Journal of Microbiological Methods 107 (2014) 92–97

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Highly efficient transformation of *Stenotrophomonas maltophilia* S21, an environmental isolate from soil, by electroporation

Xing Ye [a], Hongling Dong [a], Yu-Ping Huang [a,b,*]

[a] College of Life Sciences, Wuhan University, Wuhan 430072, China
[b] Hubei Provincial Cooperative Innovation Center of Industrial Fermentation, Wuhan 430072, China

ARTICLE INFO

Article history:
Received 7 July 2014
Received in revised form 10 September 2014
Accepted 26 September 2014
Available online 7 October 2014

Keywords:
Conjugation
Electroporation
Optimization
Stenotrophomonas maltophilia
Transformation

ABSTRACT

Stenotrophomonas maltophilia is an emerging opportunistic pathogen, which also exhibits potential of wide applications in industry, environment and agriculture. An efficient transformation method for *S. maltophilia* would be convenient to its genetic studies. In this report, we focused on developing an efficient transformation protocol for *S. maltophilia*. Gene transfer by three different methods (chemical transformation, conjugation and electroporation) indicated that electroporation was the most efficient method to transform *S. maltophilia* S21. Then, the entire electroporation process from competent-cell preparation to post-pulse incubation was optimized to get higher efficiencies. Utilizing competent cells prepared at optical density (600 nm) of 1.0, the maximal transformation efficiency of *S. maltophilia* S21 reached 1.53×10^8 transformants/μg of pBBR1MCS DNA at a field strength of 18 kV/cm, a time constant of 4.8 ms (200 Ω), a DNA amount of 100 ng and a cell concentration of 2.4×10^8 CFU/ml after 3 h incubation. Moreover, we successfully transformed the other four isolates of *S. maltophilia* using this protocol. To date, this is the first report about electroporation of *S. maltophilia* and it will facilitate the further study of this species.

1. Introduction

The gram-negative *Stenotrophomonas maltophilia* belongs to the genus *Stenotrophomonas* (Palleroni and Bradbury, 1993), which is ubiquitous in the environment. With diverse metabolic capacities, *S. maltophilia* can potentially serve as a promising candidate for wide scale applications in production of various economic enzymes (Cao et al., 2009; Wakayama et al., 2005), biodegradation (Dubey and Fulekar, 2012; Fang et al., 2013; Gao et al., 2014) and biological control (Kobayashi et al., 2002; Zhang and Yuen, 2000). In addition, *S. maltophilia* is an important opportunistic pathogen associated with fatal infections in humans, especially respiratory infections (Brooke, 2012; Fihman et al., 2012; Takahashi et al., 2011). A common problem in therapy of these infections is that it is naturally resistant to most of the currently used broad-spectrum antimicrobial agents (Alonso and Martinez, 1997; Crossman et al., 2008; Gould et al., 2006; Zhang et al., 2000).

Genetic manipulation of bacteria has become a powerful tool for elucidating fundamental biological mechanisms, which needs an efficient transformation protocol. To date, conjugation is the only method available for gene transfer of *S. maltophilia* (Huang et al., 2013; Liu et al., 2013; McCarthy et al., 2011). However, transformation efficiency of conjugation could vary according to the *S. maltophilia* strains used, and for several isolates of *S. maltophilia* in our laboratory, conjugation method is not that efficient. Thus, other efficient methods appear to be required for transforming *S. maltophilia*. Chemical transformation is a conventional transformation method used in gram-negative bacteria (Hanahan, 1983; Lederberg and Cohen, 1974; Russo et al., 2009), but not all the bacteria can be transformed effectively. Compared with chemical transformation, electroporation is an effective transformation method, which has been successfully used in both gram-positive and gram-negative bacteria (Cheong et al., 2008; Friesenegger et al., 1991; Luchansky et al., 1988), even for some bacteria resistant to transformation (Aune and Aachmann, 2010). However, no work of electroporation of *S. maltophilia* has been published as yet.

In this study, we attempted to develop an electroporation-mediated transformation protocol for *S. maltophilia*. *S. maltophilia* S21, an environmental isolate from soil, which is sensitive to commonly used antibiotics and contains no native plasmids, is a good candidate for original recipient in the construction of a genetic manipulation system for *S. maltophilia*. It could be successfully transformed by conjugation, chemical transformation and electroporation, and electroporation was proved to be the most efficient method. Various factors of electroporation were subsequently optimized, including culture density, pulse parameters, DNA amount, cell concentration and recovery time. Moreover, the generality of this method among *S. maltophilia* strains was also tested.

* Corresponding author at: Department of Microbiology, College of Life Sciences, Wuhan University, Wuhan 430072, China. Tel.: +86 27 68754533; fax: +86 27 68754833.
E-mail address: yphuang@whu.edu.cn (Y.-P. Huang).

http://dx.doi.org/10.1016/j.mimet.2014.09.010
0167-7012/

2. Materials and methods

2.1. Bacterial strains, plasmids and culture conditions

Bacterial strains and plasmids used in this study were listed in Table 1. *S. maltophilia* S21 was isolated from soil sample. *S. maltophilia* C12, C25 and C31 were isolated from throat secretion of inpatients. All the *S. maltophilia* strains were cultured at 30 °C in LB medium (peptone 10 g/l, yeast extract 5 g/l, NaCl 10 g/l) or on LB agar plates (LB medium + 1.5% agar), and *Escherichia coli* strains were grown in LB medium at 37 °C. Chloramphenicol and ampicillin were added at a final concentration of 34 μg/ml and 100 μg/ml respectively for selection when needed.

2.2. DNA isolation

Plasmids from *E. coli* DH5α and *S. maltophilia* were prepared by using the TIANprep Mini Plasmid Kit (Tiangen, China). When indicated, the plasmids were finally eluted in Milli-Q water instead of elution buffer to minimize the chance of arcing caused by salt contaminants in the electroporation step.

2.3. Preparation of chemically competent cells and transformation of *S. maltophilia* S21 using $CaCl_2$

Competent cells of *S. maltophilia* S21 were prepared and transformed using the standard $CaCl_2$ procedure described by Sambrook and Russell (2001) with some essential modifications. To prepare competent cells, 100 μl of overnight cultures of *S. maltophilia* S21 were inoculated into 5 ml of LB medium and incubated at 30 °C. When optical density at 600 nm (OD_{600}) approached 0.4, 4.5 ml of the cultures were harvested and placed on ice for 30 min, and cell pellets were collected by centrifugation at 4000 ×*g* and 4 °C for 5 min. Then cells were washed twice with 2 ml of ice-cold 100 mM $CaCl_2$. Of note, all cell suspensions at this step were placed on ice for 30 min before centrifugation. Finally, cell pellets were resuspended in 300 μl of ice-cold 100 mM $CaCl_2$.

For transformation, aliquots of 100 μl of competent cells of *S. maltophilia* S21 were mixed with 2 μl of pBBR1MCS (200 ng/μl) and placed on ice for 30 min. Subsequently, the mixtures were transferred to 42 °C for 90 s and chilled quickly on ice for 2 min, and each aliquot was added with 0.9 ml of LB and placed at 30 °C for 1 h without antibiotic selection. Finally, the cells were plated on LB agar containing 34 μg/ml chloramphenicol and incubated at 30 °C for 30 h before counting the colonies.

Table 1
Bacterial strains and plasmids used in this study.

Strain or plasmid	Relevant description	Source or reference
Strains		
S. maltophilia S21	An environmental isolate, sensitive to chloramphenicol	This laboratory
S. maltophilia C12	A clinical isolate, sensitive to chloramphenicol	This laboratory
S. maltophilia C25	A clinical isolate, sensitive to chloramphenicol	This laboratory
S. maltophilia C31	A clinical isolate, sensitive to chloramphenicol	This laboratory
S. maltophilia ATCC 13637	Type strain, sensitive to chloramphenicol	ATCC
E. coli DH5α	hsdR17(rK^- mK^+)	This laboratory
E. coli S17-1	pro res mod$^+$RP4-2 (Tet::Mu Kan::Tn7)	Milton et al. (1996)
Plasmids		
pBBR1MCS	Mobilizable broad-host-range cloning vector, Cm^r	Kovach et al. (1994)

Table 2
Comparison of three transformation methods for *S. maltophilia* S21.

Transformation method	Transformation frequency[a]	Transformation efficiency[b]
Chemical transformation	$(5.96 \pm 0.49) \times 10^{-7}$	$(1.52 \pm 0.15) \times 10^{2}$
Conjugation	$(1.16 \pm 0.08) \times 10^{-5}$	
Electroporation[c]	$(8.43 \pm 0.83) \times 10^{-2}$	$(7.59 \pm 0.37) \times 10^{6}$

[a] The frequency of chemical transformation and electroporation was expressed as transformants per cell of survivor, and the frequency of conjugation was expressed as exconjugants per cell of recipient.
[b] The efficiency of transformation was expressed as transformants per microgram of pBBR1MCS DNA.
[c] The electrocompetent cells were prepared at OD_{600} 0.4 and transformed as the initial protocol. The results are means ± SD (error bars) of three independent triplicate experiments.

2.4. Conjugation of *S. maltophilia* S21

Plasmid transferring from *E. coli* S17-1 to *S. maltophilia* S21 was performed as previously described (Milton et al., 1996) with a few modifications. Briefly, both strains were grown to the middle exponential growth phase and mixed at a ratio of 10:1 (recipient/donor). Then all the mixture was spotted on a nonselective LB agar plate and incubated overnight at 30 °C. Transformants were selected and scored according to Milton et al. (1996).

2.5. Preparation of electrocompetent cells and electroporation of *S. maltophilia* S21

Electroporation of *S. maltophilia* S21 was based on the protocol for *E. coli* (Sambrook and Russell, 2001). For preparing electrocompetent cells, 1 ml of overnight cultures of *S. maltophilia* S21 were inoculated into an Erlenmeyer flask containing 50 ml of LB medium and cultivated in a shaking incubator at 30 °C. To obtain the optimal electrocompetent cells, 5 ml of the cell cultures were successively harvested at different optical densities (600 nm) of early exponential phase and placed on ice for 30 min. Then the chilled cultures were centrifuged at 4000 ×*g* and 4 °C for 5 min, and the cell pellets were washed three times in 3 ml of ice-cold 10% glycerol (v/v). Finally, they were resuspended in different volumes of 10% glycerol respectively and the final concentration of electrocompetent cells became 2.4×10^8 CFU/ml. Cell suspensions were aliquoted in microcentrifuge tubes (50 μl per tube). If necessary, the electrocompetent cells could be stored at −80 °C.

The initial electroporation procedure was as follows: an aliquot of 50 μl of electrocompetent cells were mixed thoroughly with 2 μl plasmid DNA (200 ng/μl) and placed on ice for 5 min. Then all the mixture was transferred to a chilled 0.1-cm cuvette and electroporated by a single pulse with the Gene Pulser (Bio-Rad Laboratories, Richmond, Calif.) apparatus set at 1.8 kV, 25 μF and 200 Ω. Immediately after the pulse, the sample was suspended in 0.95 ml of LB medium and incubated at 30 °C for 1 h. Ultimately, the bacteria suspensions were serially diluted with sterile 0.9% NaCl (w/v). The transformants were assessed as described in Section 2.3.

3. Results

3.1. Comparison of three transformation methods for *S. maltophilia* S21

Three different transformation methods including conjugation, chemical transformation and electroporation were tested for *S. maltophilia* S21. The results showed that *S. maltophilia* S21 could be successfully transformed with all these methods (Table 2), and the transformation frequency was much higher with electroporation (8.43×10^{-2} transformants/survivor) than with chemical transformation (5.96×10^{-7} transformants/survivor) and conjugation (1.16×10^{-5} exconjugants/recipient). It suggested that electroporation was a relatively efficient transformation method for *S. maltophilia* S21 and

we would optimize the electroporation conditions for *S. maltophilia* S21 to get higher efficiencies.

3.2. Optimization of electroporation conditions for S. maltophilia S21

3.2.1. Effect of culture density on transformation efficiency

In the preliminary electroporation experiments of *S. maltophilia* S21, cells harvested in the early exponential phase turned to be more competent than that in middle exponential phase or stationary phase (data not shown). It suggested that the competent level of cells varied according to the time of harvest. In order to obtain the optimal electrocompetent cells, cultures were harvested at a serial of optical densities (600 nm) of early exponential phase (0.4, 0.6, 0.8, 1.0 and 1.2). As shown in Fig. 1, the optimal OD_{600} was around at 1.0, either a higher or lower culture density reduced the transformation efficiency. Therefore, all subsequent tests were conducted utilizing cells cultivated at this culture density.

3.2.2. Effect of field strength on transformation efficiency

Field strength was a crucial pulse parameter in electroporation. In our previous experiments, field strength higher than 20 kV/cm usually resulted in arcing across the cuvette (data not shown). Thus, to study the influence of field strength on transformation efficiency of *S. maltophilia*, electroporation was carried out at a field strength of 5, 10, 15, 18 and 20 kV/cm, respectively. The transformation efficiency increased with the increase of field strength (5 to 18 kV/cm) and decreased gradually when a higher field strength was used (Fig. 2). The highest transformation efficiency (1.16×10^8 transformants/μg of pBBR1MCS DNA) was obtained at 18 kV/cm, although the survival rate was only 16.9%. Based on these experiments, we chose the field strength of 18 kV/cm for the following experiments.

3.2.3. Effect of time constant on transformation efficiency

Duration of pulse was also an important parameter in electroporation. Various time constants were able to be obtained by modifying the size of the parallel resistor. The results showed that electroporation performed at a time constant of about 4.8 ms was proved to be the most efficient (Fig. 3). Any shorter and longer time constant could cause a decrease of transformation efficiency. Hence, the optimal time constant was about 4.8 ms at 200 Ω. Survival rate of competent cells always declined along with the increasing time constants. Surprisingly, time constant of 9.1 ms resulted in a sharp decrease of survival rate.

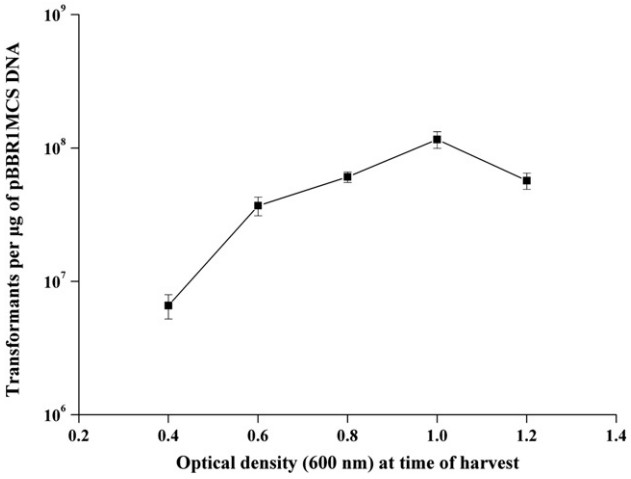

Fig. 1. Effect of culture density on transformation efficiency of *S. maltophilia* S21 with pBBR1MCS. Competent cells were prepared and electroporated as described in Materials and methods. The results are means ± SD (error bars) of three independent triplicate experiments.

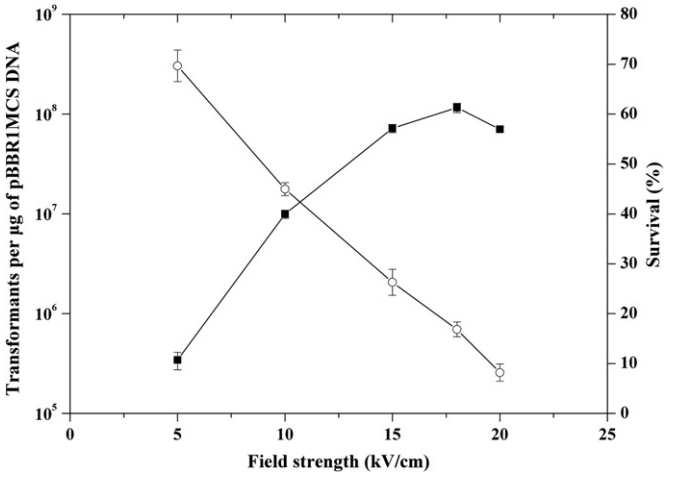

Fig. 2. Effect of field strength on transformation efficiency of *S. maltophilia* S21 with pBBR1MCS. Competent cells (2.4×10^8 CFU/ml) prepared at OD_{600} 1.0 were electroporated with 2 μl pBBR1MCS (200 ng/μl) by using different field strengths and constant capacitance (25 μF) and resistance (200 Ω). Transformation efficiency (■); survival rate (○). The results are means ± SD (error bars) of three independent triplicate experiments.

3.2.4. Effect of DNA amount on transformation efficiency

In order to determine the optimal DNA amount, we transformed *S. maltophilia* S21 with various amounts of plasmid DNA. The maximum transformation efficiency was achieved with 100 ng of pBBR1MCS. When DNA amount was more than 100 ng, the transformation efficiency decreased (Fig. 4). Consequently, DNA amount of 100 ng was used for further studies.

3.2.5. Effect of cell concentration on transformation efficiency

As shown in Fig. 5, transformation efficiency was closely related to the concentration of competent cells. At the concentration of 2.4×10^8 CFU/ml, 8.6×10^7 transformants per μg of pBBR1MCS DNA was obtained, resulting in 1.5–3.8 times higher yields than other cell concentrations. Apparently, the optimal competent cell concentration of *S. maltophilia* S21 was 2.4×10^8 CFU/ml.

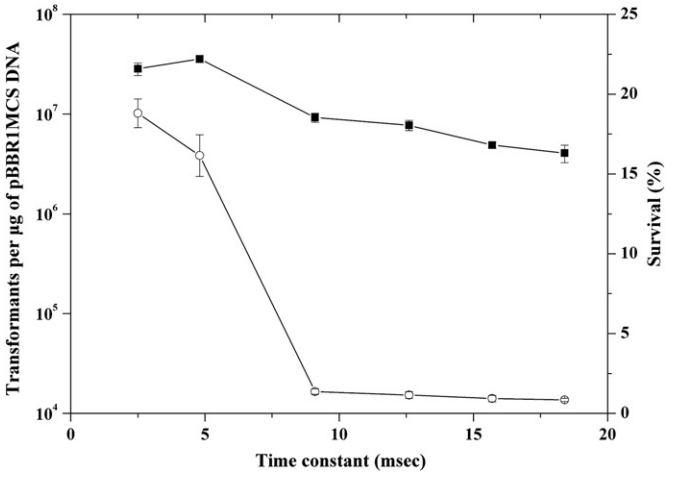

Fig. 3. Effect of time constant on transformation efficiency of *S. maltophilia* S21 with pBBR1MCS. Competent cells (2.4×10^8 CFU/ml) prepared at OD_{600} 1.0 were electroporated with 2 μl pBBR1MCS (200 ng/μl) by using different time constants (2.5 ms to 18.4 ms) and constant capacitance (25 μF) and field strength (18 kV/cm). Transformation efficiency (■); survival (%) (○). The results are means ± SD (error bars) of three independent triplicate experiments.

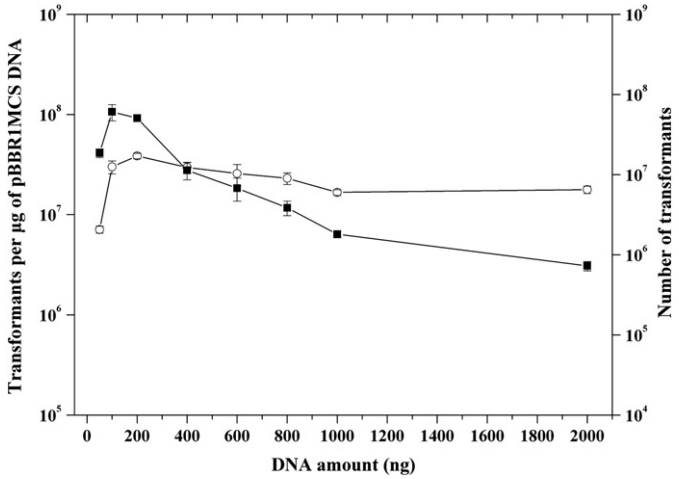

Fig. 4. Effect of DNA amount on transformation efficiency of *S. maltophilia* S21 with pBBR1MCS. 50 ng to 2000 ng of pBBR1MCS in 2 μl Milli-Q water was added into 50 μl competent cells (2.4×10^8 CFU/ml) and electroporated at 18 kV/cm, 25 μF and 200 Ω. Transformation efficiency (■); number of transformants (○). The results are means ± SD (error bars) of three independent triplicate experiments.

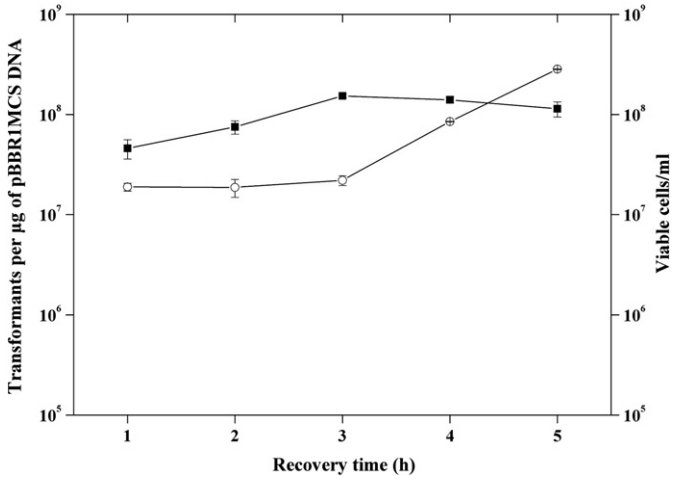

Fig. 6. Effect of recovery time on transformation efficiency of *S. maltophilia* S21 with pBBR1MCS. Fifty microliters of competent cells (2.4×10^8 CFU/ml) prepared at OD_{600} 1.0 were electroporated with 2 μl pBBR1MCS (50 ng/μl) at 18 kV/cm, 25 μF and 200 Ω and incubated for various length of time (1 to 5 h) before spreading on plates containing chloramphenicol. Transformation efficiency (■); viable cells (○). The results are means ± SD (error bars) of three independent triplicate experiments.

3.2.6. Effect of recovery time after electroporation on transformation efficiency

Generally, incubation after electroporation could facilitate the survival of transformants. The pulsed cells were incubated in LB medium for different lengths of time ranging from 1 h to 5 h prior to plating on selective plates. The results showed that transformation efficiency increased during the initial 3 h (Fig. 6), and the maximal transformation efficiency reached 1.53×10^8 transformants/μg of pBBR1MCS DNA at the recovery period of 3 h. Incubation for more than 3 h led the number of transformants to decrease gradually and the number of viable cells to increase significantly. It suggested that the optimal recovery time for *S. maltophilia* S21 was 3 h.

3.3. Plasmid isolation and digestion

To confirm transformation, eight random colonies were detected by digesting the plasmids with restriction endonuclease *Xba*I. All plasmids isolated from the examined transformants generated a 4.7 kb fragment as expected (Fig. 7), which demonstrated the presence of pBBR1MCS.

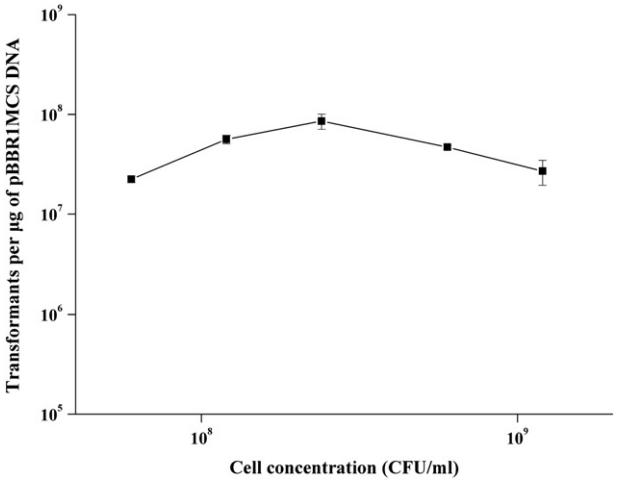

Fig. 5. Effect of cell concentration on transformation efficiency of *S. maltophilia* S21 with pBBR1MCS. Fifty microliters of different concentrations of competent cells (6.0×10^7–1.2×10^9 CFU/ml) were mixed with 2 μl pBBR1MCS (50 ng/μl) and electroporated at 18 kV/cm, 25 μF and 200 Ω. The results are means ± SD (error bars) of three independent triplicate experiments.

3.4. Application of electroporation protocol to other S. maltophilia strains

By optimizing the electroporation conditions, we got an effective transformation protocol for *S. maltophilia* S21. In order to validate the generality of the developed protocol, we further tested it with pBBR1MCS DNA to transform the other three clinical *S. maltophilia* strains and the type strain ATCC 13637. The transformation efficiency of *S. maltophilia* strain was 6.2×10^4 transformants/μg DNA for C12, 1.1×10^6 transformants/μg DNA for C25, 9.5×10^5 transformants/μg DNA for C31 and 8.5×10^5 transformants/μg DNA for ATCC 13637, respectively. Note that electroporation of these *S. maltophilia* strains was done in triplicate and repeated three times as that of S21.

4. Discussion

We transformed *S. maltophilia* S21 with plasmid pBBR1MCS by three different methods. Although electroporation was proved to be the most efficient method to transform *S. maltophilia* S21, transformation efficiency of electroporation using the initial protocol was still relatively low. To develop a more efficient electroporation protocol for *S. maltophilia* S21, factors affecting transformation efficiency, including culture density, pulse parameters, DNA amount, cell concentration and recovery time, were optimized.

The optimal culture density of harvest varies depending upon species, strains and growth conditions (Aune and Aachmann, 2010). In general, cells harvested in the exponential phase can obtain high transformation efficiencies (Tryfona and Bustard, 2006). For *S. maltophilia* S21, cells harvested in early exponential phase were more competent, which was also found in some Gram-positive species, like *Bacillus thuringiensis* and *Bacillus cereus* (Peng et al., 2009; Turgeon et al., 2006). However, for *Xanthomonas*, which are phylogenetically close to

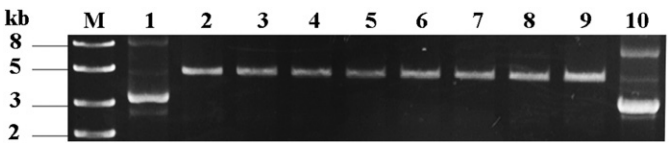

Fig. 7. Electrophoresis analysis of pBBR1MCS digested with *Xba*I. M, molecular weight marker. Lane 1, plasmid isolated from *S. maltophilia* S21; lane 2 to 9, plasmid digested by *Xba*I; lane 10, plasmid isolated from *E. coli* DH5α.

Stenotrophomonas, the optimal culture density was reported in middle exponential phase (White and Gonzalez, 1995). Similar with *Pseudomonas putida* (Iwasaki et al., 1994), a higher or lower cell concentration of *S. maltophilia* S21 reduced the transformation efficiency. Whereas, the maximal transformation efficiency was less four times than the minimum, which indicated that concentration of competent cells was not as so critical to transformation efficiency of *S. maltophilia* S21 as culture density.

At suitable pulse parameters, electric pulses can create transient pores in cell membrane and facilitate the uptake of DNA. If the intensity of electric pulses is too strong, the number of viable cells will reduce sharply and so does the transformation efficiency. Although the optimal pulse parameters of *S. maltophilia* S21 were the same as those for *E. coli* (Woodall, 2003), the survival rate of *S. maltophilia* S21 was less half than that of *E. coli* (Dower et al., 1988). In addition, time constant did not show a significant effect on transformation efficiency of *S. maltophilia* S21 as did with *E. coli* (Dower et al., 1988).

When DNA molecules are introduced into cells by electroporation, electroporation can cause damage to the cells at the same time. Incubation after electroporation allows these damaged cells to recover and express antibiotic resistance genes, which can facilitate the survival of transformants. As shown in Fig. 6, incubation for 3 h increased the transformation efficiency by more than two folds. In the meantime, the number of viable cells did not increase. However, transformation efficiency began to decrease and cells replicated simultaneously when recovery time exceeded 3 h. It could be explained that pBBR1MCS was not stable in *S. maltophilia* S21 under unselected conditions and lost gradually during cell division.

The amount of transforming DNA, of course, is an important factor in electroporation. Inadequate DNA leads to the redundancy of competent cells, while excessive DNA can cause many DNA molecules unconnected with the cells (Iwasaki et al., 1994). All these could affect the transformation efficiency of electroporation. As shown in Fig. 4, the maximal transformation efficiency and number of transformants were obtained with 100 ng and 200 ng DNA, respectively. While using plasmid amounts over 200 ng yielded less transformants and reduced the transformation efficiency accordingly. This might be caused by follow-through solutes in the DNA that interfered with the electroporation as that for *Chromobacterium violaceum* (Broetto et al., 2006).

The other three clinical isolates and the type strain of *S. maltophilia* were successfully transformed with the optimized protocol. Though their transformation efficiencies were different and lower than that of S21, the results showed that this protocol could be used to transform other *S. maltophilia* strains.

5. Conclusions

This study demonstrated that electroporation was an effective, reliable and simple method to introduce DNA into *S. maltophilia*. Meanwhile, we successfully developed an efficient transformation protocol for *S. maltophilia* S21. To our knowledge, this is the first report about the electroporation of *S. maltophilia* and will lay a foundation for the molecular genetic manipulation of this species.

Acknowledgements

This work was supported by grants from the National Infrastructure of Natural Resources for Science and Technology Program of China (No. NIMR-2014-8) and National Nature Science Foundation of China (No. 30970070).

References

Alonso, A., Martinez, J.L., 1997. Multiple antibiotic resistance in *Stenotrophomonas maltophilia*. Antimicrob. Agents Chemother. 41, 1140–1142.

Aune, T.E., Aachmann, F.L., 2010. Methodologies to increase the transformation efficiencies and the range of bacteria that can be transformed. Appl. Microbiol. Biotechnol. 85, 1301–1313.

Broetto, L., Cecagno, R., Sant'Anna, F.H., Weber, S., Schrank, I.S., 2006. Stable transformation of *Chromobacterium violaceum* with a broad-host-range plasmid. Appl. Microbiol. Biotechnol. 71, 450–454.

Brooke, J.S., 2012. *Stenotrophomonas maltophilia*: an emerging global opportunistic pathogen. Clin. Microbiol. Rev. 25, 2–41.

Cao, Z.J., Zhang, Q., Wei, D.K., Chen, L., Wang, J., Zhang, X.Q., Zhou, M.H., 2009. Characterization of a novel *Stenotrophomonas* isolate with high keratinase activity and purification of the enzyme. J. Ind. Microbiol. Biotechnol. 36, 181–188.

Cheong, D.E., Lee, H.I., So, J.S., 2008. Optimization of electrotransformation conditions for *Propionibacterium acnes*. J. Microbiol. Methods 72, 38–41.

Crossman, L.C., Gould, V.C., Dow, J.M., Vernikos, G.S., Okazaki, A., Sebaihia, M., Saunders, D., Arrowsmith, C., Carver, T., Peters, N., Adlem, E., Kerhornou, A., Lord, A., Murphy, L., Seeger, K., Squares, R., Rutter, S., Quail, M.A., Rajandream, M.A., Harris, D., Churcher, C., Bentley, S.D., Parkhill, J., Thomson, N.R., Avison, M.B., 2008. The complete genome, comparative and functional analysis of *Stenotrophomonas maltophilia* reveals an organism heavily shielded by drug resistance determinants. Genome Biol. 9, R74.

Dower, W.J., Miller, J.F., Ragsdale, C.W., 1988. High efficiency transformation of *E. coli* by high voltage electroporation. Nucleic Acids Res. 16, 6127–6145.

Dubey, K.K., Fulekar, M.H., 2012. Chlorpyrifos bioremediation in Pennisetum rhizosphere by a novel potential degrader *Stenotrophomonas maltophilia* MHF ENV20. World J. Microbiol. Biotechnol. 28, 1715–1725.

Fang, Z., Zhang, J., Liu, B., Du, G., Chen, J., 2013. Biodegradation of wool waste and keratinase production in scale-up fermenter with different strategies by *Stenotrophomonas maltophilia* BBE11-1. Bioresour. Technol. 140, 286–291.

Fihman, V., Le Monnier, A., Corvec, S., Jaureguy, F., Tankovic, J., Jacquier, H., Carbonnelle, E., Bille, E., Illiaquer, M., Cattoir, V., Zahar, J.R., 2012. *Stenotrophomonas maltophilia*—the most worrisome threat among unusual non-fermentative gram-negative bacilli from hospitalized patients: a prospective multicenter study. J. Infect. 64, 391–398.

Friesenegger, A., Fiedler, S., Devriese, L.A., Wirth, R., 1991. Genetic transformation of various species of *Enterococcus* by electroporation. FEMS Microbiol. Lett. 63, 323–327.

Gao, J., Ye, J., Ma, J., Tang, L., Huang, J., 2014. Biosorption and biodegradation of triphenyltin by *Stenotrophomonas maltophilia* and their influence on cellular metabolism. J. Hazard. Mater. 276C, 112–119.

Gould, V.C., Okazaki, A., Avison, M.B., 2006. Beta-lactam resistance and beta-lactamase expression in clinical *Stenotrophomonas maltophilia* isolates having defined phylogenetic relationships. J. Antimicrob. Chemother. 57, 199–203.

Hanahan, D., 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. 166, 557–580.

Huang, Y.W., Hu, R.M., Chu, F.Y., Lin, H.R., Yang, T.C., 2013. Characterization of a major facilitator superfamily (MFS) tripartite efflux pump EmrCABsm from *Stenotrophomonas maltophilia*. J. Antimicrob. Chemother. 68, 2498–2505.

Iwasaki, K., Uchiyama, H., Yagi, O., Kurabayashi, T., Ishizuka, K., Takamura, Y., 1994. Transformation of *Pseudomonas putida* by electroporation. Biosci. Biotechnol. Biochem. 58, 851–854.

Kobayashi, D.Y., Reedy, R.M., Bick, J., Oudemans, P.V., 2002. Characterization of a chitinase gene from *Stenotrophomonas maltophilia* strain 34S1 and its involvement in biological control. Appl. Environ. Microbiol. 68, 1047–1054.

Kovach, M.E., Phillips, R.W., Elzer, P.H., Roop, R.N., Peterson, K.M., 1994. pBBR1MCS: a broad-host-range cloning vector. Biotechniques 16, 800–802.

Lederberg, E.M., Cohen, S.N., 1974. Transformation of *Salmonella typhimurium* by plasmid deoxyribonucleic acid. J. Bacteriol. 119, 1072–1074.

Liu, J., Chen, P., Zheng, C., Huang, Y.P., 2013. Characterization of maltocin P28, a novel phage tail-like bacteriocin from *Stenotrophomonas maltophilia*. Appl. Environ. Microbiol. 79, 5593–5600.

Luchansky, J.B., Muriana, P.M., Klaenhammer, T.R., 1988. Application of electroporation for transfer of plasmid DNA to *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Listeria*, *Pediococcus*, *Bacillus*, *Staphylococcus*, *Enterococcus* and *Propionibacterium*. Mol. Microbiol. 2, 637–646.

McCarthy, Y., Dow, J.M., Ryan, R.P., 2011. The Ax21 protein is a cell–cell signal that regulates virulence in the nosocomial pathogen *Stenotrophomonas maltophilia*. J. Bacteriol. 193, 6375–6378.

Milton, D.L., O'Toole, R., Horstedt, P., Wolf-Watz, H., 1996. Flagellin A is essential for the virulence of *Vibrio anguillarum*. J. Bacteriol. 178, 1310–1319.

Palleroni, N.J., Bradbury, J.F., 1993. *Stenotrophomonas*, a new bacterial genus for *Xanthomonas maltophilia* (Hugh 1980) Swings et al. 1983. Int. J. Syst. Bacteriol. 43, 606–609.

Peng, D., Luo, Y., Guo, S., Zeng, H., Ju, S., Yu, Z., Sun, M., 2009. Elaboration of an electroporation protocol for large plasmids and wild-type strains of *Bacillus thuringiensis*. J. Appl. Microbiol. 106, 1849–1858.

Russo, R., Panangala, V.S., Wood, R.R., Klesius, P.H., 2009. Chemical and electroporated transformation of *Edwardsiella ictaluri* using three different plasmids. FEMS Microbiol. Lett. 298, 105–110.

Sambrook, J., Russell, D.W., 2001. Molecular Cloning: A Laboratory Manual, Third edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA.

Takahashi, N., Yoshioka, T., Kameoka, Y., Tagawa, H., Fujishima, N., Saitoh, H., Hirokawa, M., Enomoto, K., Sawada, K., 2011. Fatal hemorrhagic pneumonia caused by *Stenotrophomanas maltophilia* in a patient with non-Hodgkin lymphoma. J. Infect. Chemother. 17, 858–862.

Tryfona, T., Bustard, M.T., 2006. Enhancement of biomolecule transport by electroporation: a review of theory and practical application to transformation of *Corynebacterium glutamicum*. Biotechnol. Bioeng. 93, 413–423.

Turgeon, N., Laflamme, C., Ho, J., Duchaine, C., 2006. Elaboration of an electroporation protocol for *Bacillus cereus* ATCC 14579. J. Microbiol. Methods 67, 543–548.

Wakayama, M., Yamagata, T., Kamemura, A., Bootim, N., Yano, S., Tachiki, T., Yoshimune, K., Moriguchi, M., 2005. Characterization of salt-tolerant glutaminase from *Stenotrophomonas maltophilia* NYW-81 and its application in Japanese soy sauce fermentation. J. Ind. Microbiol. Biotechnol. 32, 383–390.

White, T.J., Gonzalez, C.F., 1995. Electroporation of *Xanthomonas*. Methods Mol. Biol. 47, 135–141.

Woodall, C.A., 2003. Electroporation of *E. coli*. Methods Mol. Biol. 235, 55–69.

Zhang, Z., Yuen, G.Y., 2000. The role of chitinase production by *Stenotrophomonas maltophilia* strain C3 in biological control of *Bipolaris sorokiniana*. Phytopathology 90, 384–389.

Zhang, L., Li, X.Z., Poole, K., 2000. Multiple antibiotic resistance in *Stenotrophomonas maltophilia*: involvement of a multidrug efflux system. Antimicrob. Agents Chemother. 44, 287–293.