

BACTERIAL DNA EXTRACTION METHOD FOR THE DETECTION OF *HELICOBACTER PYLORI* FROM HUMAN FECAL SAMPLES

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ABSTRACT

Objective: The work proposed the implementation of a method of DNA extraction for the detection of the pathogen from 50 stool samples.

Methods: A method of DNA extraction with Chelex resin was applied and then detected by conventional *polymerase chain reaction* (PCR).

Results: By PCR, 11 samples were positive for *Helicobacter pylori*.

Conclusions: The Chelex extraction methodology allows obtaining DNA with quality necessary to be detected by PCR, making it a fast methodology for its diagnostic application.

Keywords: DNA extraction, *Helicobacter pylori*, Polymerase chain reaction, Gastritis.

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INTRODUCTION

Helicobacter pylori is a microorganism belonging to the Helicobacteraceae family, order of the Campylobacterales, this microorganism is able to colonize the gastric mucosa of human beings naturally. Moreover, this bacterium is considered a pathogen of global interest because it is a very easy contagion microorganism between hosts or hosts [1]. *H. pylori* infection is now recognized as a problem that causes chronic gastritis, peptic ulcer disease, and lymphoproliferative disorders and is a major risk factor for gastric cancer [2].

Diagnostic methods to detect *H. pylori* are classified as direct or invasive; the directly identify is when the bacteria are obtained by biopsy of the gastric mucosa by histology endoscopy with various staining techniques. However, stool culture techniques and polymerase chain reaction (PCR) are non-invasive methods or serological tests such as the urea breath test marked with 13C (techniques least harmful for the patient) [3,4].

Each of these techniques or detection methods used has its advantages and disadvantages in its operation, they are not perfect, but they have been improving in the last decade, especially molecular techniques. In the same way, the search for alternatives such as the use of resins and new detergents facilitates the processes of extraction of genetic material, among these aspects, it stands out that Chelex is a chelating resin available as an analytical grade, this analytical resin has been dimensioned, extensively purified, and converted to be an accurate and reproducible analytical technique; something to highlight about this technique is that it does not require a previous digestion with enzymes such as proteinase K [5-7].

Considering the previously described, the objective of this work was to evaluate the application of a bacterial DNA extraction method for the detection of *H. pylori* in human fecal samples by conventional PCR.

METHODS

Selection and sampling

The research included a total of 50 patient samples, 34 (68%) women and 16 (32%) men, these samples were selected, from a clinical record

for medical importance. The experimental study was carried out in the molecular biology laboratory, belonging to the Research Department of the Universidad Estatal de Bolívar (Guaranda, Ecuador).

Bacterial DNA extraction

With the samples obtained from patients who presented with gastric problems or associated symptoms, bacterial DNA was extracted using the Chelex method [8]. For which, the strains of *H. pylori* (Hp17C2 and Hp34C1) previously confirmed by microscopy were used as controls. To identify the presence of the DNA obtained, a combination of factors was applied by the experimental design, as shown in Table 1.

Description of the method of DNA extraction with Chelex resin

The technique was carried out as follows:

- A bacterial culture (60.3 mg of biomass) is suspended in 300 μ L of 1X TAE buffer (Invitrogen, 24710-030, USA).
- The resuspension was centrifuged at 13,000 $\times g$ for 15 min at 4°C. The pellet was washed 3 times by suspending it in 1.5 mL of acetone. Each preparation was centrifuged at 13,000 $\times g$ for 10 min at 4°C.
- The supernatant was removed and the pellet was retained for later analysis.
- About 200 μ L of Chelex-100 resin (5%) and 0.2 mg of proteinase K were added to the pellets and then incubated considering the experimental design parameters (Table 1).
- Each of the preparations was homogenized and subsequently centrifuged at 100,000 $\times g$ for 5 min. Finally, 100 μ L of the supernatant was transferred to sterile tubes for further analysis.

To confirm the presence of the DNA obtained, an analysis was performed by 0.8% agarose gel electrophoresis in 1 \times TAE buffer, with the addition of 10 μ L of RedSafe, it was exposed to 100 volts for 30 min. A volume of 10 μ L of DNA previously mixed with 2 μ L of loading buffer (Promega, G190A, USA) was used. A 50 bp molecular weight marker was included. Finally, the fragments were visualized in a UV transilluminator (Dark Reader, DR-46B, USA).

Table 1: Combination of factors for DNA extraction using the Chelex method

Treatments	Description
A ₁ B ₁ (T1)	Incubated at 56°C for 20 min
A ₁ B ₂ (T2)	Incubated at 56°C for 30 min
A ₁ B ₃ (T3)	Incubated at 56°C for 40 min
A ₂ B ₁ (T4)	Incubated at 75°C for 20 min
A ₂ B ₂ (T5)	Incubated at 75°C for 30 min
A ₂ B ₃ (T6)	Incubated at 75°C for 40 min

Where factor A corresponds to incubation temperature and factor B corresponded to incubation time

Table 2: Tukey test of DNA extraction treatments

Treatment number	Treatments	Pureza (A260/A280)
5	A ₂ B ₂	1.88 a
6	A ₂ B ₃	1.66 b
3	A ₁ B ₃	1.64 bc
4	A ₂ B ₁	1.62 c
2	A ₁ B ₂	1.18 d
1	A ₁ B ₁	0.95 e

Means with a common letter are not significantly different (p>0.05)

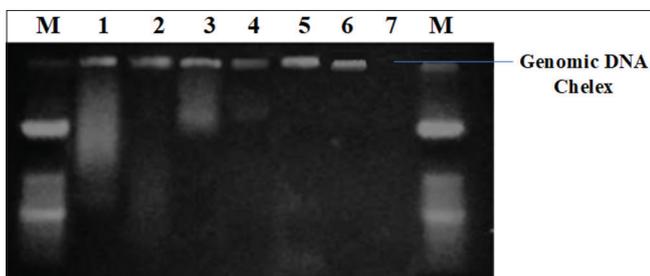


Fig. 1: Analysis of electrophoresis of DNA extracted by the Chelex method in isolate Hp17C2. Lanes M: 50-bp Ladder; Lane1, 2, 3, 4, 5 and 6: treatments 1, 2, 3, 4, 5 and 6 respectively; lane 7: negative control

Molecular analysis

The molecular analysis was performed using the PCR technique that amplifies a 394 bp fragment. Therefore, the initiators, reagents, and conditions described by Santiago *et al.* [9] were used.

RESULTS AND DISCUSSION

Of the 32 isolates under study, 2 of them as controls (Hp17C2 and Hp34C1) were selected for their plate viability for subsequent DNA extraction using the Chelex method.

After Chelex extraction, the DNA obtained showed a higher quality without contamination or inhibitors [10]. Employing the statistical analysis, it can be considered that there is a significant statistical difference between the treatments, where the treatment 5 = A₂B₂ (incubated at 75°C for 30 min) was the one that presented a higher absorbance level of 1.88 nm of λ purity, as shown in Table 2, this result proved to be within what was established to be considered the DNA of optimum quality (1.8–2 nm) according to the Carlos III National DNA Bank of the University of Salamanca [11].

Similar results to ours were obtained by Ahmed *et al.* [12], after comparing different DNA extraction methods, concluded that the Chelex 100-Microwave method was the best with an average DNA concentration of 80.1 µg/mL (A260/280, 1.82) for *Staphylococcus aureus*. In the same way, Devi *et al.* [13], with a DNA purity of A260/280: 1.76, used Chelex resin to extract the genetic material of environmental bacteria from the soil. On the other hand, it is important to emphasize that there is

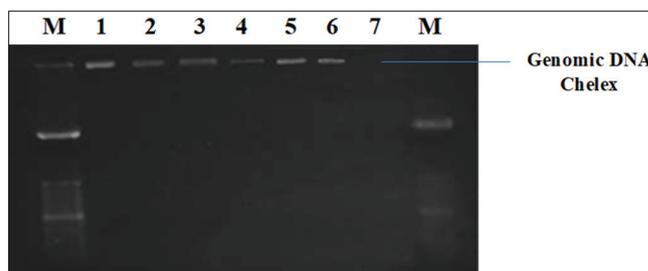


Fig. 2: Electrophoresis analysis of DNA extracted using the Chelex method in isolate Hp34C1. Lanes M: 50-bp Ladder; Lane1, 2, 3, 4, 5 and 6: treatments 1, 2, 3, 4, 5 and 6 respectively; lane 7: negative control

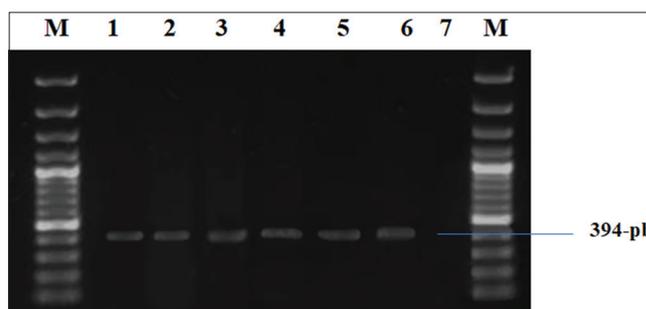


Fig. 3: Analysis of PCR amplified electrophoresis of the amplified DNA and extracted by the Chelex method (Treatment 5 "A2B2") in the isolates obtained. Lanes M: 100-bp ladder; lanes 1-6: positive isolates; lane 7: negative control

only one work carried out by García-Cuan and Miranda [14] who used the Chelex resin with modifications to extract *H. pylori* DNA, the purity of the DNA was A260/A280 of 1.6, however, this value is less to that obtained in the best treatment of our study.

Likewise, DNA quality was analyzed by agarose gel electrophoresis, high DNA quality was evident in all treatments, although with greater fluorescence in treatments 1, 3, 5, and 6; this is due to low concentration of DNA target [15] so that the isolate was continued with PCR amplification. The DNA quality of isolated controls is evidenced in Fig. 1: Hp17C2 and Fig. 2: Hp34C1.

In summary, considering that absorbance and transillumination by electrophoresis, the best DNA obtained was from the 5 "A₂B₂" treatment (incubated at 75°C for 30 min). It can be considered that time and temperatures did have a significant influence on obtaining the DNA. Furthermore, that treatment 5 was the best after electrophoretic and statistical analysis of comparison of means according to Tukey, using this best treatment, the PCR amplification was carried out. Next PCR analysis with the specific initiators for *H. pylori* VacAF and VacAR [9], 11 samples were positive 7 of women and 4 of men. From the isolates of these samples, the characteristic band of 394 pb was obtained, as shown in Fig. 3.

CONCLUSIONS

The Chelex extraction methodology allows obtaining DNA with the quality necessary to be detected by PCR, making it a fast methodology for its diagnostic application for *H. pylori*.

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AUTHORS' CONTRIBUTIONS

The author declares that this work was done by the author named in this article.

CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

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