Published online 12 November 2008

Nucleic Acids Research, 2009, Vol. 37, No. 1 ***e3***
doi:10.1093/nar/gkn884

Improvement of bacterial transformation efficiency using plasmid artificial modification

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Received July 22, 2008; Revised October 11, 2008; Accepted October 20, 2008

ABSTRACT

We have developed a method to improve the transformation efficiency in genome-sequenced bacteria, using 'Plasmid Artificial Modification' (PAM), using the host's own restriction system. In this method, a shuttle vector was pre-methylated in *Escherichia coli* cells, which carry all the putative genes encoding the DNA modification enzymes of the target microorganism, before electroporation was performed. In the case of *Bifidobacterium adolescentis* ATCC15703 and pKKT427 (3.9 kb *E. coli-Bifidobacterium* shuttle vector), introducing two Type II DNA methyltransferase genes lead to an enhancement in the transformation efficiency by five orders of magnitude. This concept was also applicable to a Type I restriction system. In the case of *Lactococcus lactis* IO-1, by using PAM with a putative Type I methyltransferase system, *hsdMS*1, the transformation efficiency was improved by a factor of seven over that without PAM.

INTRODUCTION

Recently, vast amounts of sequence information concerning bacterial genomes have become available. Currently, 670 whole-genome bacterial sequences have been published and over 1900 projects are in progress. However, much of the data has been used inefficiently in molecular biological studies since reverse genetic tools, such as convenient shuttle vectors, an efficient transformation method, gene knockout and random mutagenesis techniques, etc., have not been available. Accordingly, we have been working towards developing simple methods that would establish transformation techniques for bacteria for which the genome sequence is available.

It is well known that most bacteria carry a specific restriction modification (R–M) system which acts as a barrier against the invasion of foreign DNA by infected phages or conjugative plasmids, etc. (1). The restriction enzymes recognize a specific 4 bp–8 bp DNA sequence and cleave the DNA, but do not recognize the same sequence when modified by the sequence-specific DNA methylase (2). This prevents the degradation of the host's own DNA by the restriction enzyme. According to REBASE (3), 88% of bacterial genomes carry R–M systems and 43% carry four or more R–M systems. These multiple R–M systems, acting to prevent the incorporation of foreign DNA, make it difficult to apply reverse genetics techniques. To predict the gene-encoding modification enzyme from the genome sequence information is not difficult, since it is usually located in the region flanking that encoding the restriction enzyme, also the specific motifs of the DNA methylase have been well studied (3). It was conjectured that if all, or at least some, of modification enzymes were to be expressed in *Escherichia coli*, then a plasmid prepared in the *E. coli* would be modified as if it was replicated in the target bacterium. Thus, it would elude cleavage by restriction enzymes during the transformation of the target bacterium and greatly improve the efficiency. We term this approach 'Plasmid Artificial Modification' (PAM, Figure 1).

Bifidobacterium adolescentis is one of the dominant commensal bacteria of the adult human large intestine. We have recently analysed the whole-genome sequence of the strain *B. adolescentis* ATCC15703 (DDBJ/EMBL/Genbank Accession# AP009256). However, it was impossible to perform reverse genetic experiments using standard methodology because the transformation efficiency was at an extremely low level ($1–3 \times 10^0$ CFU/μg

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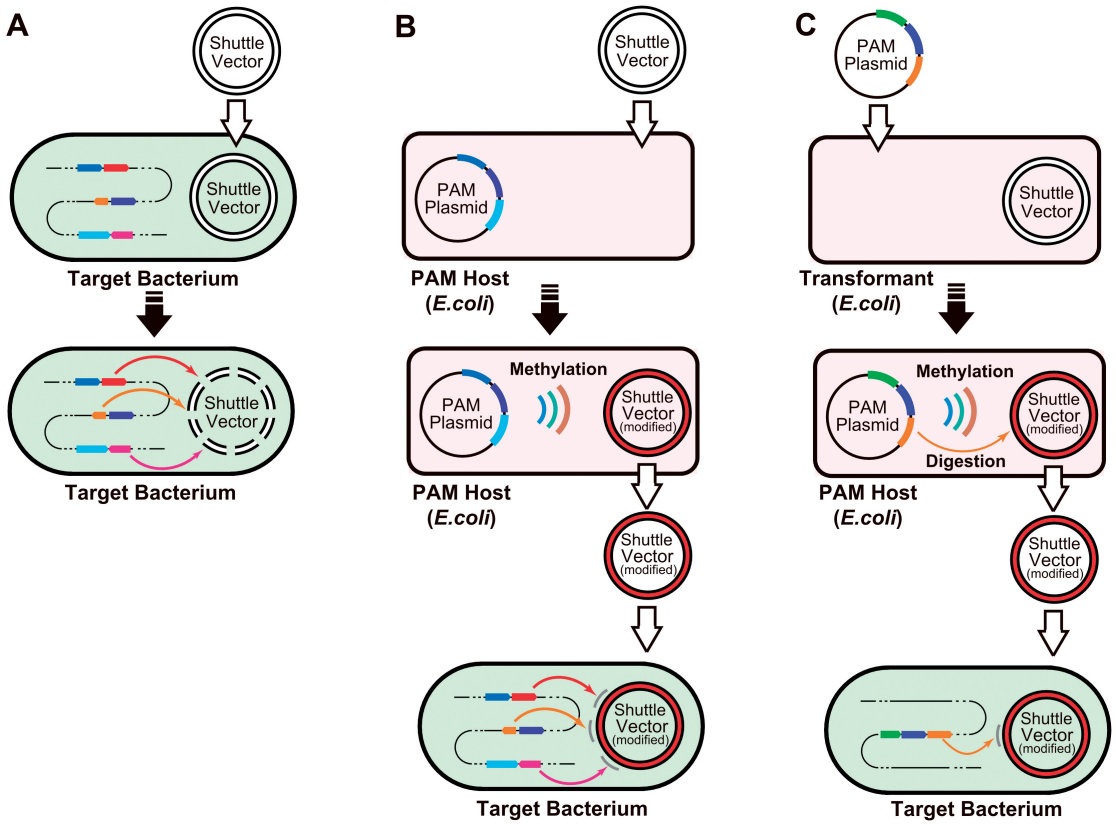

Figure 1. The PAM concept. (**A**) The conventional method for the transformation of bacteria. The introduced shuttle vector is degraded by a restriction enzyme of the target bacterium. A small amount of vector survives and replicates in the target bacterium. (**B**) A PAM plasmid carries all the modification methylase genes of the target host. A shuttle vector plasmid is introduced to *E. coli* host, which had harboured the PAM plasmid (PAM host). The shuttle vector is methylated by the modification enzyme encoded by the genes on the PAM plasmid in the *E. coli* host. The shuttle vector is then extracted and introduced into the target host by electroporation. The shuttle vector is resistant against restriction enzymes and yields higher transformation efficiency. (**C**) The R–M system has a complicated structure, such as a gene cluster that includes subunits or unknown accessory genes. Alternatively, the PAM plasmid, containing a modification gene and also unknown parts, could be introduced into the transformant harbouring a shuttle vector. The restriction enzyme acts, but some copies of the plasmid could survive in the PAM host. The plasmid is then extracted and introduced the target bacterium.

DNA, Table 3). Therefore, we used this strain to demonstrate the experimental approach using PAM.

MATERIALS AND METHODS

Vectors and bacterial strains

pBAD33 (4) was used as low copy number cloning vector. A *Bifidobactrium-E. coli* shuttle vector, pKKT427 (Figure 3), was modified from a pBRASTA101 replicon (5). The pKKT427, 3.9 kb vector, carried a spectinomycin resistance gene, a multi-cloning site and two replication origins including *repB* from *B. longum* and ColEI *ori* (Figure 3). The bacterial strains, *B. adolescentis* ATCC15703 was obtained from the American Type Culture Collection. An *E. coli* stain TOP10 (Invitrogen, Carlsbad, CA, USA) (Table 1) was used as a host for cloning and methyltransferase expression.

Culture and transformation conditions for *Bifidobacterium*

Bifidobacterium adolescentis ATCC15703 was grown anaerobically at 37°C in MRS medium (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) supplemented with 0.02% L-cysteine (Nacalai Tesque, Kyoto, Japan) and 0.34% L-ascorbic acid sodium salt (Nacalai Tesque). Spectinomycin hydrochloride (Wako, Osaka, Japan) was added to an MRS agar plate at 150 μg/ml for the transformation experiment. *Escherichia coli* transformants were grown in LB medium supplemented with 150 μg/ml of spectinomycin and/or 20 μg/ml of chloramphenicol. The electroporation of *B. adolescentis* ATCC15703 was performed as described by Matsumura *et al.* (6).

Cloning of the methyltranceferase genes

The putative methyltransferase genes of *B. adolescentis* ATCC15703 were chosen on the basis of a BLAST search at NCBI (http://www.ncbi.nlm.nih.gov/BLAST/) and REBASE (3) (http://rebase.neb.com/rebase/rebase.html). Genomic DNA of *Bifidobacterium* was extracted and purified (7), and was used as a template in PCR using KOD-Plus-DNA polymerase (TOYOBO, Osaka, Japan). PCR primers (Table 2) were designed using IMC (In Silico Biology, Inc., Yokohama, Kanagawa, Japan)

Table 1. Bacterial strains and plasmids

Bacterial strains	Genotype and Properties
Escherichia. coli TOP 10	F^-, ***mcrA***, (***mrr***, ***hsdRMS–mcrBC***), 80*lacZ* M15 *lacX*74, *recA*1, *araD*139, (*ara–leu*)7697, *galU*, *galK*, *rpsL*(Str^R), ***endA1***, *nupG*
BL21(DE3)	F^-, *ompT*, ***hsdS***$_B$(r_B^-, m_B^-), ***dcm***, *gal*, λ(DE3)
Bifidobacterium adolescentis ATCC 15703	Type strain, isolated from adult human feces
Lactococcus lactis IO-1	Isolated from household wastewater
Lactococcus lactis IL1403	Isolated from cheese starter
Plasmids	**Properties**
pKKT427	A shuttle vector between *E. coli* and *Bifidobacterium* Sp^r,3.9 kb modified of pBRATA101
pBAD33	Cm^r, *araC*, *rrnB*., 5.4 kb
pPAM1233	pBAD33 carrying **BAD_1233**
pPAM1283	pBAD33 carrying **BAD_1283**
pPAM1233-1283	pBAD33 carrying **BAD_1233** and **BAD_1283**
pGKV11	A shuttle vector between *E. coli* and *L. lactis*
pETMS1	pET21b, carrying ***hsdS*1** and ***hsdM*1** of *L. lactis* IO-1
pETNH	pET21b carrying dummy fragment

Bold face indicates nuclease genes and R–M systems.

Table 2. Primer sequence

Primer	Sequence
PMT1-F	5′-gggctagcgaattcgATGAGCAAGGAAATCAAAGT-3′
PMT1-R	5′-gatccccgggtaccgTTACCGTTTCGAATCGTTGT-3′
PMT2-F	5′-gcaggcatgcaagctATGATAAATAACCGGGAGTA-3′
PMT2-R	5′-caaaacagccaagctTCATTCCTTGCTAGCATCAA-3′
OMT-F	5′-ACAACGATTCGAAACGATGATAAATAACC GGGAGT-3′
OMT-R	5′-ACTCCCGGTTATTTATCATCGTTTCGAATC GTTGT-3′

Initiation and stop codons are underlined. Lower case shows the parts corresponding to vector cleavage sites.

and Primer 3 (8). The PCR products were ligated to pBAD33 using an In-Fusion Dry-Down PCR cloning kit (Clontech, Mountain View, CA, USA) (9).

Plasmid DNA preparations from transformed *B. adolescentis* ATCC15703

A plasmid preparation from *Bifidobacterium* was obtained based on the alkaline-SDS method (7), using the lytic enzyme, mutanolysin. A 15 ml Bifidobacterial culture transformant was centrifuged and the cell pellet was suspended in 15 ml of 0.9% NaCl, recentrifuged and then resuspended in 100 µl of TE-glucose [50 mM glucose, 10 mM EDTA, 25 mM Tris–HCl (pH 8.0)]. The suspension (100 µl) was treated with 25 µl of 25 mg/ml lysozyme, 5 µl of 10 U/µl mutanolysin (Sigma-Aldrich, St. Louis, MO, USA) and 1 µl of 10 mg/ml RNase A (Roche Diagnostics, Basel, Switzerland) at 37°C for 30 min. To this, 200 µl of an alkaline-SDS solution (0.2 N NaOH, 1% SDS) was added and the mixture was incubated for 10 min, following which it was neutralized by adding 100 µl of 3 M potassium acetate (pH 4.8) and was then centrifuged at 10 000*g* for 15 min at 4°C. The supernatant was treated with the same volume of phenol–chloroform–isoamylalcohol (25 : 24 : 1). The upper layer was collected and to this, 2.5 times its volume of ethanol was added following which the mixture was subjected to further centrifugation. The pellet was rinsed with 70% ethanol, dried and then dissolved in 50 µl of TE buffer.

Transformation of *Lactococcus lactis* IO-1

The culture and transformation conditions for *L. lactis* IO-1 were as described previously (10). The plasmid pGKV11 was used as a shuttle vector. For cloning of *hsdM*1 and *hsdS*1 of *L. lactis* IO-1, *E. coli* BL21(DE3) and pETNH were used as a host-vector system (11), where the cloned gene was tightly repressed in the absence of the inducer.

RESULTS AND DISCUSSION

Two putative R–M clusters were found in the annotated genome of *B. adolescentis* ATCC15703 (Figure 2A). Each cluster contained one putative gene-encoding DNA methyltransferase, BAD_1233 (M.*Sau*3AI homologue) and BAD_1283 (M.*Kpn*2kI, homologue). These two genes have been amplified and introduced into pBAD33 (4), a low copy number vector. Three plasmids, carrying BAD_1233 and/or BAD_1283 were constructed and designated pPAM1233, pPAM1283 and pPAM1233–1283 (Figure 2B).

There are no reported cryptic plasmids in *B. adolescentis*, accordingly a *B. longum–E. coli* shuttle vector pKKT427 (Figure 3) was used in the transformation experiments, compacted to 3.9 kb, and this gave a high transformation efficiency of the shuttle vector for *B. longum* 105-A ($1–3 \times 10^6$ CFU/µg DNA, Figure 4) (5). The plasmid, pKKT427, was introduced into PAM hosts *E. coli* TOP10 and its recombinants carrying pPAM1233, pPAM1283 or pPAM1233–1283 (Table 1). A colony resistant to the antibiotics spectinomycin and chroramphenicol was selected, the vector, pKKT427 was then extracted and introduced into *B. adolescentis* ATCC15703 by electroporation (12). It was then spread onto a MRS agar plate and cultured at 37°C under anaerobic conditions (5,6).

The shuttle vector pKKT427 acted as a replicon in the target cells, as confirmed by plasmid extraction (Figure 5). The transformation efficiency without the PAM plasmid was $1–3 \times 10^0$ CFU/μg DNA. The CFU with a single gene PAM (pBAD1233 or pBAD1283) was around 10^4 CFU/μg DNA (Table 3). Dual gene PAM (pBAD1233–1283), carrying both methylase BAD_1233 and BAD_1283, yielded 10^5 CFU/μg DNA (Table 3,

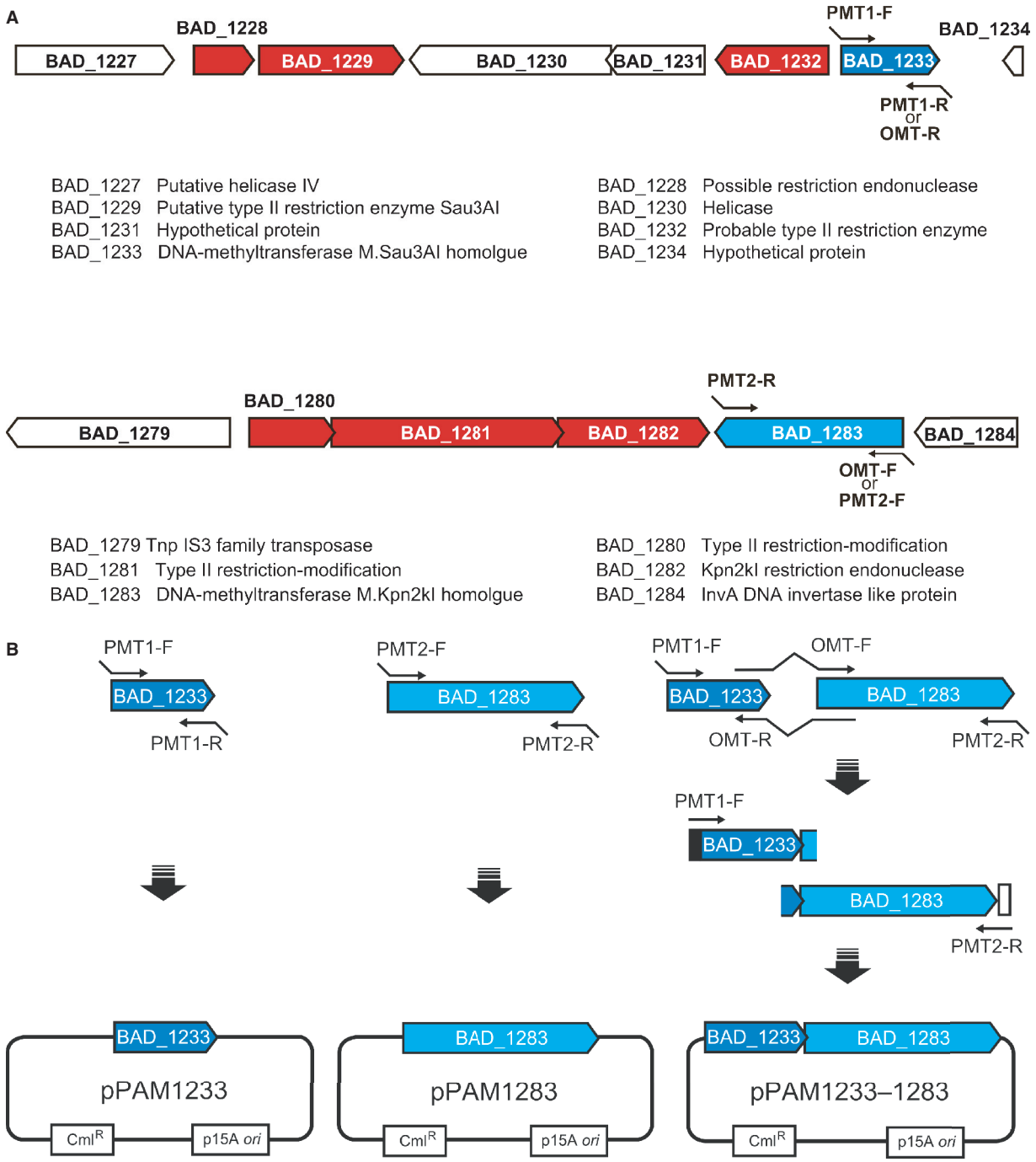

Figure 2. Construction of pPAM plasmids. **(A)** The *B. adolescentis* ATCC15703 genome includes two R–M clusters, BAD_1227–1234 and BAD_1279–1284. Red boxes show putative restriction genes. The blue boxes (BAD_1233 and BAD_1283) show putative methyltransferase genes. **(B)** The putative methyltransferase genes were amplified by PCR using primers as listed in Table 2. The PCR products were joined by *in vitro* homologous recombination to plasmid vector pBAD33, which had been cleaved by HincII, using the In-Fusion Dry-Down PCR cloning kit (Clonetech) to obtain pPAM plasmids. Overlap extension PCR was used for BAD_1233–1283. The pPAM1233–1283 plasmid was a constructed operon of BAD_1233 and BAD_1283. In the first PCR, the coding region of BAD_1233, which was added to the downstream 19 bases from the 5′-end of BAD_1283 was amplified. BAD_1283 was obtained in the same manner 20 bases from the 3′-end of BAD_1233. In the second PCR, the first PCR products were used as a DNA template and PMT1-F and PMT2-R primers were used. The amplified DNA fragment was ligated to the same vector and the plasmid pPAM1233–1283 then obtained.

Figure 4). The transformation efficiency with pPAM1233–1283 was higher than that with pPAM1233 or pPAM1283. It is postulated that the recognition sites of the two methyltransferases, encoded by BAD_1233 and BAD_1283, were different and act synergistically.

A combination of two the putative modification enzymes yielded a synergistic effect. When pKKT427 was extracted from transformed *B. adolescentis* ATCC15703 and introduced into the same cells, the efficiency was almost the same, 6–9 × 10^4 CFU/μg DNA, as for PAM with pBAD1233–1283 (Table 3). When the plasmid pKKT427 was prepared from *B. longum* 105-A, the transformation efficiency into *B. adolescentis* ATCC15703 was also improved by a factor of 10^3 compared with that from *E. coli* TOP10 without PAM plasmid, which was almost the same as that with pPAM1233 or 1283 (Table 3). It should be noted that the R–M system of *B. longum* 105-A was not determined, while that of *B. longum* NCC2705 (13) carried a BAD_1233 orthologue (78% identical to BAD_1233). It appears that 105-A carried a BAD_1233-like modification enzyme, which also improved the transformation efficiency of *B. adolescentis* by a factor of 10^3.

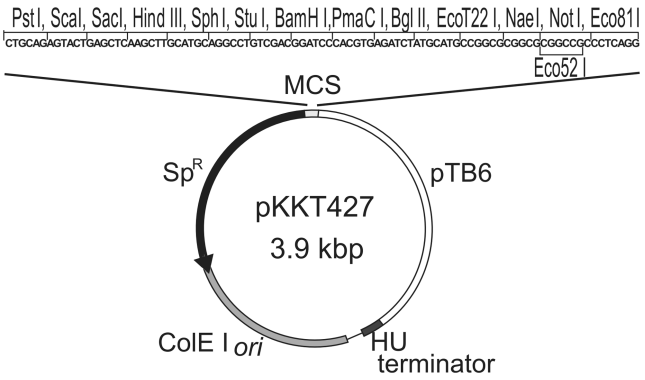

Figure 3. Molecular structure of shuttle vector pKKT427. A *Bifidobacterium–E. coli* shuttle vector, pKKT427, was a modified pBRASTA101 replicon. This shuttle vector had been constructed by modification from a previously reported shuttle vector pBRASTA101, a composite plasmid of pUC18 and MCS and it excluded the β-galactosidase and ampicillin-resistant genes.

These experiments clearly demonstrate that the PAM concept (Figure 1B) is an effective approach for constructing a transformation system for a bacterium for which the whole-genome sequence is known. A reverse sequence procedure was also tried (Figure 1C) and this also improved the efficiency.

Next, we applied this method to another bacterium, *L. lactis* IO-1 (14), which is capable to utilizing xylose and produce lactic acid efficiently. This strain has the potential to utilize biomass for lactic acid production; however, the transformation efficiency was too low to produce using the gene knockout technique. For this strain, the shuttle plasmid, pGKV11 (10), prepared from *L. lactis* IO-1(pGKV11), showed a 26-fold higher efficiency than that of *E. coli* BL21(DE3) harbouring pGKV11 (Table 4), suggesting that the strain IO-1 has R–M system(s). Primers were constructed based on the sequences

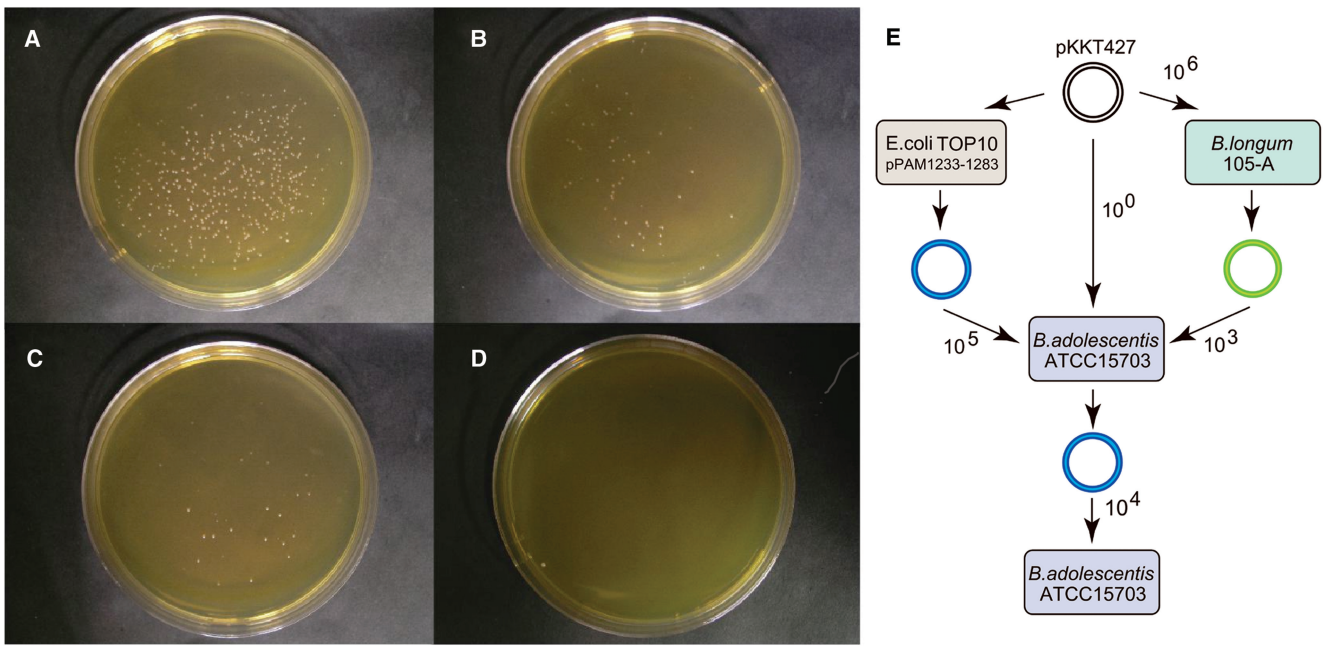

Figure 4. Comparison of PAM effects on transformation efficiencies. (**A–D**) *Bifidobacterium adolescentis* ATCC15703 was transformed by electroporation using the PAM method. The plasmid pKKT427 was prepared from *E. coli* TOP10 carrying pPAM1233-1283 (**A**), pPAM1233 (**B**), pPAM1283 (**C**) or without pPAM plasmid (**D**). An alkaline-SDS method using purification by agarose gel electrophoresis was used to isolate the PAM plasmids which were then introduced into *B. adolescentis* ATCC15703 by electroporation, as described previously (6). The electroporated samples were 100 times diluted in (A–C), but not in D. (**E**) Schematic presentation of transformation efficiencies. Plasmid pKKT427 was prepared from the PAM host (blue), *B. longum* 105-A (green) or *B. adolescentis* ATCC15703. The numbers beside arrows indicate transformation efficiencies (CFU/μg DNA).

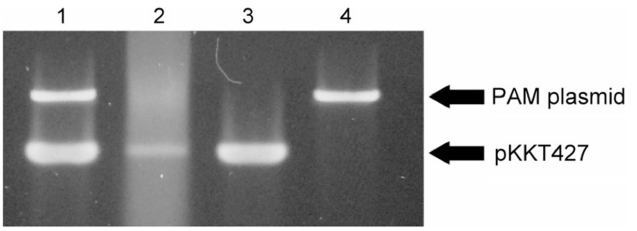

Figure 5. The transformation of *Bifidobacterium* was confirmed by plasmid isolation followed by agarose gel electrophoresis. Plasmids extracted from PAM host *E. coli* TOP10 harbouring pPAM1233–1283 (Lane 1) and from recombinant *B. adolescentis* ATCC15703 (Lane 2). Vector pKKT427 (Lane 3) and pPAM1233-1283 (Lane 4).

Table 3. Transformation efficiency of the shuttle vector pKKT427 into *B. adolescentis* ATCC15703

Modification host	PAM plasmid	Transformation efficiency (CFU/μg DNA)
E. coli TOP10	–	$1-3\times10^{0}$
	pPAM1233	$4-6\times10^{4}$
	pPAM1283	$1-2\times10^{4}$
	pPAM1233-1283	$9\times10^{4}-4\times10^{5}$
B. adolescentis ATCC15703	–	$6-9\times10^{4}$
B. longum 105-A	–	$6\times10^{3}-8\times10^{3}$

of *L. lactis* IL1403 *hsdMS* (15) and used to amplify putative *hsdMS* genes using the IO-1 genomic DNA as a template. Then, we constructed a PAM plasmid, which is carrying a methyltransferase subunit gene, designated *hsdM1* and its specificity subunit gene, *hsdS1* of the IO-1 strain, and designated pETMS1. The transformation efficiency was successfully improved by a factor of seven times under the induced conditions (in the presence of IPTG), the transformation efficiency using pGKV11 increased by a factor of 7 compared with pETNH, which is an empty vector of the PAM plasmid pETMS1 (Table 4). These results suggest that the *R* and *S* subunits of this strain were duly reconstituted and showed activity in the *E. coli* host.

From the various trails with PAM, we found three requirements for it to work effectively. First, a strong promoter, such as the *lac* promoter, should be avoided. In some cases, over-production of DNA methylase leads to cell death or diminishes the growth rate of the PAM host. In the case of *B. adolescentis*, *araBAD* was used as a promoter (4), but under a repressing condition in the absence of an arabinose inducer. Second, an *E. coli* host should be used, which lacks Type IV restriction systems. This type of enzyme degrades the DNA, which has been methylated by the R–M system of other bacterium. *Escherichia coli* K-12 carries four genes-encoding Type IV restriction enzymes (*mcrA*, *mrr* and *mcrBC*). In many cases it is not possible to clone foreign DNA methylase in the wild-type *E. coli* host ($mcrA^{+}$, mrr^{+} and $mcrBC^{+}$), this is because the cloned enzyme will methylate the host *E. coli* genomic DNA, the Type IV enzyme then cleaves its own DNA, causing cell death. From our experience TOP10 was the most effective

Table 4. Transformation efficiency of the shuttle vector pGKV11 into *L. lactis* IO-1

Host	Plasmid donor	Transformation efficiency (CFU/μg DNA)	Fold
L. lactis IO-1	*E. coli* BL21	7.6×10^{2}	1
L. lactis IO-1	*L. lactis* IO-1	2.0×10^{4}	26
L. lactis IO-1	*E. coli* BL21(DE3)/pETNH	$(5.8\pm1.8)\times10^{2}$	1
L. lactis IO-1	*E. coli* BL21(DE3)/pETMS1	$(4.2\pm2.1)\times10^{3}$	7

strain (Table 1). Third, an *E. coli* strain which is *dam, dcm* and *hsdMS* deficient should be used as the PAM host. These genes (*dam, dcm*) encode DNA methylases of the *E. coli* K-12 Type II R–M system. If the target strain carries a Type IV system, the plasmid from (dam^{+}, dcm^{+}) yields around a 10^{3} decrease in the transformation efficiency compared with that from a (dam^{-}, dcm^{-}) host. Otherwise, a strictly regulated expression system for the methylase gene was applicable for PAM plasmids.

In summary, a new method for constructing a transformation system for bacteria has been developed. With this system it is feasible to increase efficiency to $>10^{5}$ CFU/μg DNA for *B. adolescentis* ATCC15703, at which point it becomes relatively easy to set up other molecular tools, such as site-directed mutagenesis, etc. Using the *L. lactis* IO-1 strain, we also demonstrated that this system is applicable not only to a Type II R–E system but also to a Type I multi-subunit R–E system. This simple but powerful method may be generally applicable for other bacterial strains, which carry R–M systems. It could potentially promote post-genomic research into bacterial molecular biology.

ACKNOWLEDGEMENTS

Analysis of *L. lactis* IO-1 has been done as a part of a collaboration of H.Y. and M.S-K. with Prof. K.Sonomoto at Kyushu University. H.Y. and M.S-K. thank J.Oshima, N.Ikawa and T.Tozaki for their experimental contributions.

FUNDING

Grant-in-Aid for Scientific Research on Priority Areas Applied Genomics from the Ministry of Education, Culture, Sports, Science and Technology of Japan and KAKENHI (C)(20510189) from the Japan Society for the Promotion of Science Funding for open access charge: KAKENHI (C).

Conflict of interest statement. None declared.

REFERENCES

1. Arber,W. and Linn,S. (1969) DNA modification and restriction. *Annu. Rev. Biochem.*, **38**, 467–500.
2. Tock,M.R. and Dryden,D.T. (2005) The biology of restriction and anti-restriction. *Curr. Opin. Microbiol.*, **8**, 466–472.

3. Roberts,R.J., Vincze,T., Posfai,J. and Macelis,D. (2007) REBASE–enzymes and genes for DNA restriction and modification. *Nucleic Acids Res.*, **35**, D269–D270.
4. Guzman,L.M., Belin,D., Carson,M.J. and Beckwith,J. (1995) Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. *J. Bacteriol.*, **177**, 4121–4130.
5. Tanaka,K., Samura,K. and Kano,Y. (2005) Structural and functional analysis of pTB6 from *Bifidobacterium longum*. *Biosci. Biotechnol. Biochem.*, **69**, 422–425.
6. Matsumura,H., Takeuchi,A. and Kano,Y. (1997) Construction of *Escherichia coli-Bifidobacterium longum* shuttle vector transforming *B. longum* 105-A and 108-A. *Biosci. Biotechnol. Biochem.*, **61**, 1211–1212.
7. Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbour Laboratory Press, Woodbury, NY.
8. Rozen,S. and Skaletsky,H.J. (2000) Primer3 on the WWW for general users and for biologist programmers. In Krawetz,S. and Misener,S. (eds), *Bioinformatics Methods and Protocols: Methods in Molecular Biology*. Humana Press, Totowa, NJ, pp. 365–386.
9. Zhu,B., Cai,G., Hall,E.O. and Freeman,G.J. (2007) In-fusion assembly: seamless engineering of multidomain fusion proteins, modular vectors, and mutations. *Biotechniques*, **43**, 354–359.
10. van der Vossen,J.M., Kok,J. and Venema,G. (1985) Construction of cloning, promoter-screening, and terminator-screening shuttle vectors for Bacillus subtilis and Streptococcus lactis. *Appl. Environ. Microbiol.*, **50**, 540–542.
11. Watanabe,S., Sato,M., Nimura-Matsune,K., Chibazakura,T. and Yoshikawa,H. (2007) Protection of *psb*AII transcript from ribonuclease degradation *in vitro* by DnaK2 and DnaJ2 chaperones of the cyanobacterium *Synechococcus elongatus* PCC 7942. *Biosci. Biotechnol. Biochem.*, **71**, 279–282.
12. Mercenier,A. and Chassy,B.M. (1988) Strategies for the development of bacterial transformation systems. *Biochimie*, **70**, 503–517.
13. Schell,M.A., Karmirantzou,M., Snel,B., Vilanova,D., Berger,B., Pessi,G., Zwahlen,M.C., Desiere,F., Bork,P., Delley,M. *et al.* (2002) The genome sequence of *Bifidobacterium longum* reflects its adaptation to the human gastrointestinal tract. *Proc. Natl Acad. Sci. USA*, **99**, 14422–14427.
14. Tanaka,K., Komiyama,A., Sonomoto,K., Ishizaki,A., Hall,S.J. and Stanbury,P.F. (2002) Two different pathways for D-xylose metabolism and the effect of xylose concentration on the yield coefficient of L-lactate in mixed-acid fermentation by the lactic acid bacterium *Lactococcus lactis* IO-1. *Appl. Microbiol. Biotechnol.*, **60**, 160–167.
15. Bolotin,A., Wincker,P., Mauger,S., Jaillon,O., Malarme,K., Weissenbach,J., Ehrlich,S.D. and Sorokin,A. (2001) The complete genome sequence of the lactic acid bacterium *Lactococcus lactis* ssp. *lactis* IL1403. *Genome Res.*, **11**, 731–753.