A broad-host-range vector of incompatibility group Q can work as a plasmid vector in Neisseria meningitidis: a new genetical tool

Hideyuki Takahashi and Haruo Watanabe

INTRODUCTION

Neisseria meningitidis is a Gram-negative diplococcus pathogen that colonizes the nasopharynx. It eventually spreads into the bloodstream, where it causes septicaemia and, furthermore, induces meningitis when it reaches the cerebrospinal fluid. Although some of the factors involved in pathogenesis, such as Opa (reviewed by Nassif et al., 1999), Opc, pili (reviewed by Nassif et al., 1999) and the capsule (reviewed by Vogel et al., 1999), have been revealed, many problems still remain to be resolved (reviewed by Nassif & So, 1995; Nassif et al., 1999). A scarcity of genetic systems available for the meningococcus is one of the reasons hampering the development of genetic analysis (Kupsch et al., 1996).

Some R-plasmids in Neisseria gonorrhoeae have been used for the construction of vector plasmids (Stein et al., 1983; Piffaretti et al., 1988; Stein, 1989; Kupsch et al., 1996). Although it is well known that N. gonorrhoeae is genetically very closely related to N. meningitidis and a few plasmids were speculated to transfer from N. gonorrhoeae to N. meningitidis (Dillon et al., 1983; Ison et al., 1986; Knapp et al., 1988), most of the plasmids in N. gonorrhoeae were not applicable to N. meningitidis (Genco et al., 1984; Roberts et al., 1989). As far as we know, only two plasmids, a 4–5 Mb R-plasmid of N. gonorrhoeae and pMGC10, a derivative of shuttle vector pLES2, could be transferred to N. meningitidis (Ikeda et al., 1986; Nassif et al., 1991). On the other hand, some R-plasmids have been also found in N. meningitidis (Bhatti et al., 1981; Pintado et al., 1985; Rotger et al., 1986; Facinelli & Varaldo, 1987; Roberts et al., 1989). One of them was supposed to belong to the family of RSF1010, which is a broad-host-range vector of incompatibility group Q (IncQ) (Pintado et al., 1985).

IncQ plasmids such as RSF1010 and R300B (Sharpe, 1984) are found among Gram-negative bacteria (Barth et al., 1981). IncQ plasmids are not self-transmissible but mobile with the help of IncP plasmids. Since R300B is relatively small (8.68 kb; Meyer et al., 1982) and low in copy number (about 10 copies per E. coli genome; Barth & Grinter, 1974), it has been utilized for the construction of vectors (Sharpe, 1984). pGSS33 is one of

Keywords: IncQ plasmid, triparental conjugation, restriction/modification systems, complementation

Abbreviations: Amp'/Amp', ampicillin resistant/sensitive; Cml', chloramphenicol resistant; Erm', erythromycin resistant; Kan'/Kan', kanamycin resistant/sensitive; Tet'/Tet', tetracycline resistant/sensitive; Str'/Str', streptomycin resistant/sensitive.
the derivatives of R300B and has been used as a cloning vector.

In this paper, we report that pHT128, a derivative of pGSS33, can be successfully introduced into N. meningitidis.

**METHODS**

**Media and growth conditions.** *N. meningitidis* was stocked in frozen solution (Suker *et al.*, 1994) at −80 °C and routinely grown on GC agar plates [GC medium agar (Becton-Dickinson)] supplemented with 1% IsoVitaleX enrichment (Becton-Dickinson), at 37 °C in 5% CO₂. Trypticase soy broth (TSB; Becton-Dickinson) supplemented with 1% IsoVitaleX enrichment was used for the isolation of the plasmids in *N. meningitidis*. *E. coli* was grown on L plates or in L broth liquid culture at 37 °C. When required, antibiotics were added at the following concentrations. For *N. meningitidis*, chloramphenicol at 5 µg ml⁻¹, erythromycin at 4 µg ml⁻¹, ampicillin at 1 µg ml⁻¹, VCN (V, vancomycin; C, colistin; N, nystatin) inhibitor (Becton-Dickinson) was added to a final concentration of 1% to select *N. meningitidis* transconjugants. For *E. coli*, chloramphenicol was added at 10 µg ml⁻¹, kanamycin at 50 µg ml⁻¹, tetracycline at 15 µg ml⁻¹, ampicillin at 50 µg ml⁻¹ and erythromycin at 150 µg ml⁻¹. IPTG was added at a final concentration of 1 mM. The growth and concentration of bacteria were measured by monitoring the OD₆₀₀.

**Strains.** Strains used in this study are listed in Table 1. HT1037 was constructed as follows. A 1 kb fragment containing the pilE1 gene with BamHI and EcoRI linkers was amplified by PCR and cloned in the BamHI and EcoRI sites of pUC119 to construct pHT65. A blunted 1.2 kb HindIII–HindIII fragment containing the ermC gene isolated from pHT24 was inserted into the BstXI site, located at 135 bp downstream from the start codon of the pilE1 gene, to generate pHT72. pHT72 (500 ng) linearized by digestion with EcoRI was transformed into NIID57 and Erm' clones were isolated, resulting in a pilE1 insertion mutant of NIID57.

**Plasmids.** Plasmids used in this study are listed in Table 2. A Kan' Tet' derivative of RP4 (Pansegura & Lanka, 1987), pHT13, was constructed as follows. First, RP4 digested with HindIII and HpaI was blunted with a DNA blunting kit (Takara Shuzo) and self-ligated by DNA ligation kit version 2 (Takara Shuzo) to select Amp' Kan' Tet' clones. The resultant plasmid was renamed pHT13 and used as a helper for conjugation of a derivative of pGSS33 (Fig. 1a) from *E. coli* to *N. meningitidis*. pHT128 (Fig. 1b), a Stra and Amp' derivative of pGSS33, was constructed by removing the Pst–SacI region after blunting. pHT160 (see Fig. 4c) was constructed as follows. A 0.9 kb fragment containing the entire opc coding region with EcoRI and BamHI linkers was amplified by PCR and cloned in the BamHI and EcoRI sites of pTTQ18 (Stark, 1987) to construct pHT157. A 4.3 kb AlwNI–SacI fragment of pHT157 containing both the tac promoter fused to the opc gene and the lacI° gene was blunted and cloned in the PstI and SacI sites of pGSS33 after blunting. The resultant plasmid was pHT160. pHT161 (Fig. 4a) was constructed as follows. A 0.6 kb fragment containing the entire pilE1 coding region was amplified by PCR with the corresponding primers, which contained the EcoRI linker tagged by six CAC tandem repeats (His₆) and a SacI linker. The amplified fragment was cloned in the EcoRI and SacI sites of pTTQ18 to construct pHT68. A 4.8 kb AlwNI–SacI fragment of pHT68 containing both the tac promoter fused to the His₆–pilE1 gene and the lacI° gene was blunted and cloned in the PstI and SacI sites of pGSS33 after blunting. The resultant plasmid was pHT161. All of the primers to amplify *N. meningitidis* genes used in this study were designed by reference to the TIGR *N. meningitidis* database (http://www.tigr.org/).

**DNA transfer.** For transformation by natural competence, *N.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype, pedigree of strain</th>
<th>Source</th>
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<td><strong>E. coli</strong></td>
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<tr>
<td>DH5α</td>
<td>supE44 ΔlacU169 (Δ80lacZAM15) bsdR17 recA1 deoR endA1 gyrA96 thi-1 relA1</td>
<td>Takara Shuzo</td>
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<td>LE392</td>
<td>supE44 supF8 bsdR514 galK2 galT22 metB1 trpR55 lacY1</td>
<td>Promega</td>
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<td><strong>N. meningitidis</strong></td>
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<td>H44/76</td>
<td>Serogroup B, a representative isolate of the Norwegian group B epidemic</td>
<td>National Institute of Public Health, Norway</td>
</tr>
<tr>
<td>NIID57</td>
<td>Serogroup B, a clinical isolate from a Japanese healthy carrier</td>
<td>This study</td>
</tr>
<tr>
<td>HT1037</td>
<td>H44/76 Δopc::ermC</td>
<td>This study</td>
</tr>
<tr>
<td>HT1033</td>
<td>NIID57 pilE1::ermC</td>
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Table 2. Plasmids used in this study

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<th>Plasmid</th>
<th>Genotype pedigree and markers</th>
<th>Source</th>
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<tr>
<td>pUC18</td>
<td>Cloning vector; Amp&lt;sup&gt;r&lt;/sup&gt;</td>
<td>Takara Shuzo</td>
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<tr>
<td>pUC118</td>
<td>Cloning vector; Amp&lt;sup&gt;r&lt;/sup&gt;</td>
<td>Takara Shuzo</td>
</tr>
<tr>
<td>pUC119</td>
<td>Cloning vector; Amp&lt;sup&gt;r&lt;/sup&gt;</td>
<td>Takara Shuzo</td>
</tr>
<tr>
<td>pTnMax2</td>
<td>Plasmid for in vitro mutagenesis; Erm&lt;sup&gt;r&lt;/sup&gt;</td>
<td>Hass et al. (1993)</td>
</tr>
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<td>pHT24</td>
<td>Derivative of pUC18 containing a 1.2 kb HindIII–HindIII fragment that carries the ermC gene of pTnMax2; Amp&lt;sup&gt;r&lt;/sup&gt; Erm&lt;sup&gt;r&lt;/sup&gt;</td>
<td>This study</td>
</tr>
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<td>pHT3</td>
<td>Derivative of pUC118 containing a 1.3 kb BamHI–EcoRI fragment that carries the opc gene; Amp&lt;sup&gt;r&lt;/sup&gt;</td>
<td>This study</td>
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<td>pHT148</td>
<td>Derivative of pHT3 in which the ermC gene was inserted in EcoT14I sites of the opc gene; Amp&lt;sup&gt;r&lt;/sup&gt; Erm&lt;sup&gt;r&lt;/sup&gt;</td>
<td>This study</td>
</tr>
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<td>pHT65</td>
<td>Derivative of pUC119 containing a 1 kb BamHI–EcoRI fragment that carries the pilE1 gene; Amp&lt;sup&gt;r&lt;/sup&gt;</td>
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<td>pHT72</td>
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<td>This study</td>
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<td>RP4</td>
<td>Broad-host-range IncP vector; Amp&lt;sup&gt;r&lt;/sup&gt; Kan&lt;sup&gt;r&lt;/sup&gt; Tet&lt;sup&gt;r&lt;/sup&gt;</td>
<td>Pansegrau &amp; Lanka (1987)</td>
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<td>pHT12</td>
<td>Kan&lt;sup&gt;r&lt;/sup&gt; derivative of RP4 deleting its HindIII–HpaI region; Amp&lt;sup&gt;r&lt;/sup&gt; Kan&lt;sup&gt;r&lt;/sup&gt; Tet&lt;sup&gt;r&lt;/sup&gt;</td>
<td>This study</td>
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<tr>
<td>pHT13</td>
<td>Tet&lt;sup&gt;r&lt;/sup&gt; derivative of pHT12 deleting its BglII–Stul region; Amp&lt;sup&gt;r&lt;/sup&gt; Kan&lt;sup&gt;r&lt;/sup&gt; Tet&lt;sup&gt;r&lt;/sup&gt;; a helper plasmid used for conjugation</td>
<td>This study</td>
</tr>
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<td>pGSS33</td>
<td>Broad-host-range IncQ vector; Stu&lt;sup&gt;r&lt;/sup&gt; Amp&lt;sup&gt;r&lt;/sup&gt; Cml&lt;sup&gt;r&lt;/sup&gt; Tet&lt;sup&gt;r&lt;/sup&gt;</td>
<td>Sharpe (1984)</td>
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<td>pHT128</td>
<td>Stu&lt;sup&gt;r&lt;/sup&gt; Amp&lt;sup&gt;r&lt;/sup&gt; derivative of pGSS33 deleting its PstI–SacI region; Stu&lt;sup&gt;r&lt;/sup&gt; Amp&lt;sup&gt;r&lt;/sup&gt; Cml&lt;sup&gt;r&lt;/sup&gt; Tet&lt;sup&gt;r&lt;/sup&gt;</td>
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<td>Expression vector under control of the tac promoter; Amp&lt;sup&gt;r&lt;/sup&gt;</td>
<td>Stark (1987)</td>
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<td>pHT157</td>
<td>Derivative of pTTQ18 containing the opc gene under control of the tac promoter; Amp&lt;sup&gt;r&lt;/sup&gt;</td>
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<td>pHT160</td>
<td>Derivative of pGSS33 containing a 4.3 kb AlgN1–SacI fragment of pHT157; Cml&lt;sup&gt;r&lt;/sup&gt; Tet&lt;sup&gt;r&lt;/sup&gt;</td>
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<tr>
<td>pHT68</td>
<td>Derivative of pTTQ18 containing the His-tagged pilE1 gene under control of the tac promoter; Amp&lt;sup&gt;r&lt;/sup&gt;</td>
<td>This study</td>
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<td>pHT161</td>
<td>Derivative of pGSS33 containing a 4 kb AlgN1–SacI fragment of pHT68; Cml&lt;sup&gt;r&lt;/sup&gt; Tet&lt;sup&gt;r&lt;/sup&gt;</td>
<td>This study</td>
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**meningitidis** was transformed as previously described (Ryll et al., 1997). Plasmids were linearized prior to transformation unless otherwise stated.

Electroporation for *Neisseria* was performed as follows. *N. meningitidis* grown on GC agar plates at 37 °C in 5% CO<sub>2</sub> was scraped and suspended in 30 ml TSB supplemented with 1% IsoVitaleX enrichment to adjust the supension to OD<sub>600</sub> 0.1. The bacteria were cultured at 37 °C with shaking to OD<sub>600</sub> of 0.6 and then the cells were chilled and harvested by centrifugation at 4 °C. The pellets were rinsed with 1 ml ice-cold 10% (w/v) glycerol three times and the cells were resuspended to a final volume of 60 µl in ice-cold 10% glycerol. A 20 µl sample of the cell suspension was mixed with up to 1 µg of plasmid DNA and electroporation was performed under the following optimized conditions: electric field strength, capacitance and resistance were 1.25 kV, 25 µF and 400 Ω, respectively, when using a 0.1 cm electrode gap cuvette (Bio-Rad) with the Gene Pulser (Bio-Rad). After being electroporated, cells were incubated in 1 ml TSB supplemented with 1% IsoVitaleX enrichment, 10 mM MgCl<sub>2</sub> and 0.4% glucose at 37 °C for 1 h. The cells harvested by centrifugation were spread on GC agar plates with 5 µg chloramphenicol ml<sup>−1</sup> and incubated at 37 °C in 5% CO<sub>2</sub> for 18 h.

For conjugation, *N. meningitidis* grown on GC agar plates at 37 °C in 5% CO<sub>2</sub> was scraped and suspended in TSB to adjust the supension to OD<sub>600</sub> 0.25. In order to transfer a cloned vector plasmid from *E. coli* to *N. meningitidis*, we used triparental conjugation. Overnight cultures of LE392 harbouring vector plasmid pGSS33 or pHT128, LE392 harbouring a helper plasmid pHT13 and *N. meningitidis* suspension prepared as described above were mixed at a volume ratio of 1:1:2 and then filtered through 0.45 µm membrane filters (Millipore) by use of a syringe. The filters were washed with 2 ml TSB, then incubated on GC agar plates at 37 °C in 5% CO<sub>2</sub> for 8 h. Bacteria on the filters were washed out with 1 ml TSB. The bacteria, harvested by centrifugation, were then spread on GC agar plates containing 5 µg chloramphenicol ml<sup>−1</sup> and 1% VCN inhibitor and incubated at 37 °C in 5% CO<sub>2</sub> for 24 h. Transconjugants of *N. meningitidis* were picked up and streaked on the same kind of plates to isolate a single clone.

**Checking the maintenance of pHT128 in *N. meningitidis***

NIH357 harbouring pH128 grown on a GC agar plate or *E. coli* strain LE392 harbouring pHT128 on an L agar plate was scraped and suspended in 10 ml TSB supplemented with 1% IsoVitaleX enrichment at an OD<sub>600</sub> of 0.2 and cultured at

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37 °C with shaking for 24 h. One millilitre of the bacterial culture was added to 9 ml fresh medium and continuously cultured under the same conditions as described above. This step was repeated a total of four times. Each time, appropriate dilutions of the bacterial culture were spread on GC agar plates with or without 5 µg chloramphenicol ml⁻¹ for NIID57 and L agar plates with or without 10 µg chloramphenicol ml⁻¹ for LE392 to count the number of bacteria harbouring pHT128 and the total number of bacteria, respectively. The number of bacterial generations was determined graphically from the treatment of meningococcaemia, we chose to use the penicillin has been used as a first choice of antibiotic for

**Isolation of DNA.** Mini-preparation of plasmid from *E. coli* was carried out by Wizard Plus SV Miniprep DNA Purification systems (Promega). Isolation of plasmids from *N. meningitidis* transconjugants and *E. coli* transformants cultured in more than 1 ml of medium was essentially as described by Kado & Liu (1981).

For the isolation of total DNA, *N. meningitidis* was cultured in the same conditions as described in the preceding section except that chloramphenicol was added during the cultivating.

Total DNA from *N. meningitidis* transconjugants was isolated as described by Suker et al. (1994).

**Southern blotting.** One microgram of the total DNA of *N. meningitidis* was digested with *Cla* and analysed on a 0.8% agarose gel. The gel was treated with 0.25 M HCl followed by 0.5 M NaOH and then transferred to Hybond-N+ membrane (Amersham). The resultant membrane was hybridized with the cat gene of pHT128 as a probe and signals were detected by ECL direct nucleic acid labelling and detection systems (Amersham) according to the manufacturer’s protocol.

**Preparation of antibody for pili.** A peptide encompassing residues 41–55 (EGQKSAVTEYLNHG) of pilin, a region recognized by the monoclonal antibody SM1 made by Virji and co-workers (Virji & Heckels, 1983; Virji et al., 1989), was synthesized. An antiserum specific for the oligopeptide was obtained by immunization of rabbits with the peptide coupled to keyhole limpet haemocyanin using m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS).

**Western blotting.** The *N. meningitidis* cells were suspended in 1× SDS buffer (2% SDS, 5% 2-mercaptoethanol, 62.5 mM Tris/HCl pH 8.0, 10% glycerol, 0.1% Bromophenol blue) and adjusted to OD₆₀₀ 10. After boiling for 5 min, 2.5 µl samples were subjected to 12 or 15% SDS-polyacrylamide gel electrophoresis. Western blotting was performed with Sequi-Blot PVDF membrane (Bio-Rad) and Horizeblot (ATTO). The resulting membrane was soaked in PBS containing 0.1% Tween 20 (T-PBS) and 5% skim milk at room temperature for 1 h with gentle shaking. The membrane was incubated with 10-fold diluted anti-pili serum or 10-fold diluted monoclonal antibody against Opc (B306; Achtman et al., 1988) at room temperature for 1 h. After washing the membrane in T-PBS for 10 min three times, it was incubated with 2000-fold diluted horseradish-peroxidase-conjugated anti-rabbit or anti-mouse antibody (Boehringer). After washing the membrane in T-PBS for 10 min three times, signals were detected by ECL detection kit (Boehringer) and ECL film (Boehringer).

**RESULTS**

**Transconjugation of pHT128 from *E. coli* to *N. meningitidis***

To check growth inhibition by antibiotics, *N. meningitidis* strains H44/76 and NIID57 were incubated on GC agar plates containing various concentrations of antibiotics at 37 °C in 5% CO₂ for 18 h. Growth of both strains was completely inhibited by 5 µg chloramphenicol ml⁻¹, 1 µg ampicillin ml⁻¹ and 4 µg erythromycin ml⁻¹ but not clearly inhibited by 50 µg kanamycin ml⁻¹ or 2 µg tetracycline ml⁻¹ (data not shown). As penicillin has been used as a first choice of antibiotic for the treatment of meningococcaemia, we chose to use the chloramphenicol-resistance gene as a selective marker in this study.

We tried to introduce the IncQ plasmid pHT128 into *N. meningitidis* strain NIID57 by three methods: transformation by natural competence, electroporation and conjugation. Although no Cml colonies were obtained with transformation by natural competence or electroporation, some Cml colonies appeared when triparental conjugation was used to transfer pHT128 from *E. coli* to NIID57. To optimize the conditions of triparental
conjugation, we changed the concentration of the recipient strain NIID57 and found that the efficiency of conjugation seemed to be highest at a density of \( \text{OD}_{600} = 0.2-0.3 \), although the number of \( \text{Cml}^r \) transconjugants seemed to be almost equal (about \( 10^3 \) clones) independent of the concentration of the recipient strain (Fig. 2a). The efficiency of transconjugation was calculated to be about \( 10^{-5} \) (transconjugants/recipient) under the best conditions (Fig. 2b). As a similar result was obtained with \( N. meningitidis \) strain H47/76 as recipient (data not shown), pHT128 seems to be introduced into \( N. meningitidis \) serogroup B regardless of strain specificity.

Since no colonies appeared when transconjugants were selected on ampicillin-containing plates (data not shown), it was speculated that the IncP plasmid used as a helper plasmid could not be transferred or replicated in \( N. meningitidis \).

**Isolation of pHT128 from \( N. meningitidis \)**

To confirm the existence of pHT128 in \( N. meningitidis \), we isolated the plasmids from \( \text{Cml}^r \) transconjugants. Plasmids the same size as pHT128 (12 kb) were isolated from all the \( \text{Cml}^r \) clones) independent of the concentration of the recipient strain (Fig. 2a). The efficiency of transconjugation was calculated to be about \( 10^{-5} \) (transconjugants/recipient) under the best conditions (Fig. 2b). As a similar result was obtained with \( N. meningitidis \) strain H47/76 as recipient (data not shown), pHT128 seems to be introduced into \( N. meningitidis \) serogroup B regardless of strain specificity.

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**Stability of pHT128 in \( N. meningitidis \)**

To check the stability of pHT128 in \( N. meningitidis \), we successively subcultured \( N. meningitidis \) harbouring pHT128 in liquid medium and counted total c.f.u. on GC agar plates with or without 5 \( \mu \)g chloramphenicol ml\(^{-1}\) as described in Methods. Although pHT128 was maintained at a frequency of 100% in \( E. coli \) strain LE392 even in drug-free liquid medium, the plasmid was gradually lost from \( N. meningitidis \) at a rate of about 7–9% loss per generation under the same conditions (Fig. 3a). This result indicated that maintenance of pHT128 in \( N. meningitidis \) was relatively unstable in the absence of selection by chloramphenicol.
Southern blotting using the chloramphenicol acetyltransferase (cat) gene as a probe. A band corresponding to linearized pHT128 was detected from the samples at each cell generation tested and no other bands could be seen (Fig. 3b). This result showed that pHT128 was maintained in *N. meningitidis* as a plasmid.

The extraneous genes on pHT128 can be expressed in *N. meningitidis*

Next, we examined whether pHT128 vector is available for the complementation of mutations in *N. meningitidis*. A derivative of pHT128 carrying the His-tagged pilE1 or the opc gene under the control of tac promoter, pHT161 or pHT160, respectively (Fig. 4a, c), was introduced into *N. meningitidis* strain HT1033 (pilE1) or HT1037 (Δopc). Cell lysates of Cm\(^{r}\) transconjugants cultured in the presence and absence of IPTG were examined for the expression of pili and Opc protein by Western blotting with each antibody. As shown in Fig. 4b, d, pili or Opc protein were expressed in *N. meningitidis* under the control of IPTG induction. These results confirmed that the foreign genes on pHT128 could be expressed in *N. meningitidis*.

Mobilization of the plasmids is independent of neisserial natural competence

To confirm that the mobilization of the plasmids observed in this study was independent of natural competence, we performed triparental conjugation using the pilE1-defective strain HT1033 as recipient, since the product of pilE1, pili, is essential for natural competence (Jyssum & Lie, 1965). The mean conjugation efficiency to the pilE1-defective strain was 6.8 × 10\(^{−6}\), which was not lower but rather higher than that to a wild-type strain (3.0 × 10\(^{−6}\), for unknown reasons. This result showed that the conjugal transfer of plasmid from *E. coli* to *N. meningitidis* was not dependent on neisserial natural competence.

**DISCUSSION**

Genetical tools for *Neisseria* species have been mainly developed in *N. gonorrhoeae* (Stein et al., 1983; Piffaretti et al., 1988; Roberts, 1989) and there are few efficient genetic tools such as transposons and plasmids that work in *N. meningitidis*. Transposons derived from *Streptococcus faecalis*, Tn916 (Kathariou et al., 1990; Stephens et al., 1991; Erwin & Stephens, 1993) and Tn1545-A3 (Nassif et al., 1991) were applied to *N. meningitidis* for random mutagenesis. In the case of plasmids, one R-plasmid in *N. gonorrhoeae* could be introduced into *N. meningitidis* by conjugation (Ikeda et al., 1986), but no further information has been reported so far. pMGC10, a derivative of a shuttle vector between *E. coli* and *N. gonorrhoeae*, was successfully mobilized to *N. meningitidis* (Nassif et al., 1991) and this was the only plasmid that was proved to work in *N. meningitidis*. However, this plasmid was constructed to introduce Tn1545-A3 into *N. meningitidis* and has not been commonly used as a plasmid vector available in
N. meningitidis. Plasmids named pJS-A and pJS-B have been recently found in N. meningitidis (Hilsel et al., 2000; Claus et al., 2001), but they might not be suitable for plasmid vectors in N. meningitidis because these plasmids, that were release-forms from the genome, seemed to be unstable as episomes. In this study, we showed that pHT128, a derivative of an IncQ broad-host-range vector, could be introduced into N. meningitidis at a considerable frequency and that it worked as a cloning vector in N. meningitidis in the presence of antibiotic. To our knowledge this is the first observation that an artificial plasmid can work well in N. meningitidis.

The difficulties of introducing R-plasmids into N. meningitidis may be partially attributed to some restriction/modification systems in N. meningitidis because naked DNA isolated from N. meningitidis transconjugants, but not from E. coli, could be easily transformed into N. meningitidis by electroporation (unpublished data). The influence of the restriction/modification systems on the efficiency of conjugation is still controversial (Stein et al., 1988; Butler & Gotschlich, 1991). However, mobilization of plasmid by conjugation from E. coli to N. meningitidis may circumvent the barrier of modification/restriction systems by some unknown mechanism(s) because pHT128 or pMGC10 (Nassif et al., 1991) was transferred at a considerable frequency.

The efficiency of plasmid DNA transfer in triparental conjugation was variable (Fig. 2). This might have been due to the difference of living state of N. meningitidis used for the preparation of recipient cells because neisseriae have rapid autolytic properties, even if cultured under steady conditions. In order to increase the transfer frequency, it would be critical to improve the reciprocity of N. meningitidis.

To date, genetical analyses such as complementation tests in N. meningitidis have never been done because there were few useful plasmid vectors for N. meningitidis. We have shown here that pHT128 is applicable for complementation tests in N. meningitidis. We believe that our findings increase the possibilities for the molecular biological analysis of N. meningitidis.

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