

Original article

Dual profiling of *Fusicatenibacter saccharivorans* and *pks*⁺ *Escherichia coli* as stool biomarkers for colorectal cancer screening in a Thai cohort

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Abstract

Background: Fecal Immunochemical Test (FIT) is a standard non-invasive screening method for colorectal cancer (CRC) but exhibits limited sensitivity in detecting early-stage lesions, leading to delayed treatment. To improve early-stage CRC detection, it is essential to develop a non-invasive, widely applicable, and practical screening method that enhances the current FIT screening capabilities. Recent studies have shown that gut dysbiosis is associated with CRC development and therefore can serve as a source of fecal microbial biomarkers.

Objectives: To evaluate the stage-specific absolute and relative abundance profiles of candidate fecal microbial biomarkers along the colorectal adenoma-carcinoma sequence in a Thai cohort.

Methods: Stool samples were collected from three groups: healthy individuals, patients with high-risk adenomatous polyps, and early-stage CRC patients. Total DNA extraction and quantitative polymerase chain reaction (qPCR) were performed, followed by data analysis to identify fecal microbes that showed significant changes in patients with adenomatous polyps and colorectal cancer.

Results: Preliminary data revealed distinct, stage-specific quantitative alterations. The genotoxic pathobiont *pks*⁺ *Escherichia coli* demonstrated progressive enrichment, yielding a significant increase in the CRC group ($P < 0.05$). Conversely, the immunomodulatory symbiont *Fusicatenibacter saccharivorans* exhibited profound depletion upon malignant transformation ($P < 0.01$). Notably, pattern discrepancies between the absolute and relative abundance of *F. saccharivorans* underscored the impact of 16S rRNA gene copy number variations during background dysbiosis.

Conclusion: The dual profiling of these specific fecal microbial biomarkers, particularly through absolute quantification, suggests promising potential for enhancing non-invasive, early-stage CRC screening.

Keywords: colorectal cancer, fecal microbial biomarkers, *Fusicatenibacter saccharivorans*, gut dysbiosis, *pks*⁺ *Escherichia coli*, quantitative PCR (qPCR)

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Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related deaths worldwide.⁽¹⁾ According to statistics from the National Cancer Institute of Thailand, in 2022, CRC accounted for the highest number of newly diagnosed cancer cases, with a rising incidence notably observed among younger populations. CRC screening is recommended at age 45 years or older, as patient prognosis is closely linked to the timing and stage of diagnosis. Stage I CRC has a 5-year survival rate of up to 90%, while the survival rate in stage IV is reduced to less than 10%. Importantly, early-stage CRC often presents with non-specific or subtle symptoms^(2, 3), leading to delayed diagnoses.

Although colonoscopy remains the gold standard for CRC diagnosis⁽⁴⁾, its widespread implementation as a primary screening tool is limited by invasiveness, potential complications (e.g., colonic perforation and bleeding), demanding bowel preparation, and limited availability in primary care settings.⁽⁵⁾ Consequently, the Fecal Immunochemical Test (FIT) has been widely adopted due to its non-invasive nature and superior patient adherence. However, FIT has suboptimal sensitivity in detecting precancerous lesions and early-stage CRC, which restricts its efficacy in early intervention.⁽⁶⁻⁸⁾

Recent advances have highlighted the critical role of gut dysbiosis⁽⁹⁾, characterized by the depletion of beneficial microbes and the enrichment of pathogenic bacteria, in CRC tumorigenesis.⁽¹⁰⁾ Specific microbial species, including *Fusobacterium nucleatum*^(11, 12), *Parvimonas micra*⁽¹³⁾, and enterotoxigenic *Bacteroides fragilis* (ETBF)⁽¹⁴⁾, are significantly enriched in CRC patients. Emerging global evidence suggests that integrating these fecal microbial biomarkers with FIT can synergistically enhance diagnostic sensitivity.⁽¹³⁾ Concurrently, evaluating the depletion of protective gut commensals is equally crucial. Among the broader microbiome shifts, *Fusicatenibacter saccharivorans* is recognized as a short-chain fatty acid (SCFA)-producing resident of the human gut. While its exact dynamics vary across different contexts, its reduction serves as a notable indicator of structural dysbiosis and a loss of anti-inflammatory metabolic homeostasis.^(15, 16) Consequently, incorporating the depletion of this homeostasis-maintaining symbiont alongside enriched pathogenic biomarkers offers a more comprehensive framework for non-invasive early-stage screening.

Currently, research evaluating combined microbial panels and FIT within the Thai population remains strictly limited. Therefore, this study aims to identify a comprehensive panel of fecal microbial biomarkers tailored to Thai cohorts and evaluate its efficacy in combination with FIT to optimize early-stage CRC screening accuracy.

Materials and methods

Identification and selection of microbial biomarkers

Candidate microbial biomarkers were identified and selected based on a systematic review of literature reporting bacterial alterations in stool samples from colorectal cancer (CRC) and adenomatous polyp patients. The inclusion criteria required that the target species exhibited significant alterations in CRC patients compared to healthy controls, and that these microbial shifts were consistent across different geographic regions.

Stool sample collection

This study utilized leftover stool specimens from a previous study.⁽¹⁷⁾ The protocol was approved by the Institutional Review Board (IRB) of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (IRB No. 182/62).

Primer design and validation for species-specific biomarkers

Primers targeting specific regions of *F. saccharivorans*, *pks⁺ Escherichia coli*, and 16S rRNA gene were either sourced from published literature or newly designed. Nucleotide sequences were retrieved from the NCBI GenBank database and optimized using Primer-BLAST⁽¹⁸⁾ or Primer3 software to meet the following parameters: length of 18–25 base pairs, optimal annealing temperature of approximately 60°C, and minimal self-complementarity or hairpin formation.

Primer specificity was verified *in silico* via BLAST against the complete NCBI nucleotide database to eliminate cross-amplification with non-target bacteria or human host DNA. Empirical validation was subsequently conducted using quantitative PCR (qPCR) to determine amplification efficiency, linear dynamic range, and specificity through melting-curve analysis. The standard curves were constructed using serial dilutions of target DNA, and the accuracy of the amplified products was confirmed by Sanger sequencing.⁽¹⁹⁾

Table 1. Demographic and clinical data of the participants in this study.

Variable	Group			Total	P
	HC	AD	CRC		
No. of volunteers	21	31	17	69	
Age (Mean $\pm$ SD)	62.0 $\pm$ 4.7	66.4 $\pm$ 5.3	63.0 $\pm$ 6.3	64.2 $\pm$ 5.7	0.01
BMI (Mean $\pm$ SD)	23.1 $\pm$ 3.2	23.7 $\pm$ 3.6	21.8 $\pm$ 3.0	23.0 $\pm$ 3.4	0.16
Gender (n, (%))					
Male	5 (23.8)	14 (45.2)	6 (35.3)	25 (36.2)	0.232
Female	16 (76.2)	17 (54.8)	11 (64.7)	44 (63.8)	

Fecal occult blood testing

Qualitative FIT was performed using the OC-Light™ S FIT test strip (Eiken Chemical, Japan), while quantitative assessment was executed with the OC-Auto Sampling Bottle 3 analyzed on an OC-SENSOR io series analyzer (Eiken Chemical, Japan). Stool sampling was conducted by inserting the probe into multiple areas of the fecal specimen 5–6 times, prioritizing visible abnormalities such as mucus or blood. The probe was rotated to ensure adequate sample collection within the rod grooves, and subsequent processing followed the manufacturer's instructions.

DNA extraction from stool samples

Total genomic DNA was extracted from stool samples using the QIAamp PowerFecal Pro DNA Kit (Qiagen), following the manufacturer's protocol. DNA concentration and purity were evaluated utilizing a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), while genomic integrity was verified via 1.5% (w/v) agarose gel electrophoresis. All DNA samples were stored at -20°C until downstream analysis.

Preparation of standard DNA plasmids

Target-specific genes were amplified from bacterial genomic DNA templates using conventional PCR and cloned into the pGEM-T Easy Vector (Promega). The recombinant plasmids were transformed and propagated in *Escherichia coli* DH5 α . Plasmid extraction was performed using the QIAprep Spin Miniprep Kit (Qiagen), and DNA quality was assessed by measuring absorbance at 260 and 280 nm.

Quantitative real-time PCR (qPCR)

Target microbial biomarkers were quantified via SYBR Green real-time PCR using SsoAdvanced Universal SYBR Green Supermix (Bio-Rad) on a QuantStudio™ 5 Real-Time PCR System (Applied

Biosystems). Each reaction contained 200 nM of each primer and 5 μl of DNA template, subjected to 40 amplification cycles. As for absolute quantification, sample cycle threshold (C_t) values were compared to the prepared plasmid standard curves to determine absolute bacterial abundance. For relative quantification, C_t values were normalized against the universal bacterial 16S rRNA gene (representing total bacterial load) and analyzed using the $2^{-\Delta C_t}$ (Livak) method.

Results

A total of 69 participants were enrolled in this study, consisting of 25 males and 44 females. Among these, 17 patients were diagnosed with colorectal cancer (CRC group), 31 were diagnosed with colorectal polyps (AD group), and 21 participants served as healthy controls (HC group) with no abnormalities upon colonoscopy. No statistically significant differences were observed among the three groups regarding body mass index (BMI) ($P = 0.16$) and gender distribution ($P = 0.232$), indicating that these potential confounding baseline factors were well-balanced. However, a significant variation was detected in age distribution ($P = 0.01$), with the AD group exhibiting a significantly higher mean age compared to the HC and CRC groups (Table 1).

Prior to the quantification of microbial targets in clinical stool samples, the analytical performance, linearity, and amplification efficiency of the qPCR assays for *F. saccharivorans* and *pks*⁺ *E. coli* were thoroughly validated. Standard curves were constructed using 10-fold serial dilutions of plasmids harboring the respective target-specific functional genes. As demonstrated in the amplification plots, a consistent and progressive rightward shift in cycle threshold (C_t) values was observed across the dynamic range, reflecting proportional gene dilutions. The melting curve analysis for detection of both bacteria yielded a single, sharp peak with an absence

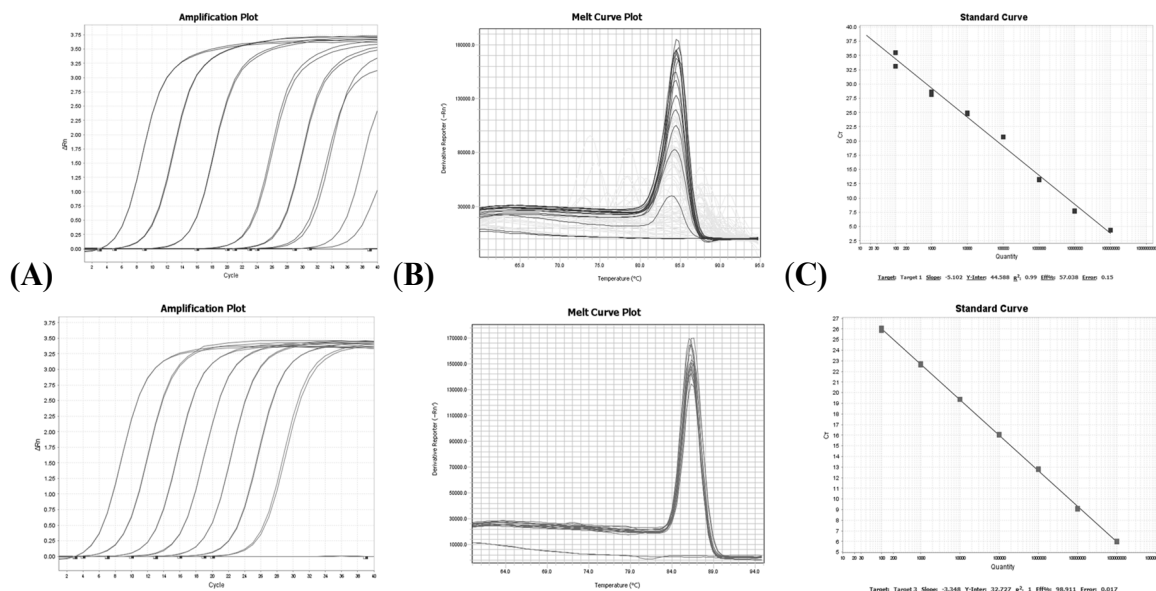


Figure 1. Quantitative PCR (qPCR) validation and standard curves for candidate fecal microbial biomarkers. The top panels represent *F. saccharivorans* and the bottom panels represent *pks⁺ E. coli*. For both assays, the analytical performance is displayed from left to right as follows: (A) amplification plots; (B) melt curve plots; and (C) linear regression standard curves.

of non-specific amplification or primer-dimer artifacts, confirming the high primer specificity for the target sequences (Figure 1). Furthermore, the generated standard curves exhibited excellent linearity. As for *F. saccharivorans*, the coefficient of determination (R^2) was 0.99, while the *pks⁺ E. coli* standard curve demonstrated an ideal value of 1.00. The amplification efficiencies derived from the slopes were within the optimal accepted biological range (90%–110%), establishing a robust and highly reproducible technical foundation for subsequent absolute abundance calculations.

To quantify the levels of each target bacterial species in the stool samples, both absolute and relative quantification methods were performed. To determine absolute abundance, standard curves were constructed using serial dilutions of recombinant plasmids containing target-specific functional genes. Relative quantitative analysis was performed and normalized to the universal 16S rRNA gene.

Regarding absolute quantification, the copy numbers of *F. saccharivorans* and *pks⁺ E. coli* showed different patterns according to clinical stage

(Figure 2). As for *F. saccharivorans*, there was no significant difference in absolute abundance between the HC and AD groups but a significant decrease was observed in the CRC cohort compared to the AD group ($P < 0.01$). Conversely, the absolute copy numbers of *pks⁺ E. coli* showed a progressive increase along the adenoma-carcinoma sequence. A statistically significant enrichment of *pks⁺ E. coli* was observed in the CRC group relative to the healthy baseline ($P < 0.05$).

Concurrently, relative quantitative profiling normalized to the universal 16S rRNA gene revealed contrasting dynamic shifts within the total gut community (Figure 3). As for *F. saccharivorans*, relative abundance was significantly enriched in the AD stage compared to healthy controls ($P < 0.01$), followed by a highly significant depletion in the CRC cohort relative to the AD group ($P < 0.001$). On the other hand, relative abundance of *pks⁺ E. coli* exhibited a significant initial elevation in the precancerous AD cohort compared to the HC group ($P < 0.05$), though its relative proportion plateaued with no statistically significant differences upon transition to the CRC stage.

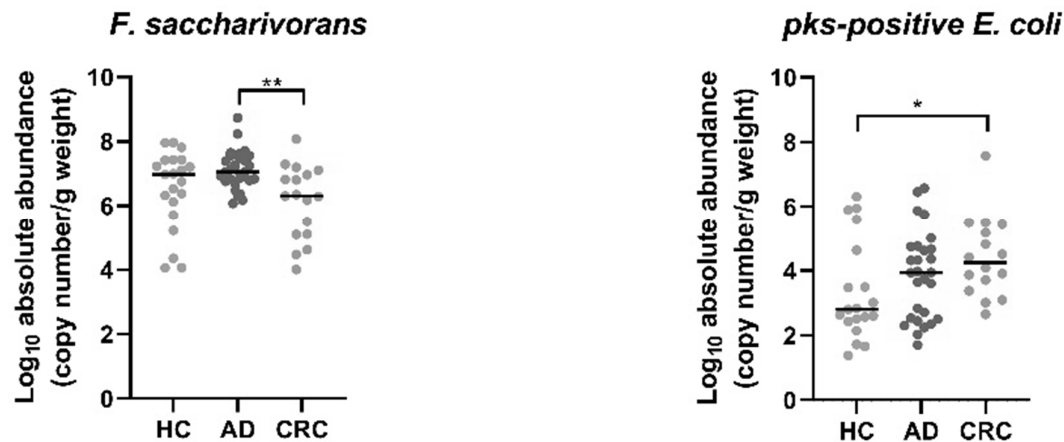


Figure 2. Absolute quantification of *F. saccharivorans* and *pks*⁺ *E. coli* in clinical samples across healthy controls (HC), adenomatous polyps (AD), and colorectal cancer (CRC) patients.

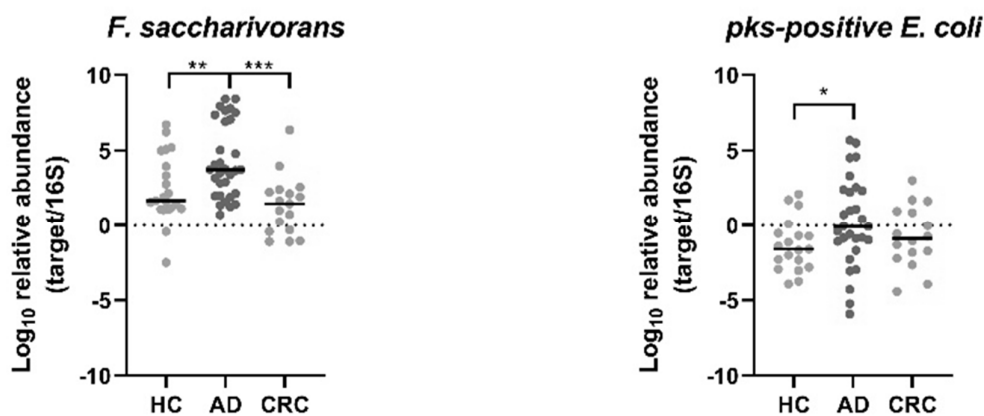


Figure 3. Relative quantification of *F. saccharivorans* and *pks*⁺ *E. coli* in the clinical samples across healthy controls (HC), adenomatous polyps (AD), and colorectal cancer (CRC) patients.

Discussion

This investigation highlights the distinct stage-specific alterations of *F. saccharivorans* and *pks*⁺ *E. coli* along the colorectal adenoma-carcinoma sequence within the Thai population. The primary finding of this study demonstrates that while the absolute and relative abundance of the beneficial symbiont *F. saccharivorans* undergoes a critical depletion during the transition to malignancy, the genotoxic pathobiont *pks*⁺ *E. coli* exhibits a progressive accumulation, peaking in the colorectal cancer (CRC) stage. Importantly, the significant enrichment of both markers, particularly the early relative increase of *pks*⁺ *E. coli* and *F. saccharivorans* in the adenomatous polyp (AD) group, underscores their potential as non-invasive screening indicators for detecting precancerous lesions, thereby offering a crucial window for early clinical intervention. ^(20, 21)

The contrasting abundance of *F. saccharivorans* and *pks*⁺ *E. coli* observed in this study aligns with current global literature regarding gut dysbiosis, where immunomodulatory commensals are displaced by genotoxic pathobionts. As an established SCFA producer, *F. saccharivorans* actively contributes to intestinal homeostasis by inducing the potent anti-inflammatory cytokine IL-10.⁽²²⁾ Recent epidemiological data suggest that this taxon exhibits context-dependent dynamics within complex microbial networks; while it functions as a protective SCFA producer under healthy conditions, its marked reduction is a documented hallmark of active ulcerative colitis and mucosal inflammation.^(22, 23) Although direct clinical evaluations of *F. saccharivorans* in colorectal cancer are still limited, its depletion in our CRC cohort aligns with this broader pathological framework. The distinct loss of this putative IL-10-inducing symbiont

within the evaluated Thai cohort highlights how a reduction in protective microbial regulators can breach the mucosal barrier and compromise local immune tolerance, thereby creating a highly permissive environment for advanced colorectal neoplasia. Conversely, the progressive enrichment of *pks*⁺ *E. coli* underscores the active acquisition of pro-tumorigenic factors within the inflammatory niche. The *pks* pathogenicity island encodes the biosynthesis of colibactin, a distinct genotoxin capable of inducing double-strand DNA breaks and genomic instability in host epithelial cells.^(24,25) The enrichment of colibactin-producing pathobiont combined with an increase in genotoxic threats, can breach the mucosal barrier and compromise local immune tolerance, thereby creating a highly permissive environment for advanced colorectal neoplasia. The epidemiological consensus between this Thai cohort and international data suggests that despite regional dietary and lifestyle variations, these specific microbial shifts remain universally predictable biomarkers.^(26,27)

Comparing absolute and relative quantification data provides crucial insights into changes in bacterial load. As for *pks*⁺ *E. coli*, the relative proportion closely aligned with the absolute copy numbers, confirming a real increase of this pathogen. In contrast, *F. saccharivorans* showed conflicting results between the two methods: its relative abundance increased significantly in the AD stage and then dropped sharply in CRC, whereas its absolute load remained stable before decreasing. This divergence can be mechanistically justified by 16S rRNA gene copy number variations and background community shifts.⁽²⁸⁻³⁰⁾ During gut dysbiosis in the CRC stage, the massive expansion of other high-copy-number opportunistic bacteria can disproportionately skew the universal 16S rRNA baseline. Consequently, this shifting reference baseline mathematically dilutes the relative ratio of *F. saccharivorans*, even when its absolute cell count per gram of stool remains stable. This finding reaffirms that incorporating absolute quantification alongside relative profiling is essential for accurate, unbiased biomarker interpretation.

Despite these promising insights, several limitations warrant acknowledgment. First, modest sample size restricts the generalizability of microbial trends and reduces statistical power for sub-group comparisons, particularly high-risk adenomas and early-stage CRC. Large-scale, multi-center prospective cohorts are needed to validate the diagnostic robustness of *F. saccharivorans* and *pks*⁺ *E. coli*. Additionally, further optimization is needed to integrate these target-

specific absolute loads into clinical diagnostic frameworks. Nevertheless, this study establishes a foundation for utilizing specific fecal microbial shifts to complement early-stage colorectal cancer screening in the Thai population.

Conclusion

In conclusion, this study demonstrates that *F. saccharivorans* and *pks*⁺ *E. coli* undergo distinct, stage-specific shifts along the colorectal adenoma-carcinoma sequence in the evaluated Thai population. While the immunomodulatory symbiont *F. saccharivorans* is depleted upon malignant transformation, the genotoxic pathobiont *pks*⁺ *E. coli* exhibits progressive enrichment in the colorectal cancer stage. The discrepancies between absolute and relative data underscore how 16S rRNA gene copy number variations and background dysbiosis affect relative calculations. Overall, these findings suggest that the dual profiling of these specific fecal microbial shifts may have potential as complementary biomarkers for non-invasive colorectal cancer screening and warrant further validation in larger cohorts.

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Conflicts of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data sharing statement

Statistical summaries are presented in the published article. The data sets generated or analyzed during the present study are available from the corresponding author on reasonable request.

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