Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 354 (2007) 90–95
BBRC
www.elsevier.com/locate/ybbrc

Assay and characterization of a strong promoter element from *B. subtilis*

Ai-Ling Zhang [a,c,1], Hui Liu [a,c,1], Ming-Ming Yang [a,b,c,*], Yue-Sheng Gong [a], Hong Chen [a]

[a] *College of Animal Sciences, Northwest A&F University, Yangling 712100, People's Republic of China*
[b] *Institute of Biochemical Engineering, College of Material Science and Chemical Engineering, Zhejiang University, Hangzhou 310027, People's Republic of China*
[c] *Yangguang-Guangji Medical R&D Co. Ltd., Wuhan 430062, People's Republic of China*

Received 12 December 2006
Available online 27 December 2006

Abstract

A new strong promoter fragment isolated from *Bacillus subtilis* was identified and characterized. Using the heat stable β-galactosidase as reporter, the promoter fragment exhibited high expression strength both in *Escherichia coli* and *B. subtilis*. The typical prokaryotic promoter conservation regions were found in the promoter fragment and the putative promoter was identified as the control element of *yxiE* gene via sequencing assay and predication of promoter. To further verify and characterize the cloned strong promoter, the putative promoter was sub-cloned and the β-Gal directed by the promoters was high-level expressed both in *E. coli* and *B. subtilis*. By means of the isolated promoter, an efficient expression system was developed in *B. subtilis* and the benefit and usefulness was demonstrated through expression of three heterologous and homogenous proteins. Thus, we identified a newly strong promoter of *B. subtilis* and provided a robust expression system for genetic engineering of *B. subtilis*.

Keywords: *Bacillus subtilis*; Expression vector; Heat stable β-galactosidase; Strong promoter; Sub-clone

Bacillus subtilis is a non-pathogenic Gram-positive bacterium and is generally regarded as safe organism (GRAS). *B. subtilis* has long been exploited for industrial and biotechnological application [1,2]. With the completion of sequencing of the *B. subtilis* genome, post-genomic studies were stimulated. Many coding gene were gradually identified and recognition of regulation machinery and element was enhanced in *B. subtilis* [3]. Undoubtedly, all these knowledge accelerated the biotechnological application of *B. subtilis* in industry field, of which the regulation element, promoter, played an important role in genetic engineering of *B. subtilis*, i.e. control element of expression vector [4].

In genetic engineering of *B. subtilis*, plasmid backbone and promoter are two basic elements. Plasmid instability was once one barrier in genetic manipulation [5], but recently many a convenient vector systems of *B. subtilis* have been developed for genetic manipulation [4,6,7]. This further makes the *B. subtilis* a potential bacillus in genetic engineering and industrial application.

A lot of information of *B. subtilis* promoter has been acquired and several of them has been exploited to be used as control element in the construction of expression vector in *B. subtilis* [8–10], of which some constitutive promoters, such as P43 promoter and phage promoter, were widely investigated and characterized. Amongst the P43 promoter was used as a common control element in the construction of expression vector in *B. subtilis* and was considered as a strong promoter [10]. It is in the interest of the biotechnological application and industry to seek new strong promoters. Here, a promoter isolated from *B. subtilis*

* Corresponding author. Fax: +86 29 87092432.
E-mail address: yangjinxin@vip.163.com (M.-M. Yang).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.12.137

exhibited high expression for reporter gene both in *Escherichia coli* and *B. subtilis*, and demonstrated higher expression strength than P43 promoter.

By using of promoter trapping vector to clone promoter fragment from the bacteria genome DNA is a frequently approach [10,11], of which the β-galactosidase (β-Gal) was one of usually used reporters [12]. In this study, a promoter fragment isolated from *B. subtilis* by using β-Gal as reporter demonstrated strong promoter activity both in *E. coli* and *B. subtilis*. The promoter fragment was sequenced and the putative promoter was predicted. The typical prokaryotic promoter conservation region was found. Sub-cloned promoter from the fragment further verified the putative promoter and used it to construct expression vector in *B. subtilis*.

Materials and methods

Strains, plasmids, and growth conditions. *Bacillus subtilis* 1A747 was kindly provided by the Bacillus Genetic Stock Center. *E. coli* DH5α was purchased from Novagen (Darmstadt, Germany). The plasmids used in this study were listed in Table 1. All strains were incubated at 37 °C in Luria–Bertani (LB) medium. When required, medium for *E. coli* and *B. subtilis* were supplemented with 50 μg/mL spectinomycin, 100 μg/mL ampicillin or 5 μg/mL chloromycetin, respectively.

General DNA manipulation. The isolation and manipulation of recombinant DNA was performed using standard techniques [13]. All enzymes were commercial preparations, and used as recommended by the manufacturers (TOYOBO, Japan).

Transformation of E. coli and *B. subtilis*. Transformation of *E. coli* cells was performed as previously described [13]. *B. subtilis* cells were transformed by electroporation [4,14].

Construction of plasmids. Using pSI3423 as template, the 320-bp and 313-bp promoter fragments were amplified through polymerase chain reaction (PCR) with the two primer pairs, P3-up/P3-down (5′-TTGGGCCCATTTAATTGAAG-3′; 5′-GCGAATTCGCTCTTCCCG-3′) and P3-up (N)/P3-down (N) (5′-TTGGGCCCATCATTTAATTGAAGC-3′; 5′-TTGAATTCCCGCCTTT CGGACTG-3′), respectively. The restriction sites *Apa*I and *Eco*RI were introduced into the amplified fragments by the primers (underline). And then the two fragments were cloned into pGEM-T vector, yielding pGJT-78 and pGJT-436. Excised from the pGJT-78 and pGJT-436 with *Apa*I and *Eco*RI, the two promoter fragments were cloned into the corresponding sites of pLJ-2, respectively, resulting in pYG78 and pGJ436. The promoter excised from pGJT-78 with *Apa*I and *Eco*RI, cloned into the corresponding sites of pGJ103, resulting in expression vector pYG123. To compare the efficiency of the expression system to that of P43 system, the *bgaB* gene was excised from pLJ-2 with *Eco*RI and *Sac*I, and then cloned into the downstream of P43 in pGJ288, respectively, resulting in pGJ-*bgaB*. Using BI1 (5′-TTGAATTCGTGACAATTGCATCGTC-3′) and BI2 (5′-TTGGATCCCTTATTCAAAAGTCACCG-3′) as primers, the *bioI* was PCR amplified from *B. subtilis* 1A747 chromosome DNA. Digested by the *Eco*RI and *Bam*HI, the amplified fragment was cloned into the pYG123, resulting in pYGI. The *vgb* gene was excised from pGJ203 with *Eco*RI and *Sac*I and cloned into the corresponding sites of pYG123, resulting in pYG-*vgb*. To demonstrate the expression difference between the system constructed in this study and P43 promoter system, the *Eco*RI- *Sac*I-treated *bioI* gene and *vgb* gene were cloned into the corresponding sites of pGJ288, respectively, yielding pGJ-*bioI* and pGJ-*vgb*.

β-Gal activity assay. The β-Gal activity assay was carried out as previously described [15,16]. Samples were prepared at different cultured time and β-Gal activity was measured. The activity was given as Miller units per mL sample (Miller U/mL).

SDS–PAGE assay. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis of the β-Gal was performed as described previously [13]. The protein fractions were analyzed on SDS/12% polyacrylamide gels that were stained with Coomassie brilliant blue R-250.

Promoter sequence predication and analysis. The promoter fragments were sequenced by Aoke Corp. (Beijing, China). The sequence analysis was performed online with NCBI blast (http://www.ncbi.nih.gov), and promoters were predicted online by using of softberry (http://www.softberry.com).

Results and discussion

Characterization of strong promoter isolated from B. subtilis

In our previous work, the *B. subtilis* promoter library was created using the promoter trapping vector with *bgaB* [16] as reporter gene, of which the DNA fragments from *B. subtilis* genome DNA digested by *sau*3AI were inserted into the *Bam*HI site upstream of β-Gal coding gene. The library was screened and obtained when the appropriate promoters had been inserted upstream of the promoterless β-Gal gene,

Table 1
Plasmids used in this study

Plasmid	Relevant characteristics	Reference or source
pLJ-2	Cm^R, promoter-less *bgaB*	Lab stock
pShuttleI	Cm^R, promoter-less *bgaB* (with its native RBS)	Lab stock
pSI3423	The cloned promoter upstream the *bgaB*	Lab stock
pGJT-78	pGEMT-vector with the sub-cloned promoter	This work
pGJT-436	pGEMT-vector with the sub-cloned promoter	This work
pYJ78	pLJ-2 with the sub-cloned promoter	This work
pGJ436	pLJ-2 with the sub-cloned promoter	This work
pGJ103	*E. coli–B. subtilis* shuttle vector	[4]
pYG123	*B. subtilis* expression vector directed by PyxiE	This work
pGJ288	pGJ103 with P43 promoter between *Apa*I and *Eco*RI	Lab stock
pGJ-*bgaB*	pGJ288 with *bgaB* downstream P43	This work
pYGI	*bioI* under control of PyxiE in pYG123	This work
pGJ203	*vgb* gene donor plasmid	[4]
pYG-*vgb*	Expression vector of *vgb* gene directed by PyxiE	This work
pGJ-*bioI*	Expression vector of *bioI* gene directed by P43	This work
pGJ-*vgb*	Expression vector of *vgb* gene directed by P43	This work