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Full Length Article

Cloning of *cbb* Gene Promoters from the Sulfur-oxidizing Bacteria *Thiobacillus thioparus* and *Halothiobacillus neapolitanus* and Assessment of their Promoter Efficacies

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Abstract

To determine the transcriptional potential of *cbb* genes involved in CO_2 fixation, the promoters of two sulfur-oxidizing bacteria, *Thiobacillus thioparus* DSM 505 and *Halothiobacillus neapolitanus* DSM 15147, were cloned. The unknown upstream sequence of the DSM 505 *cbbL* gene was successfully amplified through three rounds of thermal asymmetric interlaced PCR, yielding the fragments $UP_{505\text{-}cbbL\text{-Short}}$ and $UP_{505\text{-}cbbL\text{-Long}}$. Sequence analysis confirmed that these two fragments contained the upstream sequence of the DSM 505 *cbbL* gene and that $UP_{505\text{-}cbbL\text{-Long}}$ contained a promoter sequence. A series of fragments containing potential DSM 505 *cbbL* promoters ($P_{505\text{-}cbbL\text{-Y+I-1}}$, $P_{505\text{-}cbbL\text{-Y+I}}$, $P_{505\text{-}cbbL\text{-Y+I+1}}$, $P_{505\text{-}cbbL\text{-J+I-1}}$, $P_{505\text{-}cbbL\text{-J+I}}$ and $P_{505\text{-}cbbL\text{-J+I+1}}$) and fragments containing the known promoters of DSM 15147 *cbb* genes were inserted into the promoter-reporter plasmid pUC18-GFP, which contained the green fluorescent protein (GFP) gene. Sequencing results confirmed that all the promoter fragments were successfully cloned and transformed into *Escherichia coli* Top10. The promoter efficacies of the promoter fragments were then assessed by measuring the fluorescence intensities of the recombinant strains. For DSM 15147, the promoter efficacy of the *cbbL* promoter was much higher than that of the *cbbM* promoter, which corresponded with the transcription efficiencies of the *cbb* genes. For DSM 505, the promoter fragments $P_{505\text{-}cbbL\text{-Y+I+1}}$ and $P_{505\text{-}cbbL\text{-J+I+1}}$ were able to initiate GFP expression. The promoter efficacy of the DSM 505 *cbbL* promoter was higher than that of the DSM 15147 *cbbL* promoter, indicating that the *cbbL* gene transcription and CO_2 assimilation potentials of DSM 505 may be higher than those of DSM 15147. © 2019 Friends Science Publishers

Keywords: *cbb* Promoter; CO_2 assimilation potential; Promoting efficacy; Sulfur-oxidizing bacteria; Thermal asymmetric interlaced PCR

Introduction

Biological fixation of CO_2 holds great potential for mitigating global warming (López *et al.*, 2013). Chemoautotrophic bacteria have attracted significant attention due to their ability to fix CO_2 in the dark and in challenging environments such as barren desert soils and large-scale industrial waste gas treatment reactors (Saini *et al.*, 2011). Sulfur-oxidizing bacteria (SOB) are a major category of chemoautotrophic bacteria that are able to oxidize certain sulfur-containing compounds (e.g., S^{2-}, S^0 and $S_2O_3^{2-}$) for use as energy sources and to utilize CO_2 as their sole carbon source for growth (Baker *et al.*, 1998). Therefore, SOB are not only able to remove sulfur-containing pollutants from wastewater and industrial waste gases (Chung *et al.*, 1998; Tóth *et al.*, 2015), but also have the capability to fix CO_2, even at high CO_2 concentrations (Baker *et al.*, 1998; Wang *et al.*, 2016b).

The Calvin-Benson-Bassham (CBB) pathway is considered to be the primary carbon assimilation pathway (Badger and Bek, 2008; Berg, 2011). Ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO; encoded by the *cbb* gene) is a key enzyme in the CBB cycle (Tabita *et al.*, 2007, 2008). There are four forms of RuBisCO and most chemoautotrophic bacteria use form I and/or II, encoded by the *cbbL* and *cbbM* genes, respectively (Kusian and Bowien, 1997), to assimilate CO_2 via the CBB cycle (Berg, 2011). RuBisCO forms I and II contain key active sites in their large subunits and many SOB species use RuBisCO form I and/or II to fix CO_2 (Hernandez *et al.*, 1996; Baker *et al.*, 1998).

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The type and transcription efficiency of the *cbb* gene is the major determining factor of carbon fixation efficiency (Tabita, 1988; Yoshizawa et al., 2004; Badger and Bek, 2008). However, *cbb* gene transcription is highly sensitive to environmental conditions, such as CO_2 concentration and O_2 content (Yoshizawa *et al.*, 2004; Toyoda *et al.*, 2005). Additionally, endogenous and exogenous organic matter can repress *cbb* gene transcription, inhibiting the CO_2 assimilation process (Friedrich, 1982; Kusian and Bowien, 1997; Hu *et al.*, 2011; Wang *et al.*, 2017, 2018). Different bacteria have different sensitivities to environmental conditions and organic matter inhibition (Kusian and Bowien, 1997; Hu *et al.*, 2011; Wang *et al.,* 2017, 2018). Therefore, the apparent gene transcription efficiency of *cbb* does not necessarily represent actual RuBisCO enzyme activity or CO_2 assimilation potential in chemoautotrophs.

Cloning of the gene encoding the RuBisCO enzyme from autotrophic bacteria and expression in *Escherichia coli* have been reported (Hernandez *et al.*, 1996). However, expression levels of RuBisCO protein in heterotrophic *E. coli* are extremely low and the enzyme is generally inactive, which makes it difficult to determine the actual enzyme expression potential of RuBisCO (Kusian *et al.*, 1995; Vichivanives *et al.*, 2000). Therefore, analysis of the expression potential of the RuBisCO enzyme in chemoautotrophs has rarely been reported. However, understanding the expression potential of the RuBisCO enzyme is useful for screening bacteria with high CO_2 assimilation potentials and optimizing cultivation conditions to achieve high efficiency carbon fixation.

Promoter strength is the main factor influencing the transcription potential of structural genes (Dubbs and Tabita, 2003, 2004). Therefore, *cbb* gene promoter strength indirectly represents the transcription potential of *cbb* genes. The green fluorescent protein (GFP) gene has been widely used as a reporter for assessing promoter activity in prokaryotic organisms (Ikeno *et al.*, 2003; Wei *et al.*, 2012). However, *cbb* gene promoters in chemoautotrophs have rarely been studied and assessment of the expression potential of the RuBisCO enzyme in chemoautotrophs using promoter cloning technology has not been reported.

Our previous study showed that *Thiobacillus thioparus* DSM 505 and *Halothiobacillus neapolitanus* DSM 15147 had similar high CO_2 fixation efficiencies but that the transcription efficiency of the DSM 505 *cbb* gene was much lower than that of the DSM 15147 *cbb* gene (Wang *et al.*, 2016a). DSM 505 was also found more sensitive to inhibition by extracellular dissolved organic carbon than DSM 15147 (Wang *et al.*, 2018). These results raised the question of which strain has a higher CO_2 assimilation potential under uninhibited conditions and should be optimized. To answer this question, the promoters of DSM 505 *cbbL* and DSM 15147 *cbb* genes were cloned and the promoter efficacies of the *cbb* promoters were measured to compare the *cbb* gene transcription potential of the two strains. Our results will be helpful for identifying bacteria with high CO_2 assimilation potentials and for further optimizing cultivation conditions to improve CO_2 fixation efficiency.

Materials and Methods

Bacterial Strains, Plasmids and Culture Conditions

Two aerobic SOB (*T. thioparus* DSM 505 and *H. neapolitanus* DSM 15147) were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). *T. thioparus* DSM 505 and *H. neapolitanus* DSM 15147 are obligate autotrophic bacteria, belonging to β-Proteobacteria and γ-Proteobacteria, respectively, that possess both *cbbL* and *cbbM* genes (Wang *et al.*, 2016a). The strains were cultured using the method described by Wang *et al* (Wang *et al.*, 2016a).

The plasmids used in this study are listed in Table 1. *E. coli* Top10 was used as the host strain for all recombinant plasmids. *E. coli* Top10 was grown at 37°C in Luria broth (LB) and strains containing plasmids were grown in LB supplemented with 60 μg/mL ampicillin.

DNA Extraction

Genomic DNA was extracted from DSM 505 and DSM 15147 collected with a 0.22 μm membrane. Total bacterial DNA was extracted from the filter membranes using a PowerSoil® DNA Isolation kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's protocol. The concentration and purity of DNA were measured using a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Primers and PCR Procedures for Amplification of *cbb* Promoters

Because the full sequence of the DSM 505 *cbb* gene and its upstream sequence were unknown, a thermal asymmetric interlaced PCR (TAIL-PCR) was used to amplify the unknown upstream sequences (Liu and Chen, 2007; Qiu *et al.*, 2008). The promoters of the DSM 505 *cbbL* gene were amplified using the high fidelity PCR components and procedures (Table 2 and 3). For DSM 15147, the upstream sequences of the *cbbL* (GenBank: AF038430.1) and *cbbM* (GenBank: AF046932.1) genes were acquired from the National Center for Biotechnology Information (NCBI). The promoters of the DSM 15147 *cbbL* and DSM 15147 *cbbM* genes were amplified using the conventional PCR components and procedures (Table 2 and 3). Primers used to amplify the *cbb* gene promoters and the GFP gene are shown in Table 4.

Construction of Recombinant Plasmids

The recombinant plasmid pUC18-GFP-P_{cbb} containing the DSM 505 and DSM 15147 *cbb* gene promoters were

Table 1: Main plasmids used in this study

Plasmids	Properties	Sources
pUC18	pBR322 ori, Amp^R, Lac Z promoter, 2.7kb	Lab stock
pMD18T	ColE1 ori, Amp^R, *Lac Z* operater, 2.7kb	TaKaRa
pET28b-GFP(+)	pET28b derivative, Kan^R, containing the GFP gene, between BamH I and Hind III sites, 6.1kb	Lab stock
pUC18-GFP	pUC18 derivative, containing the GFP gene, between BamH I and Hind III sites, 3.4kb	This study
pUC18-GFP-$P_{15147\text{-}cbbL}$	pUC18-GFP derivative, containing the $P_{15147\text{-}cbbL}$ gene, between Sac I and BamH I sites, 4.7kb	This study
pUC18-GFP-$P_{15147\text{-}cbbM}$	pUC18-GFP derivative, containing the $P_{15147\text{-}cbbM}$ gene, between Sac I and BamH I sites, 4.5kb	This study
pUC18-GFP-$P_{505\text{-}cbbL\text{-}Y+I\text{-}1}$	pUC18-GFP derivative, containing the $P_{505\text{-}cbbL\text{-}Y+I\text{-}1}$ gene, between Sac I and BamH I sites, 4.9kb	This study
pUC18-GFP-$P_{505\text{-}cbbL\text{-}Y+I}$	pUC18-GFP derivative, containing the $P_{505\text{-}cbbL\text{-}Y+I}$ gene, between Sac I and BamH I sites, 4.9kb	This study
pUC18-GFP-$P_{505\text{-}cbbL\text{-}Y+I+1}$	pUC18-GFP derivative, containing the $P_{505\text{-}cbbL\text{-}Y+I+1}$ gene, between Sac I and BamH I sites, 4.9kb	This study
pUC18-GFP-$P_{505\text{-}cbbL\text{-}J+I\text{-}1}$	pUC18-GFP derivative, containing the $P_{505\text{-}cbbL\text{-}J+I\text{-}1}$ gene, between Sac I and BamH I sites, 4.6kb	This study
pUC18-GFP-$P_{505\text{-}cbbL\text{-}J+I}$	pUC18-GFP derivative, containing the $P_{505\text{-}cbbL\text{-}J+I}$ gene, between Sac I and BamH I sites, 4.6kb	This study
pUC18-GFP-$P_{505\text{-}cbbL\text{-}J+I+1}$	pUC18-GFP derivative, containing the $P_{505\text{-}cbbL\text{-}J+I+1}$ gene, between Sac I and BamH I sites, 4.6kb	This study

Amp: ampicillin; Kan: kanamycin

Table 2: PCR components for *cbb* promoters (25 μL)

PCR types	PCR components	Volume (μL)
Conventional-PCR	10×PCR Buffer (Mg^{2+} Plus)	2.50
(for DSM 15147)	dNTP Mixture (2 mM each)	1.20
	Primer-F (10 μM)	0.60
	Prime -R (10 μM)	0.60
	rTaq DNA Polymerase	0.125
	ddH_2O	18.975
	DNA template	1.00
TAIL-PCR	10×PCR Buffer (Mg^{2+} Plus)	2.50
(for DSM 505)	dNTP Mixture (2 mM each)	1.20
(Qiu *et al.*, 2008)	12N Primer (10 μM)	4.00
	Specific Primer (SP1/SP2/SP3) (10 μM)	1.00
	rTaq DNA Polymerase	0.25
	ddH_2O	15.05
	DNA template	1.00
High-fidelity PCR	5×PS Buffer	5.00
(for DSM 505)	dNTP Mixture(2 mM each)	2.00
	Primer-F (10 μM)	0.50
	Primer-R (10 μM)	0.50
	Primer HS DNA polymerase	0.25
	ddH_2O	15.75
	DNA template	1.00

Table 3: PCR procedure for *cbb* promoters

PCR procedure		Stage	Cycles	Standard thermal conditions
Conventional-PCR		1	1	94°C (3 min)
(for DSM 15147)		2	35	94°C (30 s), 55-56°C (30 s), 72°C (90s, adjusted according to the fragment length, 60s for 1kb)
		3	1	72°C (7 min)
TAIL-PCR	Primary	1	1	92°C (3 min), 95°C (1 min)
(for DSM 505)		2	10	94°C (30 s), 58°C (1 min), 72°C (2 min)
(Qiu *et al.*,		3	1	94°C (30 s), 25°C (3 min), ramping to 72°C over 3min, 72°C (2 min)
2008)		4	5	94°C (30 s), 29°C (1 min), 72°C (2 min)
		5	12	94°C (30 s), 58°C (1 min), 72°C (2 min); 94°C (30 s), 58°C (1 min), 72°C (2 min); 94°C (30 s), 29°C (1 min), 72°C (2 min)
		6	1	72°C (5 min)
	Secondary	1	12	94°C (30 s), 59°C (1 min), 72°C (2 min); 94°C (30 s), 59°C (1 min), 72°C (2 min); 94°C (30 s), 29°C (1 min), 72°C (2 min)
		2	1	72°C (5 min)
	Tertiary	1	10	94°C (30 s), 58°C (1 min), 72°C (2 min); 94°C (30 s), 58°C (1 min), 72°C (2 min); 94°C (30 s), 29°C (1 min), 72°C (2 min)
		2	1	72°C (5 min)
High fidelity-PCR		1	1	95°C (1 min)
(for DSM 505)		2	32	98°C (10 s), 56°C (10 s), 72°C (90s, adjusted according to the fragment length, 60s for 1kb)
		3	1	72°C (7 min)

constructed (Fig. 1). Gene cloning and transformation were performed using PCR amplification, PCR product purification, restriction endonuclease digestion, agarose gel electrophoresis, recovery and purification of agarose gel products, ligation, transformation, transformant PCR identification and sequencing. The reagents used for PCR amplification (TaKaRa, Dalian, China) are listed in Table 2. QuickCut *Bam*HI, QuickCut *Hind*III and QuickCut *Sac*I

Table 4: Primers used in this study

Target fragment		Primers	Sequence（5'-3'）	Restriction sites	Target fragment length（bp）
GFP gene		G1f	*CGC*GGATCCGATGGTGAGCAAGGGCGAG	BamH I	720
		G720r	*CCC*AAGCTTTTACTTGTACAGCTCGTCCA	Hind III	
Insertion fragments in recombinant plasmids		M13F	CGCCAGGGTTTTCCCAGTCACGAC	/	/
		M13R	AGCGGATAACAATTTCACACAGGA	/	
$P_{15147\text{-}cbbL}$		hnLF	*TCC*GAGCTCCACACCGGGAAGTTTGGCA	Sac I	1253
		hnLR	*CGC*GGATCCTAGTCCATGTCGGTCAGCAA	BamH I	
$P_{15147\text{-}cbbM}$		hnMF	*TCC*GAGCTCTGATCGCCTCGTTACTGTTC	Sac I	1072
		hnMR	*CGC*GGATCCCGCATGTCTTCAGTGGCTTC	BamH I	
505-*cbbL* gene		K2F	ACCAYCAAGCCSAAGCTSGG	/	495
		V2F	GCCTTCSAGCTTGCCSACCRC	/	
TAIL-PCR (DSM 505)	Primary	12N	NNNNNNNNNNNN	/	/
		V2F (SP1)	GCCTTCSAGCTTGCCSACCRC	/	
	Secondary	12N	NNNNNNNNNNNN	/	/
		505L-R-II (SP2)	CTTCAGGGTCGCTTCGTGC	/	
	Tertiary	12N	NNNNNNNNNNNN	/	/
		505L-R-I (SP3)	*CGC*GGATCCGTTCTCGTCGTCCTTGGTG	BamH I	
$P_{505\text{-}cbbL\text{-}Y+I\text{-}1}$		505L-F-YD	*TCC*GAGCTCACTGTCCGGGCTGGTTGAGG	Sac I	1518
		505L-R-I(-1)	*CGC*GGATCCTTCTCGTCGTCCTTGGTG	BamH I	
$P_{505\text{-}cbbL\text{-}Y+I}$		505L-F-YD	*TCC*GAGCTCACTGTCCGGGCTGGTTGAGG	Sac I	1519
		505L-R-I	*CGC*GGATCCGTTCTCGTCGTCCTTGGTG	BamH I	
$P_{505\text{-}cbbL\text{-}Y+I+1}$		505L-F-YD	*TCC*GAGCTCACTGTCCGGGCTGGTTGAGG	Sac I	1520
		505L-R-I(+1)	*CGC*GGATCCCGTTCTCGTCGTCCTTGGTG	BamH I	
$P_{505\text{-}cbbL\text{-}J+I\text{-}1}$		505L-F-J	*TCC*GAGCTCCTGGCGAGCACCTGTTCCTTGTTG	Sac I	1165
		505L-R-I(-1)	*CGC*GGATCCTTCTCGTCGTCCTTGGTG	BamH I	
$P_{505\text{-}cbbL\text{-}J+I}$		505L-F-J	*TCC*GAGCTCCTGGCGAGCACCTGTTCCTTGTTG	Sac I	1166
		505L-R-I	*CGC*GGATCCGTTCTCGTCGTCCTTGGTG	BamH I	
$P_{505\text{-}cbbL\text{-}J+I+1}$		505L-F-J	*TCC*GAGCTCCTGGCGAGCACCTGTTCCTTGTTG	Sac I	1167
		505L-R-I(+1)	*CGC*GGATCCCGTTCTCGTCGTCCTTGGTG	BamH I	

Note: a) M=A/C; R = A/G; W=A/T; S= G/C; Y=C/T; K=G/T; V= A/G/C; H= A/C/T; D = A/G/T; B=G/C/T; N =A/G/C/T; b) The bases with underline are restriction sites and the italics are protect bases; c) The target fragment includes the promoter region and part of *cbb* gene, and the target fragment length includes the restriction sites

restriction enzymes (TaKaRa) were used for restriction endonuclease digestion. T4 DNA Ligase (TaKaRa) was used for ligation reactions. Purification of agarose gel and PCR products was performed using a gel extraction kit (Biodev-Tech, Beijing, China) according to the manufacturer's instructions. Nucleotide sequences were determined using a 3730 Genetic Analyzer (Applied Biosystems Inc., Foster City, CA, USA). Plasmid DNA was isolated from *E. coli* host cells using a Plasmid Miniprep kit (Axygen, Union City, CA, USA).

Construction of the Promoter-reporter Plasmid pUC18-GFP

The pET28b-GFP plasmid was maintained in our laboratory (State Key Laboratory of Pollution Control and Resource Reuse, Tongji University, Shanghai, China). The GFP gene fragment of pET28b-GFP and the pUC18 plasmid were digested with *Bam*HI and *Hind*III restriction enzymes, purified and ligated using T4 DNA Ligase. The ligation product was transformed into *E. coli* Top10. Transformants were randomly selected for PCR identification using M13F and M13R universal primers (Table 4) and positive transformants were sequenced to confirm that the inserted promoter sequences were correct. The recombinant plasmid pUC18-GFP was extracted using a Plasmid UP Midiprep Purification System B kit (Biodev-Tech) and used for follow-up experiments.

Cloning of the DSM 505 cbb Promoter

The unknown upstream fragment of DSM 505 *cbbL* was amplified using TAIL-PCR (Fig. 2). First, a fragment of the DSM 505 *cbbL* gene (S-DSM 505-*cbbL*) was amplified with primers K2F and V2F. The PCR product was gel purified and ligated with the pMD18-T plasmid (TaKaRa) and the resulting vector was transformed into *E. coli* Top10. The transformants were identified by PCR and sequenced. Second, three specific reverse primers were designed based on the S-DSM 505-*cbbL* sequence (Table 4). The unknown upstream sequence was amplified with the random primer 12N and three specific reverse primers using three rounds of TAIL-PCR (Table 2 and 3). The product of each round of PCR was used as the template for subsequent round of PCR. Upstream fragments were obtained after the third round of TAIL-PCR. After gel purification, the fragments were ligated with the pMD18-T plasmid and the resulting vectors were transformed into *E. coli* Top10. The inserted fragments were identified by PCR, sequenced and named $UP_{505\text{-}cbbL}$.

Because the exact location of the initiation codon of the *cbbL* gene was unknown, two specific reverse primers were designed to amplify products either one base longer [505L-R-I(+1)] or one base shorter [505L-R-I(-1)] than 505L-R-I. Additionally, to ensure that the upstream sequence contained the promoter region, two forward primers (505L-F-YD and 505L-F-J) were designed based on

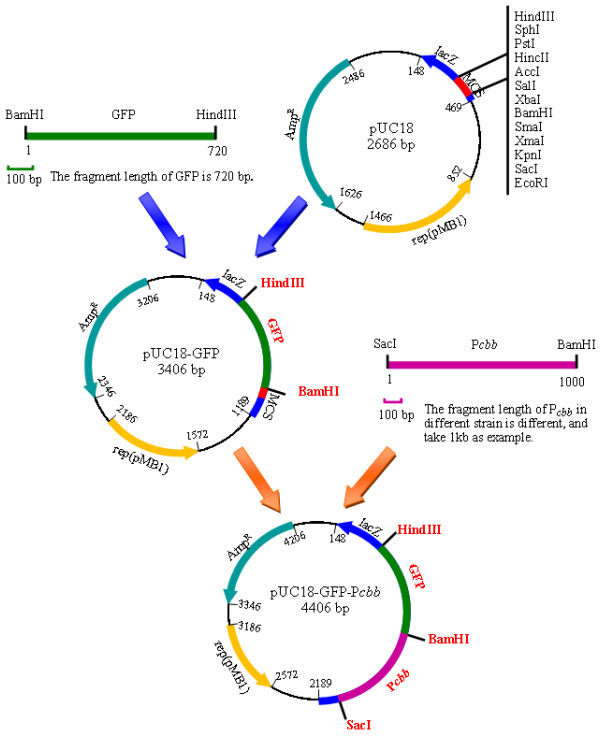

Fig. 1: Construction diagram of the recombinant plasmid pUC18-GFP-P_{cbb}. In which, the digested fragment of GFP gene was firstly inserted into the cloning vector pUC18 and formed a recombinant plasmid pUC18-GFP with *Bam*HI and *Hind*III restriction enzymes digestion. After that, the PCR amplification fragment of *cbb* gene prompter (P_{cbb}) was inserted into pUC18-GFP and formed a new recombinant plasmid pUC18-GFP-P_{cbb} with *Sac*I and *Bam*HI restriction enzymes digestion

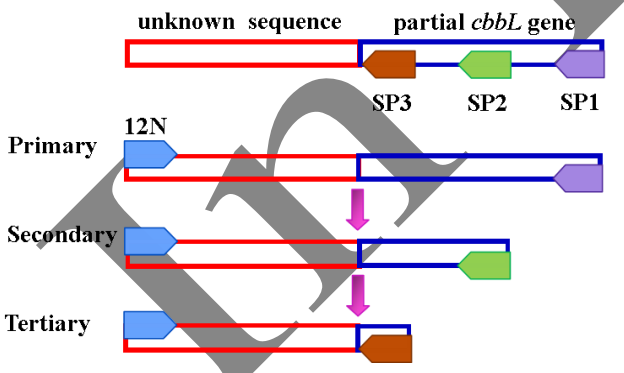

Fig. 2: Schematic diagram of amplifying the *cbbL* promoter in DSM 505 with unknown nucleotide sequence utilized thermal asymmetric interlaced PCR (TAIL-PCR) technology

the $UP_{505\text{-}cbbL}$ sequence (Table 4). Six putative promoter regions for DSM 505-*cbbL* were amplified by high-fidelity PCR and named $P_{505\text{-}cbbL\text{-}Y+I\text{-}1}$, $P_{505\text{-}cbbL\text{-}Y+I}$, $P_{505\text{-}cbbL\text{-}Y+I+1}$, $P_{505\text{-}cbbL\text{-}J+I\text{-}1}$, $P_{505\text{-}cbbL\text{-}J+I}$ and $P_{505\text{-}cbbL\text{-}J+I+1}$. The PCR reaction products and the pUC18-GFP plasmid were digested with *Sac*I and *Bam*HI, ligated and transformed into *E. coli* Top10. The transformants were identified by PCR and sequenced. Among each set of three fragments, only the fragment containing the promoter with the correct sequence can initiate expression of GFP, which can be detected by measuring the fluorescence intensity of the recombinant *E. coli* strains.

Cloning the Known DSM 15147 cbb Promoter Sequence

The *cbbL* and *cbbM* promoters were first amplified using the primers hnLF/hnLR and hnMF/hnMR shown in Table 4. The PCR reaction products $P_{15147\text{-}cbbL}$ and $P_{15147\text{-}cbbM}$ and the pUC18-GFP plasmid were digested with *Sac*I and *Bam*HI and ligated using T4 DNA Ligase. The ligation products pUC18-GFP-$P_{15147\text{-}cbbL}$ and pUC18-GFP-$P_{15147\text{-}cbbM}$ were transformed into *E. coli* Top10. Transformants were randomly selected for PCR identification using the universal primers M13F and M13R. Positive transformants were sequenced to confirm that the inserted promoter sequences were correct.

Determination of the Promoter Efficacy of the Cloned *cbb* Gene Promoter in *E. coli*

Recombinant strains were grown in LB liquid medium containing 60 μg/mL ampicillin. *E. coli* Top10 cultured in LB liquid medium without ampicillin was used as a negative control. The promoter efficacy was assayed by measuring the fluorescence intensity of cultured recombinant strains using a Synergy 4 multimode reader (BioTek Instruments, Winooski, VT, USA) at an excitation wavelength (*Ex*) of 470 nm and an emission wavelength of 510 nm. Spectral scanning was also conducted at an *Ex* of 470 nm with an expected peak at 510 nm. To eliminate interference associated with LB medium background fluorescence, the medium was discarded and bacterial cells were washed three times with ddH_2O and suspended in ddH_2O.

Results

Construction of the Promoter-report Plasmid pUC18-GFP

Double restriction enzyme-digested products were assessed by agarose gel electrophoresis. The results showed that the GFP gene fragment was excised from pET28b-GFP (Fig. 3a) and that the pUC18 plasmid was cut (Fig. 3b). PCR results showed that the GFP gene fragment was successfully inserted into the pUC18 plasmid. Results of transformant sequencing and sequence alignment showed that the sequence of the inserted GFP fragment was correct. Therefore, the recombinant bacteria *E. Coli* Top10/pUC18-GFP was obtained.

Cloning and Identification of the DSM 505 *cbb* Promoter

A 500 bp fragment of the DSM 505 *cbbL* gene (S-DSM 505-*cbbL*) was amplified with primers K2F and V2F and cloned into the pMD18-T plasmid. PCR analysis of the

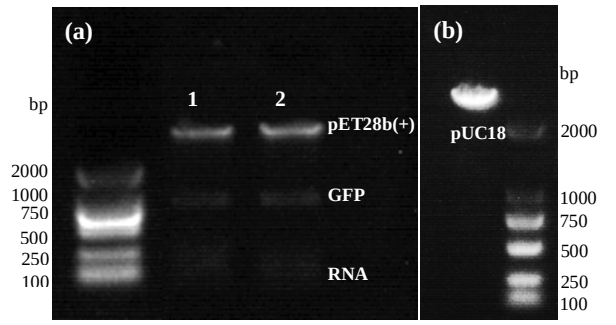

Fig. 3: Enzyme digestion results for plasmids pET28b-GFP and pUC18 with *Bam*HI and *Hind*III restriction enzymes digestion. Among them, the middle band of GFP fragment was obtained in (a) and the pUC18 band after digestion was obtained in (b). (Note: 1 and 2 are parallel samples)

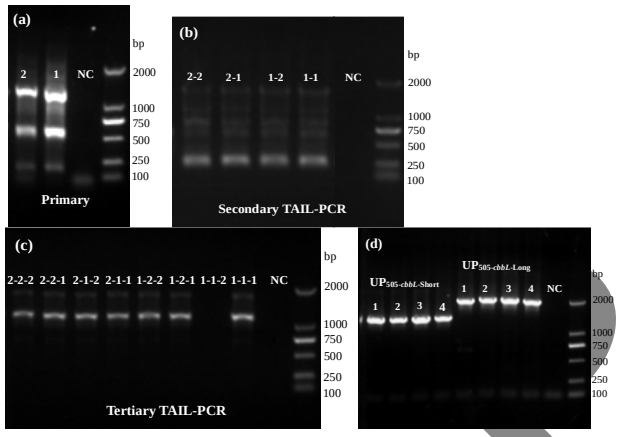

Fig. 4: Three rounds of TAIL-PCR amplification results for S-DSM 505-cbbL upstream sequence and the PCR identification results for transformants containing recombinant plasmid pMD18-UP505-cbbL-Long and pMD18-UP505-cbbL-Short, and their length were about 1.7 and 1.2 kb, respectively. (Note: NC represents the negative control, and 1, 2, 1-1, 1-2, 1-1-1, 1-1-2, 2-2-2 and 1,2,3,4 are parallel samples, respectively)

transformants confirmed that the size of the inserted fragment was correct. Sequencing analysis showed that part of the sequence at the 3'-end had 100% similarity with the NCBI Sequence Database sequence of DSM 505-*cbbL*-NCBI (gb DQ390449.1), indicating that the fragment is part of the DSM 505 *cbbL* gene. Because the cloned partial sequence aligned with the 5'-end of the NCBI sequence (gb DQ390449.1), we concluded that the sequence is located in the middle or upper regions of the *cbbL* gene. Three specific reverse primers for subsequent TAIL-PCR experiments were designed based on the obtained sequence (Fig. 2). The upstream sequence of S-DSM 505-*cbbL* was amplified through three rounds of TAIL-PCR (Fig. 2). Several fuzzy bands were observed after the first and second rounds of TAIL-PCR (Fig. 4a and b), whereas two legible bands, named $UP_{505\text{-}cbbL\text{-Short}}$ and $UP_{505\text{-}cbbL\text{-Long}}$, were visible after the third round of TAIL-PCR (Fig. 4c). $UP_{505\text{-}cbbL\text{-Short}}$ and $UP_{505\text{-}cbbL\text{-Long}}$ were approximately 1.2 kb and 1.7 kb, respectively. The results of transformant PCR identification confirmed that the inserted fragments were the correct size (Fig. 4d).

Sequencing results were analysed using NCBI BLAST. Results showed that the 3' sequence of the 1148 bp $UP_{505\text{-}cbbL\text{-Short}}$ was identical to the 5' sequence of S-DSM 505-*cbbL*, indicating that $UP_{505\text{-}cbbL\text{-Short}}$ contains the upstream sequence of S-DSM 505-*cbbL*. Moreover, the full-length sequence of $UP_{505\text{-}cbbL\text{-Short}}$ was identical to the 3' sequence of $UP_{505\text{-}cbbL\text{-Long}}$, indicating that the 1730 bp $UP_{505\text{-}cbbL\text{-Long}}$, which includes the full-length sequence of $UP_{505\text{-}cbbL\text{-Short}}$, contains a longer upstream sequence of S-DSM 505-*cbbL*. These results confirmed that both $UP_{505\text{-}cbbL\text{-Long}}$ and $UP_{505\text{-}cbbL\text{-Short}}$ contained the correct upstream sequence of S-DSM 505-*cbbL*.

Based on sequence alignment of S-DSM 505-*cbbL* and DSM 505-*cbbL*-NCBI (gb DQ390449.1), and the fact that the length of the entire DSM 15147 *cbbL* gene was 1422 bp, we determined that S-DSM 505-*cbbL* may be located in either the 0–500 bp or 600–1000 bp region of the *cbbL* gene sequence. Because the upstream sequence of $UP_{505\text{-}cbbL\text{-Long}}$ is 1730 bp, we determined that $UP_{505\text{-}cbbL\text{-Long}}$ is located approximately 1700 bp or 1100 bp upstream of the transcription start site of the DSM 505-*cbbL* gene. Therefore, $UP_{505\text{-}cbbL\text{-Long}}$ must contain a promoter sequence. As shown in Fig. 5a, b, and c, six upstream promoter region fragments of DSM 505-*cbbL* ($P_{505\text{-}cbbL\text{-Y+I-1}}$, $P_{505\text{-}cbbL\text{-Y+I}}$, $P_{505\text{-}cbbL\text{-Y+I+1}}$, $P_{505\text{-}cbbL\text{-J+I-1}}$, $P_{505\text{-}cbbL\text{-J+I}}$ and $P_{505\text{-}cbbL\text{-J+I+1}}$) were successfully PCR amplified.

Transformants containing each of the six fragments were PCR amplified with the universal primers M13F and M13R (Fig. 5d and e). Since the inserted fragments include the GFP gene and the *cbb* promoter, the lengths of the target fragments were approximately 2.2 and 1.9 kb, as expected. Sequencing of transformants confirmed that the promoter sequences were correctly inserted into pUC18-GFP. Therefore, the recombinant strains *E. coli* Top10/pUC18-GFP-$P_{505\text{-}cbbL\text{-Y+I-1}}$, *E. coli* Top10/pUC18-GFP-$P_{505\text{-}cbbL\text{-Y+I}}$, *E. coli* Top10/pUC18-GFP-$P_{505\text{-}cbbL\text{-Y+I+1}}$, *E. coli* Top10/pUC18-GFP-$P_{505\text{-}cbbL\text{-J+I-1}}$, *E. coli* Top10/pUC18-GFP-$P_{505\text{-}cbbL\text{-J+I}}$ and *E. coli* Top10/pUC18-GFP-$P_{505\text{-}cbbL\text{-J+I+1}}$ were obtained.

Cloning and Identification of the DSM 15147 *cbb* Promoter

The PCR reaction products $P_{15147\text{-}cbbL}$ and $P_{15147\text{-}cbbM}$ were visualized using agarose gel electrophoresis (Fig. 6a) and found to be approximately 1.2 kb and 1.0 kb, respectively, as expected. Transformant PCR identification results are shown in Fig. 6b. Since the inserted fragments include the GFP gene and the *cbb* promoter, the lengths of the target fragments were approximately 2.0 and 1.8 kb, as expected. Sequencing of positive transformants confirmed that the sequences of the inserted promoters and the open reading frame containing the GFP gene were correct.

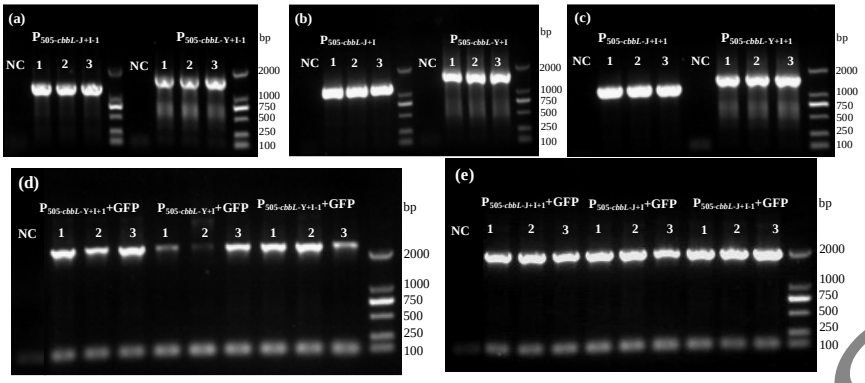

Fig. 5: PCR amplification results for the six fragments of DSM 505-*cbbL* gene upstream promoter (a, b and c); and PCR identification results for transformants containing their corresponding recombinant plasmids (d and e). The three long fragments of $P_{505\text{-}cbbL\text{-}Y+I\text{-}1}$, $P_{505\text{-}cbbL\text{-}Y+I}$ and $P_{505\text{-}cbbL\text{-}Y+I+1}$ were about 1.5 kb, and the three shout fragments of $P_{505\text{-}cbbL\text{-}J+I\text{-}1}$, $P_{505\text{-}cbbL\text{-}J+I}$, and $P_{505\text{-}cbbL\text{-}J+I+1}$ were about 1.2 kb. The long fragments of $P_{505\text{-}cbbL\text{-}Y+I\text{-}1}$+GFP, $P_{505\text{-}cbbL\text{-}Y+I}$+GFP and $P_{505\text{-}cbbL\text{-}Y+I+1}$+GFP were about 2.2 kb, and short fragments of $P_{505\text{-}cbbL\text{-}J+I\text{-}1}$+GFP, $P_{505\text{-}cbbL\text{-}J+I}$+GFP, and $P_{505\text{-}cbbL\text{-}J+I+1}$+GFP were about 1.9 kb. (Note: NC represents the negative control, and 1, 2 and3 are parallel samples)

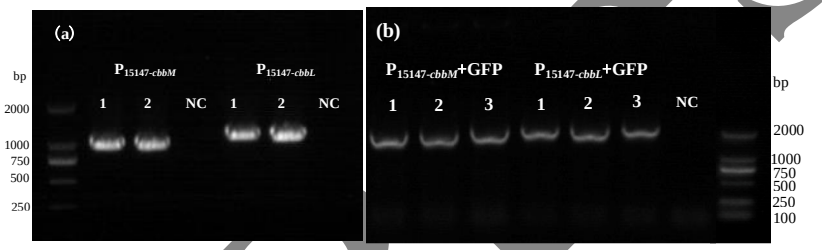

Fig. 6: PCR amplification results for *cbb* gene upstream promoter regions from *cbbL* and *cbbM* in DSM 15147 with fragments length of about 1.2 and 1.0 kb (a), and the PCR identification of transformants *E. coli* Top10/pUC18-GFP-$P_{15147\text{-}cbbL}$ and *E. coli* Top10/pUC18-GFP-$P_{15147\text{-}cbbM}$ (b) with length of about 2.0 and 1.8 kb. (Note: NC represents the negative control, and 1, 2 and 3 are parallel samples)

Thus, we expected the GFP gene to be expressed if the cloned promoter-containing fragment had promoter activity. Therefore, the recombinant bacteria *E. coli* Top10/pUC18-GFP-$P_{15147\text{-}cbbL}$ and *E. coli* Top10/pUC18-GFP-$P_{15147\text{-}cbbM}$ were obtained.

Comparison of the Promoter Efficacy of *cbb* Promoters in *E. coli*

The fluorescence intensities and emission spectra of the recombinant bacteria in suspension are shown in Fig. 7. For DSM 15147, an obvious emission peak at 510 nm was observed for *E. coli* Top10/pUC18-GFP-$P_{15147\text{-}cbbL}$ at an *Ex* of 470 nm. The fluorescence intensity of *E. coli* Top10/pUC18-GFP-$P_{15147\text{-}cbbL}$ was also significantly higher than that of *E. coli* Top10/pUC18-GFP-$P_{15147\text{-}cbbM}$ (Fig. 7a and b).

For DSM 505, six recombinant strains containing different promoter fragments were constructed. Of the three recombinant bacteria containing fragments differing in sequence by one or two base pairs, only the one containing the promoter with the correct sequence could exhibit GFP fluorescence. Thus, the fluorescence intensities of the recombinant bacteria were measured to determine which promoter effectively initiated GFP expression. The fluorescence intensities and emission spectra of the recombinant bacteria in suspension at an *Ex* of 470nm are shown in Fig. 7c-f. Obvious emission peaks at 510 nm were observed for *E. coli* Top10/pUC18-GFP-$P_{505\text{-}cbbL\text{-}Y+I+1}$ and *E. coli* Top10/pUC18-GFP-$P_{505\text{-}cbbL\text{-}J+I+1}$ at an *Ex* of 470 nm. Higher fluorescence intensities were observed for *E. coli* Top10/pUC18-GFP-$P_{505\text{-}cbbL\text{-}Y+I+1}$ and *E. coli* Top10/pUC18-GFP-$P_{505\text{-}cbbL\text{-}J+I+1}$. And the fluorescence intensity of the recombinant bacteria *E. coli* Top10/pUC18-GFP-$P_{505\text{-}cbbL\text{-}Y+I+1}$ was higher than that of *E. coli* Top10/pUC18-GFP-$P_{505\text{-}cbbL\text{-}J+I+1}$.

Fluorescence intensities of the recombinant bacteria *E. coli* Top10/pUC18-GFP-$P_{505\text{-}cbbL\text{-}Y+I+1}$ and *E. coli* Top10/pUC18-GFP-$P_{505\text{-}cbbL\text{-}J+I+1}$ in suspension were higher than those of *E. coli* Top10/pUC18-GFP-$P_{15147\text{-}cbbL}$ (Fig. 7g and h). These results indicate that the promoter efficacy of the DSM 505 *cbbL* promoter was higher than that of the DSM 15147 *cbbL* promoter.

Discussion

In DSM 15147, the promoter strength of the *cbbL* gene was much higher than that of the *cbbM* gene. Similarly, the transcription efficiency of the *cbbL* gene was much higher

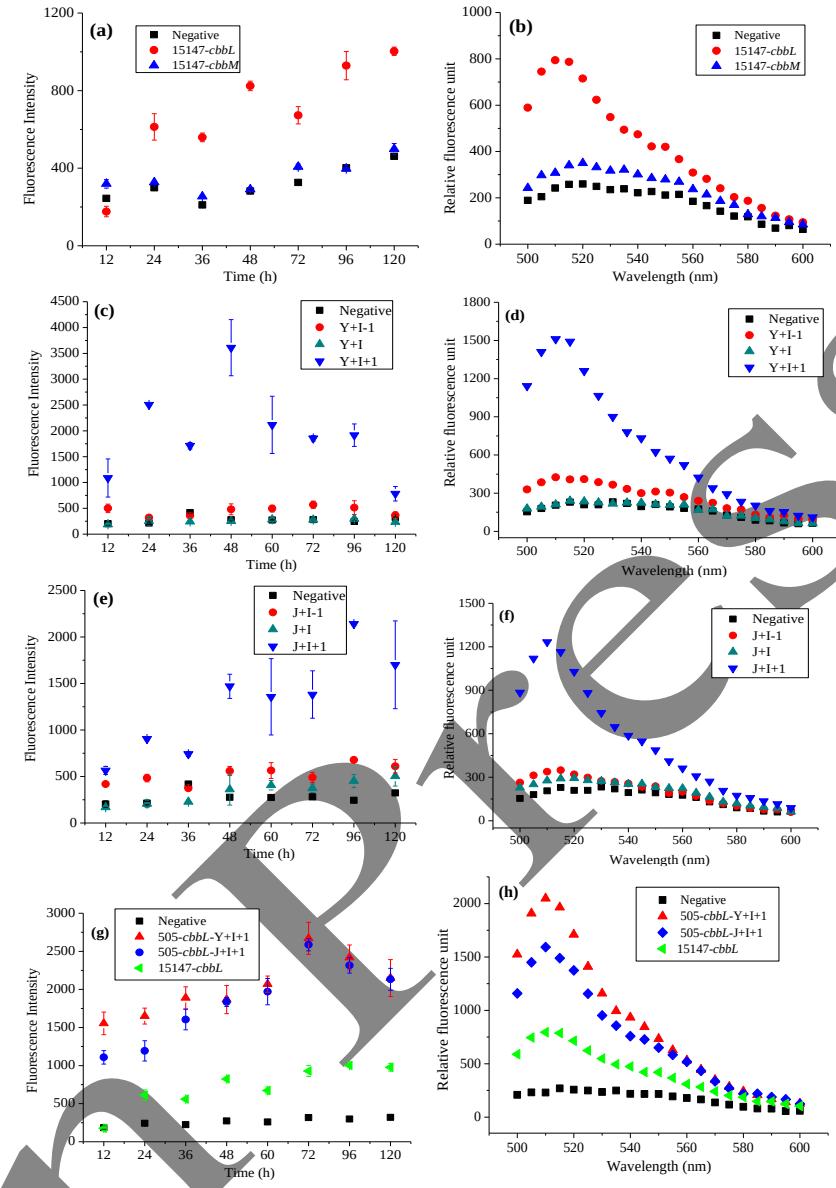

Fig. 7: Fluorescence intensity and emission spectra of recombinant bacteria in suspension at an excitation wavelength of 470 nm. (a): Fluorescence intensity of *E. coli* Top10/pUC18-GFP-P$_{15147\text{-}cbbL}$ and *E. coli* Top10/pUC18-GFP-P$_{15147\text{-}cbbM}$; (b): emission spectra of *E. coli* Top10/pUC18-GFP-P$_{15147\text{-}cbbL}$ and *E. coli* Top10/pUC18-GFP-P$_{15147\text{-}cbbM}$ at 72 h during cultivation; (c)(d): Fluorescence intensity and emission spectra of the three recombinant bacteria strains containing long fragments of promoter (*E. coli* Top10/pUC18-GFP-P$_{505\text{-}cbbL\text{-}Y+I\text{-}1}$, *E. coli* Top10/pUC18-GFP-P$_{505\text{-}cbbL\text{-}Y+I}$ and *E. coli* Top10/pUC18-GFP-P$_{505\text{-}cbbL\text{-}Y+I+1}$); (e)(f): Fluorescence intensity and emission spectra of the three recombinant bacteria strains containing short fragments of promoter (*E. coli* Top10/pUC18-GFP-P$_{505\text{-}cbbL\text{-}J+I\text{-}1}$, *E. coli* Top10/pUC18-GFP-P$_{505\text{-}cbbL\text{-}J+I}$ and *E. coli* Top10/pUC18-GFP-P$_{505\text{-}cbbL\text{-}J+I+1}$); (g)(h): the fluorescence intensity of the recombinant bacteria in suspension of E. coli Top10/pUC18-GFP-P505-cbbL-Y+I+1, E.coli Top10/pUC18-GFP-P505-cbbL-J+I+1 and E. coli Top10/pUC18-GFP-P15147-cbbL, and the excitation spectra at 96 h during cultivation

than that of the *cbbM* gene (Wang *et al.*, 2016a), indicating that the *cbbL* gene might play a more significant role in CO_2 assimilation than the *cbbM* gene in DSM 15147.

For DSM 505, only the fragments amplified with the reverse primer 505-R-I (+1) contained the promoter with the correct sequence and were able to initiate GFP expression. These results were strengthened by the fact that both the long and short fragments amplified with the reverse primer 505-R-I (+1) were able to initiate GFP expression. The longer promoter fragments had higher promoter efficacy, this was likely due to the fact that the longer promoter fragments contain positive regulators; similar results had been reported for the *cbbL* promoter of *Rhodobacter capsulatus* (Paoli *et al.*, 1998).

The promoter efficacy of the DSM 505 *cbbL* promoter was higher than that of the DSM 15147 *cbbL* promoter, suggesting that the transcription potential of the DSM 505 *cbbL* gene should be higher than that of the DSM 15147 *cbbL* gene. However, the transcription efficiency of DSM 505 *cbbL* was lower than that of DSM 15147 *cbbL* (Wang *et al.*, 2016a). This indicated that the *cbbL* gene transcription and CO_2 assimilation potentials of DSM 505 might be higher than those of DSM 15147 but that the CO_2 assimilation potential of DSM 505 might not be fully realized.

The transcription activity of the *cbb* gene is known to be influenced by environmental factors (Yoshizawa *et al.*, 2004; Toyoda *et al.*, 2005), including CO_2 concentration (Wang *et al.*, 2016a; Wang *et al.*, 2016b) and exogenous organic matter (Friedrich, 1982; Kusian and Bowien, 1997; Wang *et al.*, 2017). Previously, it was found that DSM 505 was more sensitive to inhibition by extracellular dissolved organic carbon than DSM 15147 (Wang *et al.*, 2018). Therefore, optimization or elimination of such adverse factors could improve the transcription efficiency of the DSM 505 *cbbL* gene, resulting in higher CO_2 fixation efficiencies.

Conclusion

Regarding DSM 15147, the promoter efficacy of the *cbbL* promoter was much higher than that of the *cbbM* promoter, which corresponded with the *cbb* gene transcription efficiency. However, in contrast to the actual expression efficiency, the promoter efficacy of the DSM 505 *cbbL* promoter was higher than that of DSM 15147. Our results suggest that the *cbbL* gene transcription and CO_2 assimilation potentials of DSM 505 are higher than those of DSM 15147 but that the gene transcription potential of DSM 505 *cbbL* is not fully realized.

Acknowledgments

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References

Badger, M.R. and E.J. Bek, 2008. Multiple rubisco forms in proteobacteria: their functional significance in relation to CO_2 acquisition by the CBB cycle. *J. Exp. Bot.*, 59: 1525–1541

Baker, S.H., S. Jin, H.C. Aldrich, G.T. Howard and J.M. Shively, 1998. Insertion mutation of the form I *cbbL* gene encoding ribulose bisphosphate carboxylase/oxygenase (RuBisCO) in *Thiobacillus neapolitanus* results in expression of form II RuBisCO, loss of Carboxysomes, and an increased CO_2 requirement for growth. *J. Bacteriol.*, 180: 4133–4139

Berg, I.A., 2011. Ecological aspects of the distribution of different autotrophic CO_2 fixation pathways. *Appl. Environ. Microbiol.*, 77: 1925–1936

Chung, Y.C., C. Huang, J.R. Pan and C.P. Tseng, 1998. Comparison of autotrophic and mixotrophic biofilters for H_2S removal. *J. Environ. Eng.,* 124: 362–367

Dubbs, J.M. and F.R. Tabita, 2004. Regulators of nonsulfur purple phototrophic bacteria and the interactive control of CO_2 assimilation, nitrogen fixation, hydrogen metabolism and energy generation. *FEMS Microbiol. Rev.*, 28: 353–376

Dubbs, J.M. and F.R. Tabita, 2003. Interactions of the *cbb*II promoter-operator region with cbbR and RegA (PrrA) regulators indicate distinct mechanisms to control expression of the two *cbb* operons of *Rhodobacter sphaeroides*. *J. Biol. Chem.*, 278: 16443–16450

Friedrich, C.G., 1982. Depression of hydrogenase during limitation of electron donors and derepression of ribulose bisphosphate carboxylase during carbon limitation of *Alcaligenes eutrophus*. *J. Bacteriol.*, 149: 203–210

Hernandez, J.M., S.H. Baker, S.C. Lorbach, J.M. Shively and F.R. Tabita, 1996. Deduced amino acid sequence, functional expression, and unique enzymatic properties of the form I and form II ribulose bisphosphate carboxylase/oxygenase from the chemoautotrophic bacterium *Thiobacillus denitrificans*. *J. Bacteriol.*, 178: 347–356

Hu, J.J., L. Wang, S.P. Zhang, Y.Q. Wang and X.F. Xi, 2011. Inhibitory effect of organic carbon on CO_2 fixing by non-photosynthetic microbial community isolated from the ocean. *Bioresour. Technol.*, 102: 7147–7153

Ikeno, S., C. Ogino, T. Ito and N. Shimizu, 2003. Detection of benzene derivatives by recombinant *E. coli* with Ps promoter and GFP as a reporter protein. *Biochem. Eng. J.*, 15: 193–197

Kusian, B., R. Bednarski, M. Husemann and B. Bowien, 1995. Characterization of the duplicate ribulose-1,5-bisphosphate carboxylase genes and *cbb* promoters of *Alcaligenes eutrophus*. *J. Bacteriol.*, 177: 4442–4450

Kusian, B. and B. Bowien, 1997. Organization and regulation of *cbb* CO_2 assimilation genes in autotrophic bacteria. *FEMS Microbiol. Rev.*, 21: 135–155

López, J.C., G. Quijano, T.S. Souza, J.M. Estrada, R. Lebrero and R. Muñoz, 2013. Biotechnologies for greenhouse gases (CH_4, N_2O and CO_2) abatement: state of the art and challenges. *Appl. Environ. Microbiol.*, 97: 2277–2303

Liu, Y.G. and Y. Chen, 2007. High-efficiency thermal asymmetric interlaced PCR for amplification of unknown flanking sequences. *Biotechniques*, 43: 649–656

Paoli, G.C., P. Vichivanives and F.R. Tabita, 1998. Physiological control and regulation of the *Rhodobacter capsulatus cbb* operons. *J. Bacteriol.*, 180: 4258–4269

Qiu, Y., J. Tian, R. Ge, B. Zhao, Y. Shen and Z. Huang, 2008. A modified TAIL-PCR and its application in isolating gene promoter of wheat. *Chin. J. Biotechnol.,* 24: 695–699

Saini, R., R. Kapoor, R. Kumar, T.O. Siddiqi and A. Kumar, 2011. CO_2 utilizing microbes - a comprehensive review. *Biotechnol. Adv.*, 29: 949–960

Tóth, G., N. Nemestóthy, K. Bélafi-Bakó, D. Vozik and P. Bakonyi, 2015. Degradation of hydrogen sulfide by immobilized *Thiobacillus thioparus* in continuous biotrickling reactor fed with synthetic gas mixture. *Intl. Biodete. Biodegr.,* 105: 185–191

Tabita, F.R., 1988. Molecular and cellular regulation of autotrophic carbon dioxide fixation in microorganisms. *Microbiol. Rev.*, 52: 155–189

Tabita, F.R., T.E. Hanson, H. Li, S. Satagopan, J. Singh and S. Chan, 2007. Function, structure, and evolution of the RubisCO-like proteins and their Rubis CO homologs. *Microbiol. Mol. Biol. Rev.*, 71: 576–599

Tabita, F.R., S. Satagopan, T.E. Hanson, N.E. Kreel and S.S. Scott, 2008. Distinct form I, II, III, and IV Rubisco proteins from the three kingdoms of life provide clues about Rubisco evolution and structure/function relationships. *J. Exp. Bot.*, 59: 1515–1524

Toyoda, K., Y. Yoshizawa, H. Arai, M. Ishii and Y. Igarashi, 2005. The role of two cbbRs in the transcriptional regulation of three ribulose-1,5-bisphosphate carboxylase/oxygenase genes in *Hydrogenovibrio marinus* strain MH-110. *Microbiology*, 151: 3615–3625

Vichivanives, P., T.H. Bird, C.E. Bauer and F.R. Tabita, 2000. Multiple regulators and their interactions in vivo and in vitro with the *cbb* regulons of *Rhodobacter capsulatus*. *J. Mol. Biol.*, 300: 1079–1099

Wang, Y.N., Y.F. Tsang, L. Wang, X.H. Fu, J.J. Hu, H. Li and Y.Q. Le, 2018. Inhibitory effect of self-generated extracellular dissolved organic carbon on carbon dioxide fixation in sulfur-oxidizing bacteria during a chemoautotrophic cultivation process and its elimination. *Bioresour. Technol.*, 252: 44–51

Wang, Y.N., Y.F. Tsang, L. Wang, X.H. Fu, H. Li, J.J. Hu and Y.Q. Le, 2017. Influence of reduced sulfur on carbon fixation efficiency of *Halothiobacillus neapolitanus* and its mechanism. *Chem. Eng. J.*, 326: 249–256

Wang, Y.N., L. Wang, Y.F. Tsang, X.H. Fu, J.J. Hu, H. Li and Y.Q. Le, 2016a. Response of cbb gene transcription levels of four typical sulfur-oxidizing bacteria to the CO_2 concentration and its effect on their carbon fixation efficiency during sulfur oxidation. *Enzyme Microb. Technol.*, 92: 31–40

Wang, Y.N., L. Wang, Y.F. Tsang, X.H. Fu, J.J. Hu, H. Li and Y.Q. Le, 2016b. The variability in carbon fixation characteristics of several typical chemoautotrophic bacteria at low and high concentrations of CO_2 and its mechanism. *Intl. Biodete. Biodegr.*, 113: 105–112

Wei, M., Z. Kong, L. Zhong, L. Qiu, Y. Li, L. Zhao, X. Li and W. Zhong, 2012. Construction of a native promoter-containing transposon vector for the stable monitoring of the denitrifying bacterium *Pseudomonas stutzeri* LYS-86 by chromosomal-integrated gfp. *Plasmid*, 68: 61–68

Yoshizawa, Y., K. Toyoda, H. Arai, M. Ishii and Y. Igarashi, 2004. CO_2-responsive expression and gene organization of three ribulose-1,5-bisphosphate carboxylase/oxygenase enzymes and carboxysomes in *Hydrogenovibrio marinus* strain MH-110. *J. Bacteriol.*, 186: 5685–5691

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