Detection of the glmM gene in *Helicobacter pylori* isolates with a novel primer by PCR

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ABSTRACT

A novel reverse primer (GLM MR1) was designed for detection of the glmM gene in *Helicobacter pylori* by Polymerase Chain Reaction (PCR). The percentage of amplification in clinical isolates using GLM MR1 was 100 %, for ureA gene was 86.36 %. The primer designed is useful for the identification of *H. pylori*.

*Helicobacter pylori* is the human specific and etiologic agent of chronic active gastritis as well as gastric and duodenal ulcers (1,6). Several assays have been developed to diagnose *H. pylori*, including microbiologic culture, histological examination, rapid gastric biopsy urease tests, urea breath tests, serologic tests, and the detection of *H. pylori* by DNA and RNA probe technology (9).

Polymerase Chain Reaction (PCR) based-methods have been developed to detect the organism directly in clinical specimens. The targets of these PCR methods include the 16S rRNA gene (8), the
random chromosome sequence (15), the 26-kDa species-specific antigen (SSA) genes (7,11), urease (ureA) gene (3), and glmM (ureC) gene (2).

The ureC gene encodes for a phosphoglucomannose mutase, this gene is unrelated to urease production so it was renamed as glmM gene. This gene is considered a “housekeeping” gene, and participates directly in cell wall synthesis (4).

The aim of this work was to design a reverse primer for detecting the glmM gene by Polymerase Chain Reaction (PCR) from clinical strains of Helicobacter pylori isolated from gastric biopsy specimens for confirming the presence of the bacterium.

The amplification of the ureA gene in this work was for using it as a positive control, since this gene is widely used for identifying H. pylori by PCR (3,9). The design of this reverse primer (GLM MR1 5'-GCA TTC ACA AAC TTA TCC CCA ATC-3') for detecting the glmM gene from H. pylori was done because the original primers described by Tomasini et al., 2003 were used in other studies of our research group for the amplification of the this gene as well as vacA, cagA and others in a PCR multiplex (data not shown) but when these primers were used, we observed non-specific bands as well as in a non-multiplex assay using the original glmM primers as shown in Figure 1(5).

GLM MR1 was designed based on sequences reported at the “GeneBank” with No. Access: AE001446 of the Database at the NCBI (National Center of Biotechnology Information). A bioinformatics analysis was done using Bioinformatics programs (DNAMAN v 5 and Bioedit v 7.0.9) to confirm no interactions existing between GLM MR1 and the GLM MF primer reported by Tomasini et al., 2003 (Table 1). Both primers did not show complementarities or other types of interactions. GLM MR1 amplifies a product of 140 bp as shown in Figure 2.

To validate the design of the GLM MR1 primer, the product of PCR was sequenced. PCR was done using Helicobacter pylori ATCC 43504 DNA, the product was purified with a purification kit (Marligen
Bioscience®); according to the manufacturer and it was sequenced at the Unidad de Síntesis y Secuenciación IBT of the Universidad Nacional Autónoma de México (UNAM) from Cuernavaca-Morelos, México. The alignment of the resulting sequences from sequencing was done at the National Center for Biotechnology Information (NCBI) Database with the Basic Local Alignment Search Tool (BLAST). The results of the alignment showed a high alignment score (>= 200) of the primers and it only aligns with *H. pylori* strains (data not shown).

PCR using *glmM* (GLM MR1 and GLM MF) and *ureA* primers was applied to 101 *H. pylori* clinical isolates obtained from gastric biopsy specimens from 14 pediatric patients who had undergone endoscopy for diagnosis of abdominal recurrent pain or discomfort at the Department of Pediatric Endoscopy of the Hospital General Centro Médico “La Raza” of the Instituto Mexicano del Seguro Social (IMSS), México. All the clinical isolates (101) were rapid urease test (RUT) and culture positive.

*H. pylori* ATCC 43504 DNA was used to standardize both PCRs, as well as DNA from *Proteus mirabilis* as negative control and no template as negative control reaction. DNA from 101 pure clinical isolates was obtained using the thiocyanate guanidine method (Ochoa, 2006). Primer sequence, sizes of the products from PCR and references are summarized in Table 1. Briefly, the template DNA from reference strain (80 ng), 5.0 pM of each primer (*glmM* and *ureA*) were added to a PCR mixture tube containing 1 U of Taq DNA polymerase (Invitrogen®), 2.5 mM of each deoxynucleoside triphosphate, 1.5 mM Tris-HCl (pH 8.3), 1.5 mM MgCl₂; volume was adjusted with distilled water to 25 µL. Reaction mixture was subjected to 35 amplification cycles (10 min at 94°C, 1 min at 94°C, 1 min at 45°C (*ureA*;12) and 58°C (*glmM*; this work), 1 min at 72°C (model 9600 Thermocycler; Perkin-Elmer Cetus). The amplicons were visualized with ethidium bromide after electrophoresis was done.
Results from: PCR amplification of *ureA* (*ureAPCR*) and *glmM* (*glmMPCR*) genes, isolation of clinical strains (culture) and rapid urease test (RUT) of clinical isolates are shown in table 2; the *ureAPCR* amplified 86.36% (94 of 101) even though all clinical isolates were positives when rapid urease test (RUT) was done from culture. Moreover, *glmMPCR* fully amplified in the 101 clinical isolates (100%) and the presence of non specific bands or other products was not observed in *P. mirabilis* DNA amplification confirming the high specificity of the primer for amplification by PCR.

We highly recommend the use of the *glmM* gene as well as the GLM MR1 primer designed together with GLM MF described by Tomasini *et al.*, 2003 for the diagnostic and identification of *H. pylori* in clinical isolates from gastric biopsy specimens.

As it is observed in our results, the “gold standard” method for diagnostic of *H. pylori* infection is still being the culture of the microorganism but this is still complex, tedious and expensive. Development of molecular biology techniques for the detection of DNA by PCR methods of several fastidious microorganisms such as *H. pylori* has been highly recommended (3). Development of specific primers based on “housekeeping genes” such as 16S rRNA, *glmM* and *ureA* genes is useful (15). In the present work, both genes used are “housekeeping” however the amplification of the *ureA* (86.36%) gene was lower than with the *glmM* (100%) gene; this results may be due to sequence polymorphism in the *ureA* loci, or by the diversity of clones within a same patient (2,14,15). The *glmM* gene is essential for the development of wall cell in bacteria as well as for the growth of the microorganism and this gene has been extensively used for confirming the presence of *H. pylori*. We recommend the use of the *glmM* gene, and the use of the GLM MR1 primer for the detection of *H. pylori* by PCR because of the high specificity obtained in this study. It would be interesting to develop assays using these primers with other samples such as biopsies or saliva than from pure culture strains.
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REFERENCES


**FIG 1.** Product from PCR amplification of *glmM* gene. Primers reported by Tomasini *et al.*, 2003, non-specific bands (BI) are shown. Lane 1, 100-bp ladder; lane 2 no template control; lane 3, shows the amplicon for *glmM* gene.

**FIG 2.** Amplification of *glmM* with the primer designed GLM MR1 and GLM F described by Tomasini *et al.*, 2003. There are no nonspecific bands; a product of 140 bp was obtained. Lane M: 100-bp ladder; lanes 1-2 no template controls; lane 3, amplicon of *glmM* gene.

**TABLE 1.** Oligonucleotide sequence of primers and PCR product sizes.

<table>
<thead>
<tr>
<th>Amplified gene</th>
<th>Primer</th>
<th>Sequence(5’-3’)</th>
<th>PCR product size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>ureA</em></td>
<td>URE AR</td>
<td>CTC CTT AAT TGT TTT TAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>URE AF</td>
<td>GCC AAT GGT AAA TTA GTT</td>
<td>411</td>
<td>12</td>
</tr>
<tr>
<td><em>glmM</em></td>
<td>GLM MR</td>
<td>GCA TTC ACA AAC TTA TCC CCA ATC*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GLM MF</td>
<td>GGA TAA GCT TTT AGG GGT GTT AGG GG</td>
<td>140</td>
<td>13</td>
</tr>
</tbody>
</table>

* Primer designed at this work.
TABLE 2. Diagnosis of *H. pylori* from biopsy specimens and clinical isolates.

<table>
<thead>
<tr>
<th>TEST</th>
<th>Culture</th>
<th>RUT</th>
<th><em>ure</em>APCR</th>
<th><em>glm</em>MPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>100 (101/101)</td>
<td>100 (101/101)</td>
<td>86.36 (94/101)</td>
<td>100 (101/101)</td>
</tr>
</tbody>
</table>

RUT: Rapid Urease Test.