Journal of Applied and Natural Science
16(1), 118 - 122 (2024)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Detection of *Bacteroides fragilis* LuxR gene, involved in quorum sensing, among colitis patients in Mosul, Iraq

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Article Info
https://doi.org/10.31018/jans.v16i1.4716
Received: June 2, 2023
Revised: January 17, 2024
Accepted: January 30, 2024

How to Cite
Saad, A. *et al.* (2024). Detection of *Bacteroides fragilis* LuxR gene, involved in quorum sensing, among colitis patients in Mosul, Iraq. *Journal of Applied and Natural Science*, 16(1), 118 - 122. https://doi.org/10.31018/jans.v16i1.4716

Abstract
Bacteroides fragilis is the most anaerobic bacteria that infect humans, particularly in the abdominal cavity. Its pathogenesis is linked to numerous virulence factors. Understanding these factors and exploring alternative options for the use of antibiotics in the treatment of this bacterium, molecular techniques offer several advantages over traditional culture techniques because they are easier and more specific. The present study aimed to use specific primers for the 16sRNA and *Lux*R genes to identify *B. fragilis*. Genetic identification of the *B. fragilis* isolates was performed using the 16SrRNA gene, and the obtained sequences were submitted to National Centre for Biotechnology Information (NCBI) with accession numbers (OQ448827, OQ448828). Each strain was assigned a unique strain name, AS. AWB94 and AS. AWB79. From the total of all samples, it was found that the growth of various types of bacteria constituted (76%), and the samples that did not have growth formed (24%). It was noted that Bacteroidetes constituted only two isolates (2.7%), and these two isolates possessed the gene for quorum sensing (luxR gene), while the results confirmed that they do not possess the sialidase (nanH) enzyme gene. Both isolates possessed the quorum sensing gene (*Lux*R) out of one hundred samples. This suggests that the isolates have a quorum-sensing mechanism responsible for cell-to-cell communication, multidrug resistance, and biofilm formation.

Keywords: 16sRNA gene, *Bacteroides fragilis*, Colitis, LuxR gene, Quorum sensing

INTRODUCTION

Bacteroides fragilis is a Gram-negative, rod-shaped and pleomorphic shape, bile-resistant, esculin hydrolysis and non-spore-forming strict anaerobe that is predominantly found in the human colon and less abundant in the digestive organs of other animals and in the environment. spread of *B. fragilis* to the bloodstream or surrounding tissues results in clinically significant infection. This bacterium is known to cause infections such as intra-abdominal sepsis, abscess formation, soft tissue infection, bacteremia, colorectal cancer, brain, lungs, and liver (Kierzkowska *et al.,* 2023).

The human digestive system, especially the gut, is a complex environment with diverse bacterial populations. The significance of identifying the 16S rRNA gene in diagnosing the microbial community in fecal samples from patients is crucial. Researchers often rely on this method due to its high accuracy, ensuring precise microbial analysis. This method has been widely used to investigate microbial diversity in various environments, with factors such as sampling time, targeted rRNA region, the strategy of sequence, machinery, depth, and read length all potentially impacting the results (Reitmeier *et al.*, 2020 and Saeed and Sulaiman, 2023).

Quorum sensing (QS) is the means of microbial communication between bacterial species and has a role in the production and response to exogenous stimulating molecules called autoinducers AIs (Maddela *et al*., 2019 ; Sulaiman, 2020).

When the density of the microbial community increases, this will lead to an increase in the secretion of autoinducers in the microbial community. Once the concentration of autoinducers exceeds a critical threshold (known as the quorum), it triggers the transcription of various genes that extracellular products contribute to antibiotic resistance, virulence, cell growth, and biofilm formation (Subramani and Jayaprakashvel, 2019).

In *B. fragilis*, Positive lux R and luxI-negative type I pre-

sent in QS systems have been described. These bacterial QS systems are mediated by a related structural analog of homoserine lactones (HSLs) and controls a number of different activities such as antibiotic production, sporulation, bioluminescence, competence, virulence factor secretion, and biofilm formation (Yekani *et al.*, 2020). The study aimed to isolate bacteria, genetically diagnose them, and determine the role of the quorum-sensing gene and its virulence on colon cancer patients in Mosul's hospitals.

MATERIALS AND METHODS

Sample collection

For this study, a total of 100 colon biopsies were collected from 50 male and female patients, ranging in age from 18 to 65 years old, all of whom were diagnosed with colon cancer. The samples of the patients with symptoms of anemia, visible blood in stools, and changes in bowel habits were obtained from patients attending Ibn-Sena General Hospital in Mosul City, Iraq, between October 2022 and February 2023.

Isolate bacteria

The sampling procedure was performed by a specialist doctor under sterile conditions. All the collected samples were cultured on aerobic and anaerobic media (anaerobic media, tryptose soy broth, brain heart infusion broth) and then sub-cultured onto Bacteroides bile esculin agar and blood agar base incubated by using an anaerobic generator system (gas bag) at 37°C for 12-24 h. Suspected colonies were selected, and the morphological characteristics of the growing bacterial colonies were observed on the bile esculin medium. The colonies were collected from swabs and then stained using a Gram stain. They were examined under a microscope to determine their phenotypic characteristics. Additionally, biochemical tests such as to identify the IMVIC, catalase, and oxidase tests were conducted for bacterial isolates obtained from colon biopsies. Further confirmation for the isolates was achieved by the molecular method utilizing polymerase chain reaction (PCR).

DNA extraction of *Bacteroides fragilis*

Genomic DNA from the *B. fragilis* strains was extracted using commercially available molecular biology laboratory equipment and the Gene Aid DNA extraction kit (Taiwan), following the manufacturer's instructions (Starkey *et al*., 2014). The presence of *B. fragilis* is detected using 16sRNA gene, while the virulence factor (quorum sensing QS) detected by LuxR gene was studied using PCR amplification with specific primers, as outlined in Table 2. The standard PCR reaction consisted of 20 µl, including 10 µl of the master, 2 µl of forward and reverse primers, 1µl and 2 µl of Deoxy ribonucleotide acid as a template, and nuclease-free water as necessary for achieving the final volume (Calfee *et al*., 2001), as indicated in Table 1. The PCR programs used for analysis are detailed in Table 3, as specified in the relevant references.

RESULTS AND DISCUSSION

Phenotypic and biochemical identification

The results of the phenotypic and biochemical tests, conducted for the isolates under study, were identical to what was mentioned in the approved diagnostic systems (Collee *et al.,*1996*;* Macfaddin ,2000; Prescot and Harley,2002*;* Snyder and Atlas, 2006). The results of bacterial culture showed the bacteria in the form of circular colonies, convex, with distinct edges and a dark brown color tilted to black, and the staining results showed that the bacteria are Gram-negative bacilli, as

Table 1. Oligonucleotide primers sequences and PCR product utilized in the identification of *Bacteroides fragili*

Genes name	Primers sequencs (5´-3´)	Size (bp)	Reference
16srRNA	F-5' TTCGCTTTTCTGTTTTCTGTGT3' R-5'CAGCAACCACCCAAACATTATT3'	555	GeneBank: HE608156.1
LuxR	F-5' GGTCCAGAACCTCAGAAGCA 3' R-5' CATGGTCGCAGCATGCATTT 3'	334	NCBI Ref.Seq. NC_006347.1

Table 2. Program used for gene amplification

Primer	PCR step	Temp.	Time	Repeat	Reference
16srRNA	Initial Denaturation	95C	5min	1	The study's protocol involved utilizing the NCBI Gene-Bank and Optimize Writer Protocol online to create the procedure.
	Denaturation	95C	30sec	30 cycles	
	Annealing	60C	30sec		
	Extension	72C	1 min		
	Final extension	72C	5min	1	
LuxR	Initial Denaturation	95C	5min	1	
	Denaturation	95C	30sec.	30 cycles	
	Annealing	60C	30sec.		
	Extension	72C	30sec.		
	Final extension	72C	1min	1	

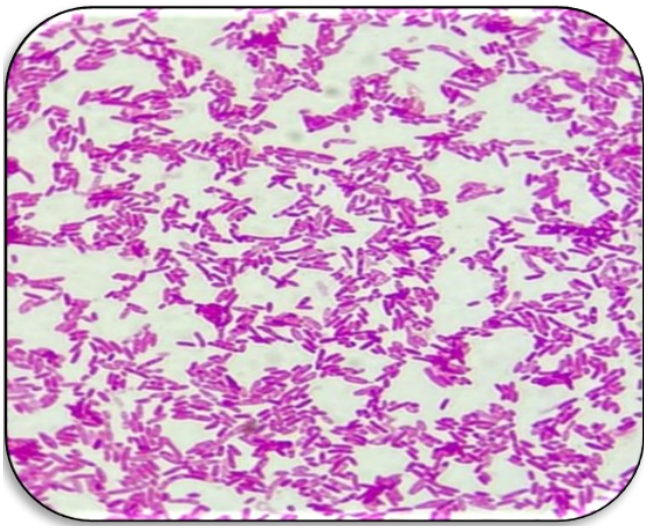

Fig. 1. *Bacteroides fragilis colonies under microscope*

Table 3. Showing results of biochemical tests for *B. fragilis*

Species	Biochemical test	Result
B. fragilis	Oxidase	-
	Catalase	+
	Indole	-
	MR	-
	Urease	-
	Motility	-
	Bile salt tolerance +esculin hydolises	+
	Glucose	+
	Lactose	+
	Sucrose	+

shown in Fig. 1 and biochemical results are shown in Table 3.

The results showed that 98% were positive for bacterial growth, but only 2% belonged to *Bacteroides fragilis*. The low ratio was compared with another study in Lebanon enrolled and the prevalence rate was found in one isolate (1.965%) (Al-Buhilal *et al*., 2022), in Brazil (AL-Sabaawy *et al*., 2022), used 16s-23srRNA for identification, the frequency of *B. fragilis* was 9.5 % totally. This rate in isolation of *B. fragilis* belonged to the obligate anaerobic group. The growth of this group required special equipment, which was time-consuming and highly costly.

Diagnosis of bacteria using PCR technology

The presence of *B. fragilis* was confirmed by amplifying a 1500bp Fragment using specific primers targeting the 16sRNA gene, as shown in Fig. 2.

In the present study, two isolates of *B. fragilis* were identified using molecular methods with specific primers based on the 16sRNA gene (GenBank code: OQ448828.1 and OQ448827.1), a crucial gene for bacterial differentiation. The use of PCR and 16s RNA sequencing plays a significant role in accurately identifying bacterial isolates and discovering new bacterial species, which is a significant development in clinical

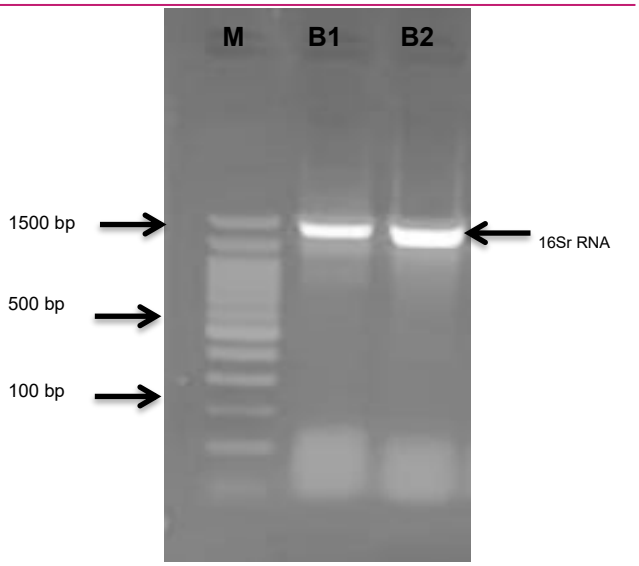

Fig. 2. *Visualization of the 16s RNA gene PCR products after staining with red safe stain and observing under UV light at 280 nm. Marker (100bp) and lanes B1 and B2 demonstrate positive isolates with an amplicon size of 1500bp*

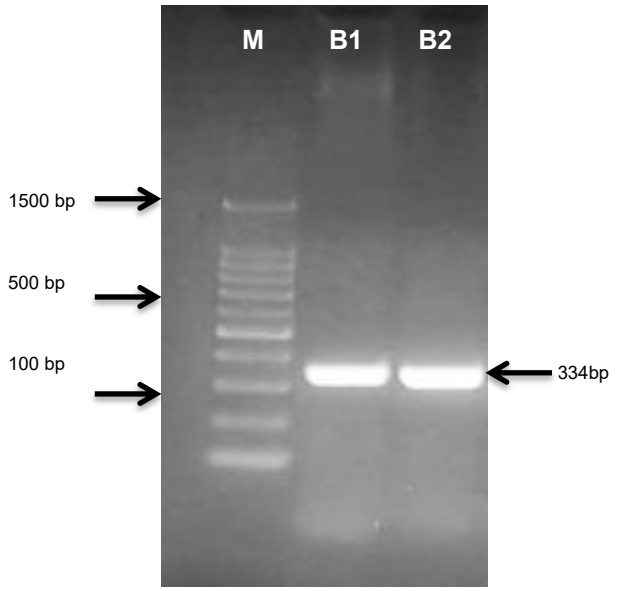

Fig. 3. *Visualization of the LuxR gene PCR products after staining with red safe stain and observing under UV light at 280 nm. The marker (100bp) and lanes B1 and B2 demonstrate positive isolates with an amplicon size of 334bp*

microbiology, particularly in laboratory settings.

The 16S RNA is important for identifying rare, slow-growing, and culture-negative bacteria and those with unusual phenotypic profiles or that are not easy to cultivate. This approach provides valuable insights into the etiology of infectious diseases, which can assist clinicians in selecting appropriate antibiotics, determining treatment duration, and implementing infection control measures (Al-Buhilal *et al*., 2022)

The positive isolates were examined for the presence of the LuxR QS gene through two rounds of analysis.

Amplification of a sample using a primer resulted in a 334bp fragment, as illustrated in Fig. 3.

The study revealed that the two identified *Bacteroides fragilis* isolates tested positive for the *Lux*R gene. The quorum sensing (QS) of *B. fragilis* was used to regulate the density of bacterial cells with other cells and their environmental surroundings, and the *Lux*R is commonly present in G^- bacteria. The gene *Lux*R encodes a transcriptional activator and is responsible for acyl-homoserine lactone (type I) autoinducers, such as N-[3-oxohexanoyl] homoserine lactone and hexanoyl homoserine lactone [C6-HSL] (AL-Sabaawy *et al*., 2022).

Previous research has shown that the *Lux*R transcriptional regulator plays a crucial role in QS, coordinating gene expression encoding of antibiotics, motility, virulence factors, bioluminescence, biosynthesis, biofilm formation, nodulation and plasmid transfer (Woo *et al*., 2008 and Sulaiman and Salih, 2020). The LuxR system has also been demonstrated to influence biofilm formation during the exponential growth phase (Pumbwe *et al*., 2008; Yang et al., 2017).

Conclusion

Many bacteria use quorum sensing (QS) as a regulatory mechanism to manage collective traits that help them to exploit specific niches. Colorectal disease is a significant infection that results from fastidious anaerobic microorganisms. The 16sRNA gene was a useful tool for promptly identifying *B. fragilis*. In addition to the 16sRNA gene, the LuxR gene was critical as it played a vital role in biofilm formation in antibiotic-resistant and recurrent infections caused by bacteria.

ACKNOWLEDGEMENTS

The researchers would like to acknowledge and thank the Biology Dept. at the Science Coll., Mosul Uni., for their valuable support in carrying out this study.

Conflict of interest

The authors declare that they have no conflict of interest.

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