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Original article

Isolation and characterization of a competence operon associated with transformation and adhesion in *Helicobacter pylori*

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Abstract

To identify adhesion-associated loci, we screened 1500 mutant strains of a *Helicobacter pylori* mutant library. A mutant that showed decreased adhesion to two gastric epithelial cell lines was identified. Inverse polymerase chain reaction (PCR) revealed that the interrupted locus of this mutant was an *hp0015* homolog of *H. pylori* strain 26695. DNA sequencing and reverse-transcription PCR revealed that *hp0015* and two downstream genes (*hp0016* and *hp0017*) were a transcriptional unit. Deletion and complementation constructs revealed that *hp0016* and *hp0017* were involved in natural transformation, but not in adhesion. *Hp0015* was associated with both adhesion and natural transformation. The reduction of adhesion to human gastric tissues in the *hp0015* mutant was similar to that of the *babA2* knockout mutants and greater than that of the *sabA* knockout mutants. Co-culture of a wild-type strain with AGS cells revealed that 19 genes in AGS cells were upregulated; however, five of the 19 genes were not induced by co-culture with *hp0015* mutants. These results indicate that *hp0015* is associated with adhesion, and hp0015, *hp0016*, and *hp0017* are associated with natural transformation. *Hp0017* has been named as *virB4*/*comB4*. Therefore, *hp0015* and *hp0016* are named as *comB2* and *comB3*, respectively.

Keywords: *Helicobacter pylori*; Mutant library; Adherence; Natural transformation

1. Introduction

Helicobacter pylori is an important gastric pathogen in humans. It is a causative agent of type B gastritis and peptic ulcer, and a risk factor for gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma (MALToma) of the stomach [1–5]. Several virulence factors have been identified, such as vacuolating cytotoxin and CagA, but the pathogenesis of *H. pylori* is still not fully understood.

The interaction of bacteria and host cells through the binding of adhesin(s) and host cell receptor(s) is the first step in bacteria colonization and contributes to persistent infection. Adherence may be advantageous to *H. pylori*, helping to stabilize it against mucosal shedding into the gastric lumen. The most well defined adhesin in *H. pylori*, BabA, was identified by the interaction with Lewis b (Le^b) blood group antigen [6,7]. The interaction between the *H. pylori* adhesin SabA and the cellular receptor sialylated-Lewis x (sLe^x) may promote persistent infection [8]. Le^b antigens are only abundant in individuals with blood type O. Seroepidemiologic studies have revealed that infection rates are the same in patients with different blood types [9]. Although a recent study further demonstrated that the BabA adhesin of most *H. pylori* strains can bind to A-Le^b and B-Le^b glycans [10], there are some clinical *H. pylori* strains do not bind to the Le^b, A-Le^b or sLe^x antigens [8]. These results suggest that different adhesins might be involved in the adhesion of different strains. Therefore,

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there could be other adhesins besides BabA and SabA in *H. pylori*. In the current study, we adopted an *H. pylori* mutant library to identify the genes involved in cell adhesion.

2. Materials and methods

2.1. Bacterial strains and culture conditions

An *H. pylori* clinical isolate, an NTUH-C1 strain that was *cagA*$^+$, *vacA*$^+$, with a highly natural transformation efficiency and well genetic characterization in our laboratory, was obtained at the National Taiwan University Hospital (NTUH), as previously described [11–14]. The NTUH-C1 strain was used to identify the strain-specific genes [14], rather than the sequenced 26695 and J99 strains. *H. pylori* strains were grown on Columbia agar plates (Oxoid Unipath Ltd., Hampshire, England) containing 5% sheep blood and chloramphenicol (4 μg/ml) or kanamycin (10 μg/ml) and incubated for 2 or 3 days in microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2) at 37 °C. *Escherichia coli* strains were grown on LB agar plates or in LB broth containing appropriate antibiotics.

2.2. Cell adherence assay

Human gastric cancer epithelial cell lines (SC-M1; 5×10^5) [15] were grown in a 24-well culture plate with cover slides. Five hundred microliters of *H. pylori* (1×10^9 CFU/ml) were labeled with 5 μl FITC (10 mg/ml) for 1 h at room temperature. The bacterium was washed three times with 0.05% Tween-20/1× PBS. The FITC-labeled bacteria were plated to prove that the bacteria were still alive after these experimental procedures. The FITC-labeled *H. pylori* were incubated with SC-M1 cells for 1 h (MOI = 1:100). Nonadherent bacteria were removed by washing 2 × 5 min with PBS buffer on a horizontal shaker. The entire cover slides with SC-M1 cells and FITC-labeled adherent *H. pylori* were observed under a fluorescence microscope. Each mutant strain was compared with the wild-type strain [16,17]. The adherence of *H. pylori* with another human gastric cancer epithelial cell line (AGS) was also observed. The expression of Le^b and sLe^x antigens on SC-M1 and AGS cells was detected by immunofluorescence assay with Le^b (Seikagaku, Tokyo, Japan) and sLe^x (Chemicon, Temecula, CA) monoclonal antibodies. The SC-M1 cell was Le^b negative but sLe^x positive, whereas the AGS cell was negative for both Le^b and sLe^x antigens.

2.3. Inverse PCR and DNA sequencing

To identify the loci that were interrupted by the transposon, genomic DNAs of mutant strains were extracted to perform inverse PCR and DNA sequencing as previously described [11].

2.4. Reverse-transcription PCR

Total RNA of *H. pylori* NTUH-C1 wild-type strain and M17-84 were extracted as previously described [12], and reverse transcribed by a Superscript II reverse transcriptase (Invitrogen, San Diego, CA) with an HP0017-5R primer (5′-CTTCTTGGTTGTGGAAATTG-3′). The cDNA was amplified by PCR using HP0015-F (5′-ATGTCCGCTCATT TTTTAAG-3′) and HP0016-R (5′-CGCTCTATAACATTTGG TTG-3′) as primers. For each experiment, genomic DNA was used as a positive control, whereas RNA without reverse transcription served as a negative control to exclude DNA contamination.

2.5. Complementation of M17-84

Because *hp0015-hp0016-hp0017* appeared to be an operon, we complemented *hp0015*, *hp0015-hp0016*, and *hp0015-hp0016-hp0017* separately into M17-84 by a shuttle vector pHel2 (a gift from Dr. R. Haas, Max-Planck-Institute für Biologie, Tübingen, Germany) [18] and chromosomal integration. PCR products of *hp0015*, *hp0015-hp0016*, and *hp0015-hp0016-hp0017* contained the predicted promoter region (5′ end, 141 base pairs from the *hp0015* coding sequence) and were cloned into a pGEM-T easy vector (Promega, Madison, WI). The *cat* gene (chloramphenicol-resistant gene, a gift from Dr. D.E. Taylor, University of Alberta) [19] was then cloned into the *Sal*I site of these plasmids. The complementation constructs were naturally transformed into a wild-type NTUH-C1 strain first, then the original *hp0015* was mutated with M17-84 genomic DNA. The complemented gene was shown to integrate at the same locus by PCR. *Hp0015*, *hp0015-hp0016*, and *hp0015-hp0016-hp0017* were also cloned into the *Eco*RV site of the pHel2 vector. Because the M17-84 mutant was profoundly impaired ($<2 \times 10^{-9}$) in natural transformation, complementation plasmids were transformed into wild-type strains by electroporation first, and then the *hp0015* in chromosome was mutated by recombination with genomic DNA from M17-84. The gene alignments of these complementation strains were confirmed by PCR using different combinations of primers.

2.6. Constructions of hp0015 and hp0016 deletion mutants

Using the *hp0015-hp0016-hp0017* operon in a pGEM-T easy plasmid, *hp0015* deletion construct was generated by inverse PCR with HP0015(−1)R (5′-CAAAACCCTT TTGTTTAATT-3′) and HP0016(+2)F (5′-TGATTATCCTG TCAGCGA-3′) primers. Primers HP0015(+281)R (5′-TA AAAAATTCCCATAAACC-3′) and HP0017(−1)F (5′-AATG TTAGAAAAGCTTTTAAG-3′) were used to generate *hp0016* deletion construct. A blunt-end PCR product of *cat* coding region was also amplified by a Pfu polymerase with CAT(+1)F (5′-ATGCAATTCACAAAGATTG-3′) and CAT(+624)R (5′-T TATTTATTCAGCAAGTC-3′) primers and then phosphorylated by a polynucleotide kinase (New England Biolabs, Beverly, MA). The inverse PCR products and blunt-end *cat* were ligated. The orientation of the *cat* gene was the same as the direction of the operon. We obtained two plasmids *hp0015* and *hp0016* that were replaced by a *cat* coding region. These plasmids were transformed into a wild-type *H. pylori*