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Gene Expression of col Gene of *Bactericides fragile* and *splA* Gene of *Lactobacillus acidophilus* Associated with Pinworm Patients

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Abstract

Background: The intestinal gut has a complex microcosm, as commensal microbiota plays a role in the establishment and survival of invading helminths such as *Enterobius vermicularis* and helps provide direct competitive protection from pathogen bacteria. **Objective:** The aim of the study was to investigate the role of the col gene, which produces collagenase which damage a mucosa intestinal gut and the *slpA* gene of *Lactobacillus acidophilus*, which encodes a protein for density and biological mechanisms of adhesion, colonization, and closes a receptor on epithelial intestinal cells to prevent *Enterobius vermicularis* as a pinworm parasite. **Methodology:** This was designed as a follow up study. About 100 patients in this follow-up study suffering from pinworm had signs and symptoms, and it was conducted in the Maysan Governorate Laboratory in Qalat Saleh District, Iraq, from May, 2025 to March, 2026 before treatment, 24 sample as a control patients without pinworm disease, and 24 samples were cultured on MRS and Bacteroides agar under anaerobic conditions. Microscopic examination and biochemical tests were used for all isolates. Quantitative PCR (qPCR) was employed to determine the adhesion and colonization genes for Lactobacillus acidophilus. **Results:** The results were reported *Bacteroides fraglis* was produce a sharp increase in collagenase enzyme and was associated with high metabolic gene expression against intestinal epithelial cells. After qPCR a fold change ranged between 1.04 and 7.29, with upregulation due to the presence of Enterobius vermicularis and increased upregulation in a specific gene, *splA*, of *Lactobacillus acidophilus*, interacting with the mucosa to provide protective functions. Conclusion: This study concluded upregulation in col gene, which encoded collagen protein in *Bacteroides fragilis*, and increased upregulation of the *splA* gene in *Lactobacillus acidophilus* in patients with pinworm disease. *[Bangladesh Journal of Infectious Diseases, June 2026;13(1):57-62]*

Keywords: Gut microbiome; *Lactobacillus acidophilus*; *Enterobius vermicularis*; *splA* gene; col genes; *Bactericides fragile*

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Introduction

In a birth increase the density of bacteria and during with Aging 5 years stable of this population[1]. Many groups of complex density of bacteria, viruses, fungi, bacteriophage, and protozoa, and different types in the intestinal tract[2]. Imbalance in microbial populations depends on some factors such as density changes, infections, chronic stress, antibiotics, and aging; this is called dysbiosis[3]. The human gut microbiome ranges from 10^4 and about

1000 bacterial species and bacteriophage[4]. This ecosystem affects human health, which helps with digestion and makes vitamins, reducing the immune system to protect pathogenic bacteria[5]. *Enterobius vermicularis* is an intestinal parasite which found in public institutions as schools, psychiatric clinics, and kindergartens, or in low socioeconomic states with poor hygiene[6]. *Bactericides fragile* is a more abundant genus in the gut tract, with 0.5% to 1.0% of the total gut bacterial population[7]. It is a gram-negative anaerobe in the gut microbial ecosystem[8].

Collagenase is an enzyme encoded from collage gene (Col) secreted from aerobic and anaerobic pathogen of analysis collagen as a source of nutrients[9]. *Bactericides fragile* secreted a key source of collagenase in the intestinal tract. A gap in research in this manuscript is investigating the effect of pathogenesis of *Bactericides fragile* by studying the role of collagenase in the intestinal tract in the presence of *Lactobacillus acidophilus*.

This study aimed to investigate the pathogenesis of *Enterobius vermicularis* and *Bactericides fragile* on the collagen layer of intestine tract and the role of the *Lactobacillus acidophilus splA* gene to prevent this pathogenicity in the presence of *Enterobius vermicularis*.

Methodology

Study Design and Settings: About 100 patients in this follow-up study were conducted in the Maysan Governorate laboratory in Qalat Saleh District, Iraq, from May, 2025 to March, 2026, from patients with *Enterobius vermicularis* before treatment. It is one of the laboratory experiments investigating the Importance of *Bactericides fragile* associated with the intestinal parasite pinworms.

Study Population: In this study, two sample groups were used: a control group 24 sample individuals without pinworms and a disease group of 24 sample patients with pinworms, according to signs and symptoms before treatment. All samples were collected on culture media as a blood agar under aerobic conditions, at 37-48º C, MRS agar with CO_2, and Bacteroides agar under anaerobic conditions. For colony-forming units, the number was determined by serial dilution methods. Healthy control was in the inclusion criteria (Age, sex), no antibiotic use within 2 weeks, and no chronic gastrointestinal diseases.

Sample Collection and Processing: All stool samples were collected in sterile containers and transported in media and processed within 2 to 4 hours for the activity of bacteria to stain, cultured on blood media and *Bactericides* agar as selective media at 37º C under anaerobic conditions.

Laboratory Analysis: All samples were cultured immediately for diagnosis by microscopic examination and biochemical tests. Direct examination was performed to diagnosis bacteria isolates by wet mount technique. A smear was prepared from each colony on a clean glass slide and heat fixation with stained by Gram staining procedure; all smears were examined under oil immersion (1000x) to diagnose bacterial isolates. Enterobius vermicularis examination for detection by the Scotch tape method, then examination under a microscope.

DNA Extraction: From all stool samples, genomic DNA was extracted by Q1Aam DNA Stasis Mini kit. Based on the manufacturer's instructions[11]. Then, DNA Quantitation for measure the concentration and purity of DNA (A260/A250 a ratio) by a Nanodrop spectrophotometer as a standard protocol[10].

Real-Time PCR (qPCR): For quantifying bacterial abundance and virulence gene expression according to the procedure of NIQE guidelines[12]. At the end of amplification, a melting curve analysis was performed after end of amplification. The temperature was increased from 65ºC to 95ºC to obtain specific and amplified products. All virulence genes were calculated by 2-ΔΔ equation against the 16S rRNA gene as an internal control[11].

Statistical Analysis: The Statistical Package for the Social Sciences (SPSS) software version 26.0 was used to analyze all collected data[13]. The data were expressed as a percentage, mean, SD and T-test for detecting demographic variables with a p-value of 0.05. The fold change in tables of all gene

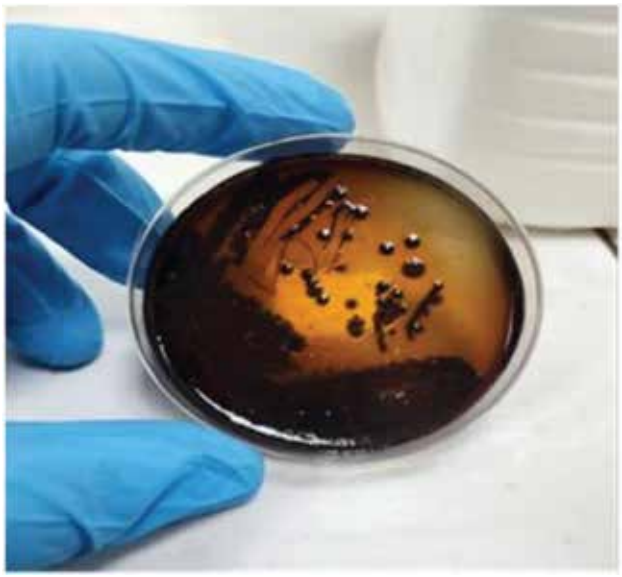

Figure I: *Bacteroides fraglis* on *Bacteroides* Agar

expression was calculated by the following equations: ΔCT = CT of target gene – CT of reference gene; ΔΔCT = ΔCT of each sample - average control ΔCT; Fold change = 2-ΔΔCt.

Ethical Consideration: Ethical approval for this study was obtained from the Research and Knowledge Management Unit, Training and Human Development Center, Misan Health Directorate, Ministry of Health, Republic of Iraq (Approval No. 253; Date 21/4/2025).

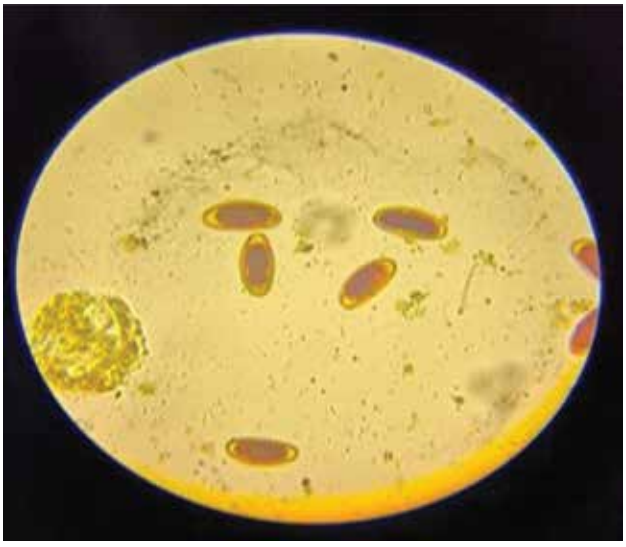

Figure II: Ova of *Enterobious vermicularis* under 40X

Figure II demonstrates the characteristic ova of *Enterobius vermicularis* observed under 40× magnification by light microscopy. The eggs are oval to elongated with a distinctive asymmetrical, planoconvex ("D-shaped") appearance, having one side flattened and the opposite side convex. They possess a smooth, transparent, double-layered shell enclosing a developing larva or embryonated embryo. These morphological features are diagnostic of *Enterobius vermicularis* and facilitate its identification in laboratory examinations. The ova typically measure approximately 50 to 60 µm in length and 20 to 30 µm in width, and their recognition is essential for the diagnosis of enterobiasis. Therefore, in this study, a sharp increase in collagenase enzyme during the pinworm infection was reported, which acts as an inducer of *Enterobius vermicularis* ova.

The analysis results revealed the significantly shift in RT-qPCR in the transcription of the collagenase gene in the fold-change range between 1.047 and 7.29, with upregulation of gene expression 1.01 compared with the control 6.66 (Table 1).

In a qPCR Amplification plot for six bacterial isolates to detect gene expression of the collagen gene and a melting curve for the collagen gene product without any dimer, all samples have the same gene (Figure III).

Table 1: Amplification of qPCR for Collagen Gene in *Bacteroides fragilis*

COL	16SRNA	ΔCT	ΔΔCT	2-ΔΔCt	Folding -change		
34.8	22.9	11.9	-0.06667	1.047296543	1.047296543	Control	1.014676
34.1	22.4	11.7	-0.26667	1.203027816	1.203027816		
34.5	22.2	12.3	0.33333	0.79370236	0.79370236		
32.2	23.1	9.1	-2.86667	7.293796761	7.293796761	Expression gene	6.664345
32	22.7	9.3	-2.66667	6.349618879	6.349618879		
31.9	22.6	9.3	-2.66667	6.349618879	6.349618879		

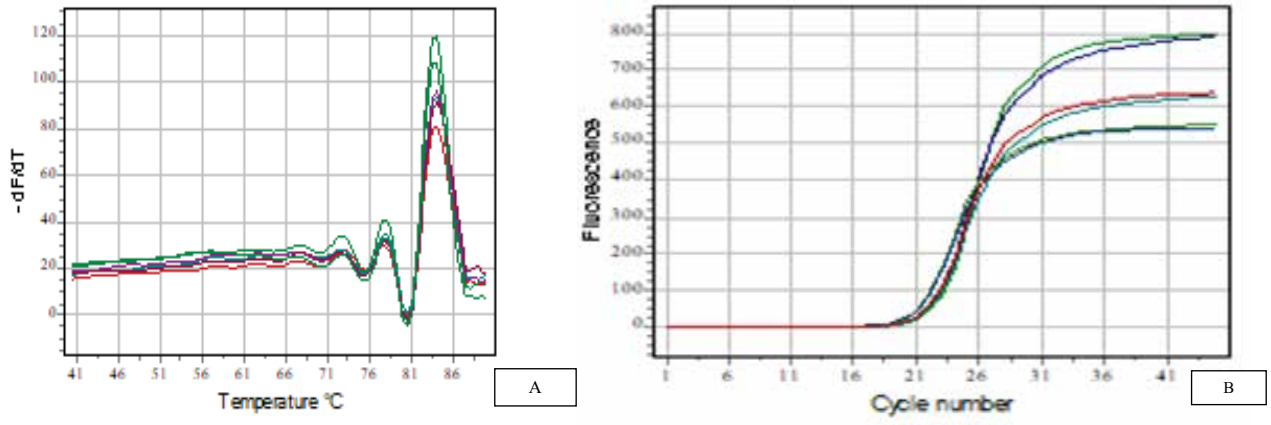

Figure III: (A) qPCR Amplification Plot for Col gene, (B) Melting Curve for Product Gene

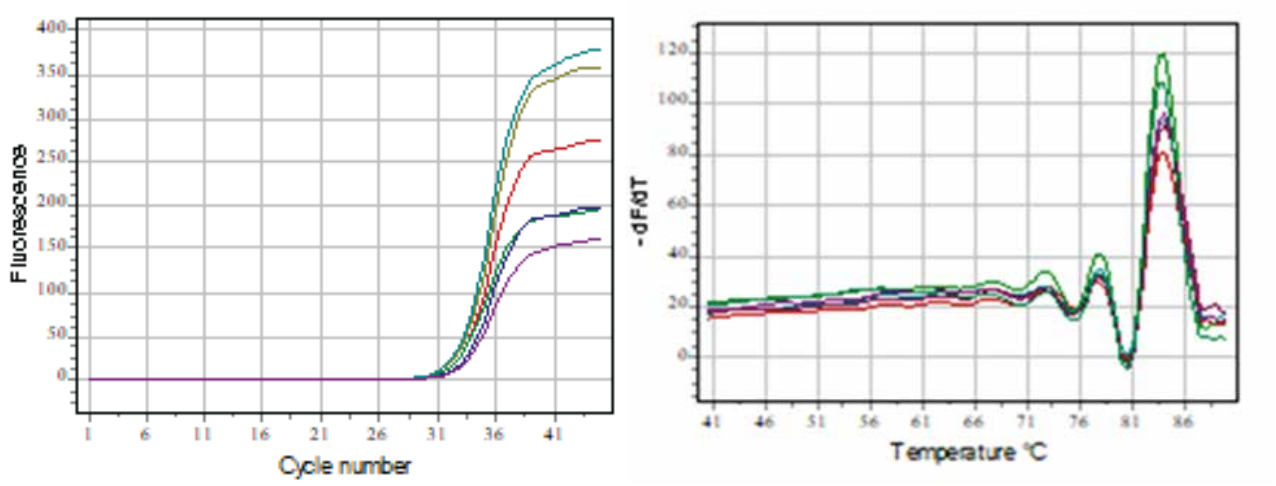

Figure IV: Amplification Curves of 16S rRNA (Housekeeping Gene) used as an Internal Control for Normalization

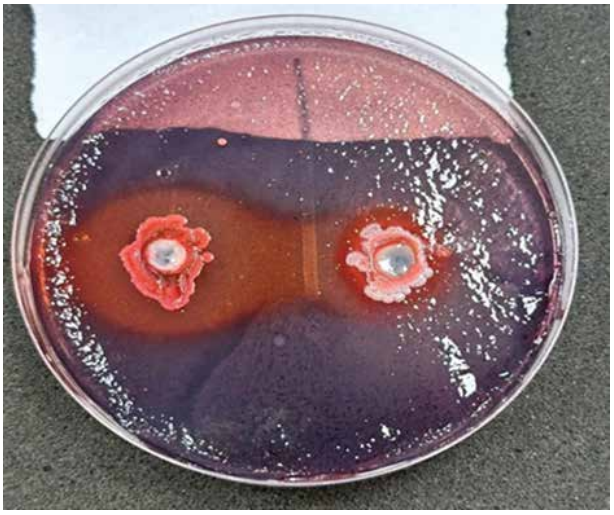

Figure V: Collagenase Enzyme Clear Zone on Collagen agar of *Bactericides fragile*

Figure IV represents amplification of the housekeeping gene 16srRNA.

Figure V shows a collagenase enzyme secreted by Bacteroides fraglis on collagen agar. The nearly sixfold upregulation in the collagen gene, encoding collagenase, refers to more metabolic activity of gene expression toward collagen in intestinal tissue. Also, upregulation of the splA gene to 5.202414066 with control 7.55. Lactobacillus acidophilus has a specific gene interact with the mucosa called surface layer protein A, which interacts with intestine host and Enterobius vermicularis to provide a protective function, as shown in Table 2, amplification of the splA gene, which encodes upregulation of the host cell surface protein (Table 2, Figure VI).

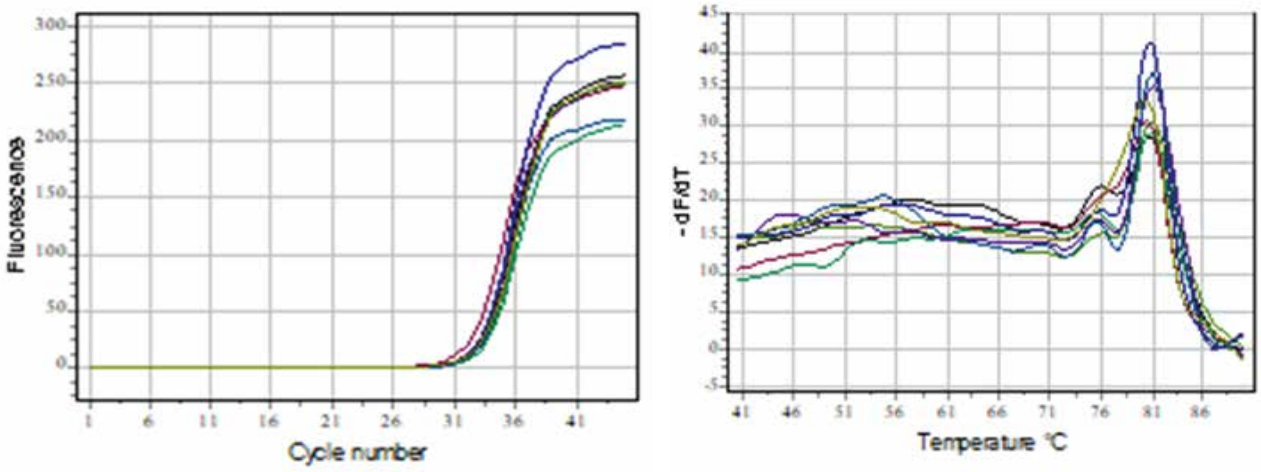

Figure VI: (A)qPCR Amplification Plot for SPIA gene; (B) Melting curve for product gene

Table 2: Amplification of qPCR for SPLA Gene in *Lactobacillus acidophilus*

splA	16SRNA	ΔCT	ΔΔCT	2-ΔΔCt	Folding -change	Gene expression	Control
33.4	25.5	7.9	0.35	0.784584098	0.784584098	5.202414066	7.55
32.5	25.9	6.6	-0.95	1.931872658	1.931872658		
33.8	25.8	8	0.45	0.732042848	0.732042848		
33.5	25.8	7.7	0.15	0.901250463	0.901250463		
31.2	25.9	5.3	-2.25	4.75682846	4.75682846		
31	25.8	5.2	-2.35	5.098242509	5.098242509		
31.2	26.2	5	-2.55	5.856342784	5.856342784		
30.8	25.6	5.2	-2.35	5.098242509	5.098242509		

Discussion

Bacteroides fragilis is a normal colonic microbiota in humans. It has a collagenase enzyme encoded by col gene to penetrate the collagen layer in the intestine. The analysis results revealed the significantly shift in RT-qPCR in the transcription of the collagenase gene in the *fold-change* range between 1.047 and 7.29, with upregulation of gene expression 1.01 compared with the control 6.66. The nearly sixfold upregulation in the collagen gene, encoding collagenase, refers to more metabolic activity of gene expression toward collagen in intestinal tissue. Therefore, in this study, a sharp increase in collagenase enzyme during the pinworm infection was reported, which acts as an inducer of *Enterobius vermicularis* ova.

The amplification of the housekeeping gene 16srRNA was recorded. A qPCR Amplification plot for six bacterial isolates to detect gene expression of the collagen gene and a melting curve for the collagen gene product without any dimer, and all samples have the same gene.

A collagenase enzyme secreted by *Bacteroides fragilis* on collagen agar has been detected. The upregulation of the splA gene to 5.202414066 with control 7.55 has been recorded. *Lactobacillus acidophilus* has a specific gene interact with the mucosa called surface layer protein A, which interacts with intestine host and *Enterobius vermicularis* to provide a protective function. The *splA* gene, which encodes upregulation of the surface protein.

Enterobius vermicularis causes irritation and inflammation in the mucosa of the intestine, but the *splA* gene in *Lactobacillus acidophilus* is encoded to mediate binding to mucosa[13] by blocking all receptors on intestinal cells. Lactobacillus acidophilus attaches to intestinal cell surface proteins to enhance the host immune response[14].

In a study[13], it has been demonstrated a collagen adhesion of *Bacteroides fragilis* outer membrane and identified a collagen protein, these agree with this study six isolates of *Bacteroides fragilis* that have a collagen gene. *Enterobius vermicularis* causes irritation and inflammation in the mucosa of the intestine, but the *splA* gene in *Lactobacillus acidophilus* is encoded to mediate binding to mucosa[12] by upregulation and block all receptors on intestinal cells. *Lactobacillus acidophilus* attaches to intestinal cell surface proteins to enhance the host immune response[15]. *Lactobacillus acidophilus* attaches to the surface of intestinal cell proteins to enhance the host immune response[15].

This study agrees with Altermann et al[13] and has documented a carried gene, phospholipase, in *Lactobacillus acidophilus* and the role of probiotic defense in the gut. High upregulation of genes to survive motility. Hynonen and Palva[14] reported a protective barrier in intestinal mucosa function and the role of proteins and enzymes in *Lactobacillus acidophilus*. Accurate studies disagree with Reynolds et al[15], who suggested that infection with *Enterobius vermicularis* will decrease Lactobacillus acidophilus count, and disagree with these qPCR results[11]. Finally, the increased gene expression in the FpBA gene in contrast with the MUB and *SplA* genes in this study refers to the FpBA gene's use in adhesion and colonization, with an increase in carbohydrate metabolism to prevent *Enterobius vermicularis*.

Conclusion

An accurate study focuses on upregulation of the col gene, which encodes collagen protein in Bacteroides fraglis and increased upregulation splA gene in Lactobacillus acidophilus in patients with pinworm disease. Collagen degradation of intestine basement membrane by upregulation of collagenase enzymes and forming a molecular explanation and increased risk of secondary complication as a chronic inflammation in pinworm cases.

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Conflict of interest:
The authors declare that there is no conflict of interest regarding the publication of this study.

Financial Disclosure
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Authors' contributions
Baneen Ali Abdulhussein contribution in methodology, sample collection, and laboratory work; Zahrah Adnan Al-Shammarri: Supervision, Formal Analysis, Writing – Review & Editing. All the authors approved the final manuscript.

Data Availability
Any questions regarding the availability of the study's supporting data should be addressed to the corresponding author, who can provide it upon justifiable request.

Ethics Approval and Consent to Participate
Ethical approval for the study was obtained from the Institutional Review Board. As this was a prospective study the written informed consent was obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations.

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