 10 identical to the consensus sequences, causing an increment of the promoter strength (Arsenijevic and Topisirovic 2000). This optimized promoter caused an increment in chloramphenicol resistance when introduced, together with the correspondent gene, in *L. reuteri* and *L. plantarum*, but a decrease of the resistance in *L. acidophilus*.

The development of engineered promoters for lactobacilli is still a field to explore. In addition, regulatory sequences, as the cre elements described above, can be a target for modification or elimination in order to change the promoter activity (Krüger and Hecker 1995).

Key elements for choosing promoters

The straightforward approach for expressing a gene with high production yield could be choosing a strong constitutive promoter, which allows the stable production of a high level of protein in large-scale fermentations without the need for the addition of inducing compounds, avoiding the consequent additional cost. On its part, the libraries of engineered constitutive promoters could offer a wide range of activities of these promoters, allowing the fine adjustment of gene expression and conferring advantages over other promoters in metabolic engineering (Jensen and Hammer 1998a).

However, many times, an unregulated promoter does not provide the desired effect because, while the gene of interest is being expressed at a high level, resources for the rest of metabolic routes of the cell are also being subtracted, hindering the bacterial growth. Moreover, the heterologous protein may have a toxic effect on the host cell. Therefore, it is advisable to use inducible promoters, allowing the activation of expression only when it is necessary or viable. Inducible expression can be preferable in cases where the aim is to overproduce a recombinant protein at high levels in a specific moment, while avoiding deleterious effects during growth phase (Terpe 2006). The toxicity of the heterologous protein can also take to choose a weak promoter, especially if high levels of expression are not required.

It is also necessary to take into account that inducible promoters usually only work within the same genus, as the case of the promoter inducible by bile (Martínez-Fernández et al. 2019), and in many cases, they only work within the same species or the same strain. This is caused many times by the need of the adequate two-component systems. Therefore, inducible promoters are of much more restricted use, whereas the constitutive promoters usually have a wider application. Nevertheless, exceptions can be found, such as the promoter of *Lc. lactis ilv*BN genes, which also work in *L. casei* (Gosalbes et al. 2000). Even so, inducible promoters can be applied in other bacteria, if the genes involved in its regulation are also transferred, an example is the transfer of the NICE system of *Lc. lactis* to strains of *Lactobacillus* that allows the induction by nisin in *Lactobacillus* strains when the promoter of nisin is present (Wu et al. 2006).

Conversely, constitutive promoters have many times a wide range of suitable hosts. Constitutive promoters of *Lactobacillus* have been used in other LAB such as *Lactococcus*, *Enterococcus*, or *Streptococcus*, other Gram-positive bacteria such as *Bifidobacterium* and *Listeria* (Landete et al. 2017), and even in *E. coli* (Klein et al. 1994). In the same way, constitutive promoters of other species or genus have been applied in *Lactobacillus* (Table 3). Regarding engineered promoters, Rud et al. (2006) observed

similar levels of expression in both *L. plantarum* and *L. sakei* for the synthetic promoters developed. Nevertheless, a constitutive promoter does not necessarily have the same activity in different organisms (Jensen and Hammer 1998b). Even among *Lactobacillus*, some promoters have been reported to be species dependent (Chen and Steele 2005).

Applications of promoters from *Lactobacillus* strains

The main objective of searching for promoters is the expression of genes of interest under the regulation of these promoters, through replicative vectors or chromosomal integration. Tables 1 and 2 show examples of the different applications of constitutive and inducible promoters from *Lactobacillus* strains. *Lactobacillus* strains have the potential as delivery systems for valuable proteins like antibodies and antigens. Numerous promoters from *Lactobacillus* strains have been used in oral vaccines to deliver different types of antigens (Tables 1 and 2). In the same way, IL-10 has been successfully expressed using different recombinant *Lactobacillus* using constitutive and inducible promoters. Both tables show the use of promoters in vectors, as well as promoters that have been integrated into the bacterial chromosome. The use of promoters in the development of food-grade vectors or integrative food-grade expression system is also encompassed.

There are constitutive promoters that have been used for the expression of various proteins of interest, such as the promoter of the elongation factor Tu of *L. reuteri* CECT925. This and other constitutive promoters of *Lactobacillus* have been used for the fluorescent labeling of other LAB, *Bifidobacterium* and even *Listeria* (Landete et al. 2015, 2017).

The pSIP system is the inducible system most extensively used, and it has been employed in the recombinant overproduction of heterologous proteins such as β-glucosidase, β-galactosidases, aminopeptidases, and β-glucuronidase, which were expressed in *L. sakei* and *L. plantarum* strains (Sørvig et al. 2003; Böhmer et al. 2013). Moreover, the selection of promoters from *Lactobacillus* strains for biotechnological applications, such as the production of aminopeptidases, β-glucuronidase, βs-galactosidases, esterases, or diacetyl production by LAB strains has a great potential for the metabolic engineering applied to dairy fermentation (Nguyen et al. 2015).

Perspectives

The growing knowledge of the genome of *Lactobacillus* strains will allow detecting natural promoters for the expression of genes of interest and the improvement of its biotechnological and probiotic properties. Although promoters from *Lactobacillus* are adequate for food-grade vectors, most studies used non-food-grade vectors. Therefore, developing food-grade vectors and integrative food-grade expression system has a great potential in the development of food and in the production of different enzymes used in food, human, or animals.

An interesting field is the identification of inducible promoters for the creation of biosensors. Those promoters regulate the increase of the reporter signal level according to the concentration of the effector molecule, such as metals, contaminants, or specific molecules from microorganisms.

Finally, engineered promoters allow for the fine-tuning of gene expression, which is important for biotechnological applications. Therefore, more efforts should be made in the development of new engineered promoters generated for *Lactobacillus* and other GRAS bacteria.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

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