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Note

Improving the efficiency of plasmid transformation in *Shewanella oneidensis* MR-1 by removing *Cla*I restriction site

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ABSTRACT

Here we demonstrate that elimination of *Cla*I restriction site from the sequence of a plasmid DNA increases the efficiency of transformation of *Shewanella oneidensis* MR-1 significantly. To achieve reliable transformation of *S. oneidensis* MR-1 plasmids either lacking *Cla*I site or isolated from primary transformants of *S. oneidensis* should be used.

Shewanella oneidensis MR-1 is a Gram-negative facultative anaerobic γ-proteobacterium that possesses a natural ability to produce hydrogen and has a potential for bioremediation of radionuclides and heavy metals contaminated areas (Hau and Gralnick, 2007; Kolker et al., 2005; Moser and Nealson, 1996; Myers and Myers, 1993; Myers and Nealson, 1988; Nealson et al., 2002; Scott and Nealson, 1994). The *Shewanella*'s respiratory capabilities with an electrode as an electron acceptor have gained great interest in the emerging bioengineering discipline of bioelectrochemical systems (Bretschger et al., 2007; Harris et al., 2010; Watanabe et al., 2009). *S. oneidensis* MR-1 is able to provide an efficient expression and maturation of heterologous hydrogenases. In this regard, *S. oneidensis* could be a promising basis for the development of an applied system for producing biohydrogen (Sybirna et al., 2008).

For further studies on *S. oneidensis* MR-1 it is important to have reliable vectors and methods for the introduction of the exogenous DNA. The technique of electroporation using standard laboratory equipment was well adapted for this strain (Ozawa et al., 2001). Despite that, we observed significant differences in the efficiency of transformation of *S. oneidensis* MR-1 with different plasmids, even if these plasmids were based on the same origin of replication and do not differ significantly. To study that we conducted several transformation experiments.

Escherichia coli DH5α (φ80d*lacZ*ΔM15, *recA*1, *endA*1, *gyrA*96, *thi*-1, *hsdR*17(r_K^-, m_K^+), *supE*44, *relA*1, *deoR*, Δ(*lacZYA-argF*)U169) and *S. oneidensis* MR-1 (wild type) strains were grown on LB medium supplemented with chloramphenicol (12 μg/ml) or neomycin (40 μg/ml) if required. In some cases *S. oneidensis* or *E. coli* transformants were selected on LB medium supplemented with 10 μg/ml or 100 μg/ml of ampicillin, respectively (Yin et al., 2013).

Primers used in this study are listed in Table 1. Plasmids used in this work and their relevant features are listed in Table 2. DNA manipulations were done using standard protocols. All DNA was additionally purified via phenol–chloroform extraction protocol (Birnboim and Doly, 1979; Maniatis and Sambrook, 1982).

A 4.1 kbp fragment of pBAD33 vector excluding *araC* regulatory gene and promoter of *araBAD* operon was amplified with *Pfu* polymerase using primers H1N and H2N and self-ligated. The resulting vector was designated as pB331.

The amplification and site-directed mutagenesis of *E. coli lacZ* gene were done following the overlap extension PCR method (Ho et al., 1989) using primers H9, H10, H11, and H12 and total DNA of *E. coli* K12 as a template. The PCR products that carried the gene without internal *Nde*I site were cloned in *Nde*I-*Bam*HI sites of pB331 giving the plasmid pB-GAL.

A DNA fragment bearing promoter region of *S. oneidensis hyaB* gene was amplified using primers HS49 and HS50 and cloned in *Bgl*II-*Nde*I sites of pB331 giving the plasmid pBHyaB.

A modification and amplification of the gene *adi1* of *Mycoplasma hominis* were done as described above for *lacZ* gene using primers HD45, HD46, HD47, and DiBam and plasmid pET3d-ADI as a template (Fayura et al., 2013). Obtained PCR product was cloned into *Nde*I-*Bam*HI sites of pBHyaB resulting in the plasmid designated as pB-ADI. All DNA constructs were sequenced before use.

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Table 1
Primers used in this study.

Primer	Sequence 5′–3′
H1N	GTCTCATATGAAGAGCTCGGTACCCGGGGA
H2N	CTGAGATCTGTCAAACATGAGCAGATCCT
H9	CGACGGTTTCCACATGGGGATTGGTGGCGA
H10	CCCCATGTGGAAACCGTCGATATTC
H11	ATGGTCATATGACCATGATTACGGATTC
H12	TGTGGATCCACAGCAGGTATTTGCG
HS49	TTTATCATATGGGTCAACCTTTTGTTATGG
HS50	TTGGATCCATAGGTTACCCTTACATCAC
HD45	TGACACATATGGCTGTATTTGACAGTAAAT
HD46	AGTTGCTGAAACTTATGATTTAGCATCAAAAGCAG
HD47	GCTAAATCATAAGTTTCAGCAACTAGGTCAGTT
DiBam	TAGGATCCTACCACTTAACATCTTTACGTG

To eliminate *Cla*I restriction sites target plasmids were digested with *Cla*I restriction endonuclease, filled-in with T4 DNA polymerase and self-ligated. As a result new *Nru*I restriction site was generated. Digestion of obtained constructs with *Nru*I restriction endonuclease was used to prove desired modifications.

Transformation of *S. oneidensis* MR-1 cells was performed as described previously (Ozawa et al., 2001) with some modifications. Briefly, a fresh colony of bacterial cells was inoculated into 2 ml of LB liquid medium and grown aerobically overnight at 28 °C. The obtained culture was diluted 100 times with fresh LB medium and grown aerobically at 28 °C to $OD_{600} = 0.5$. Cells were chilled on ice and pelleted at 3000 *g* for 10 min at 4 °C, washed 3 times with sterile ice cold distilled water (50 ml) and re-suspended in distilled water to $OD_{600} = 100–120$, then ice cold 50% (w/v) solution of glycerol was added to final concentration of 12.5%. The obtained competent culture was poured in aliquots of 50 μl in sterile 1.5 ml polypropylene tubes and stored at −70 °C. Electroporation of bacterial cells was performed using 0.2 cm cuvettes and 0.4–0.5 μg of plasmid DNA at 2.5 kV, 200 Ω, and 25 μF with a Gene Pulser® II from Bio-Rad Laboratories (Hercules, CA, USA). After electroporation cells were washed out from cuvettes with 0.95 ml of SOC medium (2% tryptone, 0.5% yeast extract, 0.05% NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 20 mM glucose) and incubated for 90 min at 28 °C. Then cells were poured on LB plates supplemented with appropriate antibiotic and incubated for 24–36 h at 28 °C.

We observed that the plasmid pB-ADI transformed *S. oneidensis* MR-1 much more efficiently than the plasmid pB-GAL despite the fact that both plasmids are derivatives of the same vector, pB331 (Table 3). Notably, the vector pB331 transformed this bacterium much more efficiently than the parental vector pBAD33 (Table 3). Our attempts to transform *S. oneidensis* MR-1 with a pUC-derived plasmid bearing kanamycin resistant gene or with vector pBR322 also resulted in obtaining just few transformants (Table 3). However, the same plasmids isolated from primary *S. oneidensis* transformants re-transformed *S. oneidensis* MR-1 with efficiency of about 1–3 orders higher than that for DNA isolated from *E. coli* cells (Table 3). Restriction analysis of plasmids purified from primary *S. oneidensis* transformants did not reveal any structural changes (data not shown). It could be supposed that endogenous DNA restriction–modification systems could influence transformation efficiency significantly (Elias et al., 2005; Heidelberg et al., 2002; Romine, 2011).

Table 3
Efficiency of transformation of *S. oneidensis* MR-1 with plasmid DNA isolated from different host strains.

Plasmids	Presence of single *Cla*I site	Efficiency of transformation, CFU/μg DNA[a]	
		DNA isolated from *E. coli* DH5α	DNA isolated from *S. oneidensis* MR-1
pBAD33	Yes	1×10^2	1×10^4
pB331	No	3×10^3	2×10^4
pB-ADI	No	2×10^3	5×10^4
pB-GAL	Yes	5×10	2×10^4
pUC-kanMX	Yes	2×10	4×10^4
pBR322	Yes	5×10	ND
pBR322_ΔClaI	No	3×10^3	ND
pB-GAL_ΔClaI	No	4×10^3	6×10^4

ND — not determined.
[a] Results of the typical representative experiment are shown.

Earlier a gene (SO_A0003) coding for a putative type II restriction endonuclease which is homologous to *Cla*I restriction endonuclease had been found in the genome sequence of *S. oneidensis* MR-1 (Heidelberg et al., 2002). Analysis of the nucleotide sequences of plasmids used revealed a single *Cla*I site in all plasmids that transform *S. oneidensis* MR-1 strain with low efficiency when isolated from *E. coli* DH5α (Table 3). In contrast, this site was absent in the sequence of plasmids that transformed *S. oneidensis* MR-1 strain with high efficiency. Thus, presented data indicate a possible effect of *Cla*I-type restriction–modification system on the efficiency of transformation of *S. oneidensis* MR-1. To check this hypothesis we removed *Cla*I site from sequences of pB-GAL and pBR322 plasmids. Being purified from *E. coli* cells, newly constructed plasmids designated as pB-GAL_ΔClaI and pBR322_ΔClaI transformed *S. oneidensis* MR-1 much more efficiently than the initial plasmids (Table 3). Taken together, presented data suggest that the sequence of *Cla*I site, ATCGAT, being non-methylated correctly decreases efficiency of *S. oneidensis* MR-1 transformation significantly and most likely due to a faster degradation of introduced DNA by the endogenous restriction–modification system. It is obvious that further study of the DNA restriction–modification systems is necessary to check this hypothesis and to simplify molecular-genetic procedures in *S. oneidensis* MR-1 like it was done in *E. coli* (Palmer and Marinus, 1994).

At present, there are two ways to routinely achieve efficient transformation of this bacterium: either elimination of *Cla*I sites from plasmids or using plasmid DNA purified from primary transformants of *S. oneidensis* MR-1 which can be obtained with low efficiency even with plasmids bearing *Cla*I sites. Until a derivative strain of *S. oneidensis* MR-1 defective in SO_A0003 gene is constructed these approaches would provide a considerable rise of number of transformants obtained in a particular experiment.

Table 2
Plasmids used in this study.

Plasmids	Relevant features	References
pBAD33	Vector based on p15A origin, containing *araC* regulatory gene and P_{BAD} promoter of *araBAD* operon of *E. coli*, Cm^r	Guzman et al. (1995)
pB331	Derivative of pBAD33 without *araC* regulatory gene and P_{BAD} promoter of *araBAD* operon of *E. coli*	This study
pBHyaB	Derivative of pB331 with promoter of *hyaB* gene of S. oneidensis	This study
pB-ADI	Derivative of pBHyaB bearing *adi1* gene of *M. hominis*	This study
pB-GAL	Derivative of pB331 bearing *lacZ* gene of *E. coli*	This study
pET3d-ADI	Expression vector, bearing *adi1* gene of *M. hominis*	Fayura et al. (2013)
pUC-kanMX	pUC57 derivative, pMB1 origin, Km^r, Ap^r	Semkiv (unpublished results)
pBR322	Cloning vector, pMB1 origin, Tet^r, Ap^r	Bolivar et al. (1977)
pBR322_ΔClaI	pBR322 without *Cla*I restriction site	This study
pB-GAL_ΔClaI	pB-GAL without *Cla*I restriction site	This study

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