eppendorf

APPLICATION NOTE No. 424

Long-Range PCR Optimization with Eppendorf Mastercycler® X50

Hidenori Matsui, Kitasato Institute for Life Sciences, Kitasato University

Abstract

There is a strong association between the *Helicobacter suis* infection and gastric diseases in animals and humans. The H. suis genome sequences can contribute to the understanding of these pathogens' virulence mechanism.

Here, we succeeded in the long-range PCR amplification of the genome of non-culturable *H. suis* strains using Mastercycler® X50 prior to nanopore sequencing.

Introduction

Helicobacter pylori (H. pylori), is a Gram-negative micro-aerophilic bacterium usually found in the stomach and typically associated with various gastroduodenal diseases such as chronic gastritis and gastric ulcers. Infection with *H. pylori* has also been linked to risk of developing stomach cancer [1, 2]. Other than *H. pylori*, several other *Helicobacter* species [collectively called *H. heilmannii*-like organisms (HHLOs) including *H. suis* (type 1 *H. heilmannii*), with *H. suis* being the most prevalent gastric non-*H. pylori Helicobacter* species in humans] are also associated with multiple gastric pathological changes [3, 4]. While *H. heilmannii* gastritis is much rarer than *H. pylori* gastritis, both *H. heilmannii* and *H. suis* infection have been associated with a high rate of gastric mucosa-associated lymphoid tissue (MALT) lymphoma [4, 5, 6].

Diagnostic tests for Helicobacter infection include urease test (RUT), urea breath test (UBT), *in vitro* culture, serological and immunohistochemical assays. However, these tests were originally developed and optimized according to *H. pylori* characteristics, vastly reducing sensitivity and specificity of these tests in the detection of HHLOs, especially for human targets. Not only does differentiating immunohistochemical assays lack HHLO-specific antibodies and antigens, HHLOs typically populate the mucus layer above the surface and foveolar epithelial cells, further limiting the suitability of these methods in diagnosing HHLO infection from biopsies. Furthermore, *in vitro* culture of HHLOs by traditional *H. pylori* culture techniques was found to be highly difficult [6]. Hence, there is a crucial need in developing a sensitive, easy, reliable and cost-effective method for the detection of these HHLOs in patient biopsy specimens.

The polymerase chain reaction (PCR) technique was first developed in 1983, and has since become one of the most important basic methods in biological science. Genetic diagnosis by PCR is specific, robust, inexpensive, and does not require high technical expertise to operate, making it a highly suitable method for simple clinical diagnosis. This application note reports the establishment of an end-point PCR-based method using *H. suis*-specific primer pair for detecting *H. suis* in gastric biopsy specimens.

Materials and Methods

Uncultivable *Helicobacter suis* strains were individually maintained in the stomachs of C57BL/6 mice by repeated inoculations of gastric mucosal homogenates from infected to uninfected mice at intervals of around six months. Total DNA was extracted from gastric biopsy and mucosa homogenate specimens using QIAGEN® DNeasy tissue kit. For the preparation of gastric mucosa homogenate, the mouse's stomach was cut along the greater curvature from esophagus through proximal duodenum. The gastric mucosa was mashed by placing between two ground glass slides, then homogenized with phosphate buffered saline (PBS). The final DNA samples were stored at -20 °C until further use.

The following primers were used for the amplification of a 10 kb fragment including an outer membrane protein gene:

Forward Primer
5'-ATAAAGCCCATGAATTCTTAGGCATGCGTGCTCTG-3'

Reverse Primer
5'-TATTCAAGGAAAGTCCCTGGAGAAACTCCAGAGAC-3'

Extracted DNA was used as template for the long-range PCR protocol with KOD FX DNA Polymerase (Toyobo®). PCR reaction master mix was prepared in 0.2mL PCR tubes using 2x PCR Buffer, 2 mM dNTP mix, 5 µM of each primer, 10 ng of DNA template and 1 U of DNA polymerase. The final 50 µL volume per reaction was run in Mastercycler® X50s according to the settings and cycling conditions in Table 1 below.

Table 1: PCR cycling conditions and Mastercycler® X50s settings for the amplification of 10 kb gene fragment.

Header (Eppendorf settings)	Lid	105 °C
	TSP/ESP	ON
	Lid auto-off	ON
	Temperature mode	Fast
Initial Denaturation		94 °C/2 min
Cycles 5x	Denaturation	98°C/10 s
	Annealing + elongation	74°C/5 min
Cycles 5x	Denaturation	98°C/10 s
	Annealing + elongation	72°C/5 min
Cycles 5x	Denaturation	98°C/10 s
	Annealing + elongation	70°C/5 min
Cycles 20x	Denaturation	98 °C/10 s
	Annealing + elongation	68 °C/5 min
Post-Cycle Elongation		68 °C/ 10 min
Storage	Hold	*22 °C*

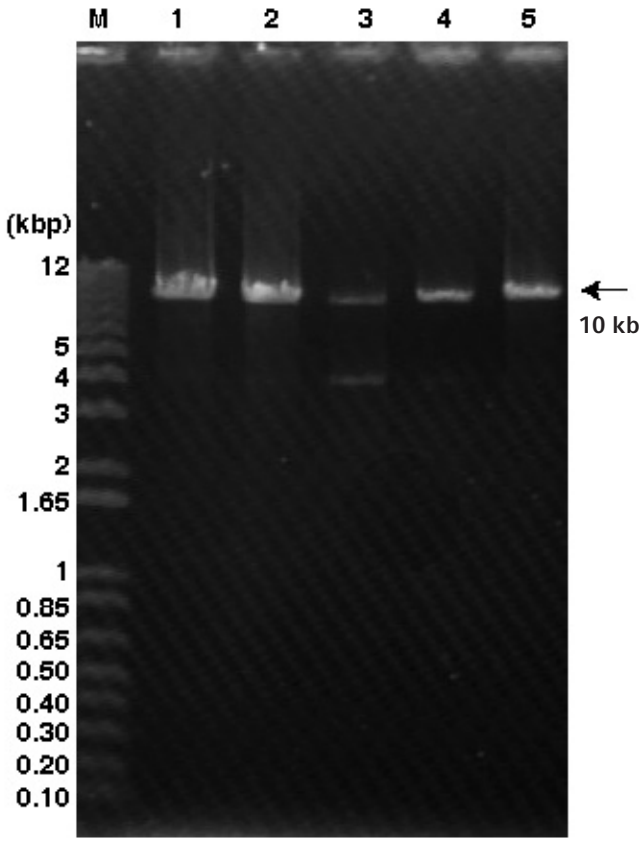
The PCR products were cleaned using phenol extraction and ethanol precipitation method prior to gel electrophoresis and detected via 0.6% of agarose gel electrophoresis using DNA stain with Ethidium bromide and visualized using the FAS-III system (Toyobo).

Results and Discussion

To date, there is a dearth of established genetic-based method in the diagnosis of HHLOs from human gastric biopsies. The most recent one was published by Blaecher *et al.* (2017) [7], which was a probe-based real-time PCR (RT-PCR) method and reported to exhibit a high degree of diagnostic specificity and analytical sensitivity.
In this paper, we report a robust detection method using end-point PCR technique with a *H. suis*-specific primer pair. Figure 1 shows that a highly specific 10 kb-long PCR product was amplified using touch-down PCR strategy. This PCR system successfully amplifies all different strains of *H. suis* isolated from inoculated C57BL/6 mice.

Conclusion

The methods described above can be implemented for detection or isolation of *H. suis* from human gastric biopsies.



Legend:
1: *Helicobacter suis* TKY
2: *Helicobacter suis* SNTW101
3: *Helicobacter suis* SH8
4: *Helicobacter suis* SH10
5: *Helicobacter suis* HS5

Figure 1: Long-range amplification of five different H.suis strains

Literature

[1] Kusters JG, van Vliet AH, Kuipers EJ (July 2006). "Pathogenesis of *Helicobacter pylori* Infection". Clin Microbiol Rev. 19(3): 449–90.
[2] Suerbaum S, Michetti P (October 2002). "*Helicobacter pylori* infection". N. Engl. J. Med. 347 (15): 1175–86.
[3] Zhang G, Ducatelle R, Mihi B, Smet A, Flahou B, Haesebrouck F. (2016) *Helicobacter suis* affects the health and function of porcine gastric parietal cells. Vet Res.; 47(1): 101.
[4] Stolte M, Kroher G, Meining A, Morgner A, Bayerdörffer E, Bethke B. (1997 Jan). A comparison of *Helicobacter pylori* and *H. heilmannii* gastritis. A matched control study involving 404 patients. Scand J Gastroenterol. 1997 Jan; 32(1): 28-33.
[5] Morgner A, Lehn N, Andersen LP, Thiede C, Bennedsen M, Trebesius K, Neubauer B, Neubauer A, Stolte M, Bayerdorffer E (2000). *Helicobacter heilmannii*-associated primary gastric low-grade MALT lymphoma: complete remission after curing the infection. Gastroenterology 118: 821–828.
[6] Goji S, Tamura Y, Sasaki M, Nakamura M, Matsui H, Murayama SY, Ebi M, Ogasawara N, Funaki Y, Kasugai K (2015). *Helicobacter suis*-Infected Nodular Gastritis and a Review of Diagnostic Sensitivity for *Helicobacter heilmannii*-Like Organisms. Case Rep Gastroenterol 9: 179–187.
[7] Blaecher C, Bauwens E, Tay A, Peters F, Dobbs S, Dobbs J, Charlett A, Ducatelle R, Haesebrouck F, Smet A (June 2017). A novel isolation protocol and probe-based RTPCR for diagnosis of gastric infections with the zoonotic pathogen *Helicobacter suis. Helicobacter* 22(3). DOI: 10.1111/hel.12369

Ordering information

Description	Order no. international	Order no. North America
Eppendorf PCR Tube Strips 0.1 mL		
PCR clean without Lids	0030 124.804	3303124804
PCR clean, with Cap Strips, domed	0030 124.812	0030124812
PCR clean, with Cap Strips, flat	0030 124.820	0030124820
Mastercycler® X50s	6311 000.010	6311000010
Mastercycler® X50i*	6301 000.012	6301000012

*** without touchscreen**

Your local distributor: www.eppendorf.com/contact
Eppendorf AG · Barkhausenweg 1 · 22339 Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com

www.eppendorf.com

QIAGEN® is a registered trademark of Qiagen GmbH. Toyobo® is a registered trademark of Toyobo Co., Ltd. Corporation.
Eppendorf®, the Eppendorf Brand Design and Mastercycler® are registered trademarks of Eppendorf AG, Germany. All rights reserved, including graphics and images.
Copyright © 2019 by Eppendorf AG.