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Molecular Approach for Screening and Identification of Food Containments using Colony PCR

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Abstract

Food is the primary cause for diseases in humans and carries high risk pathogens. Assessment of the safety in foods is needed to validate the presence of pathogenic bacteria. We used colony PCR for this approach to detect foodborne pathogens such as *Escherichia coli*, *Lactobacillus* and *Bacillus cereus*. Suitable primers were selected based on specific gene 1040 for *Escherichia coli*, gene S2 for *Lactobacillus*, and gene NVF for *Bacillus cereus*. Agarose gel electrophoresis is used for the detection of amplified products against a suitable marker. ImageJ is used for DNA band analysis, enabling precise quantification, normalization, and statistical comparisons. These studies have established a promising role in the detection of pathogens in various environmental samples. The insights gained from this study may serve as the foundation for rapid detection of foodborne diseases in the food industry.

Keywords: Colony PCR, Primers, *Escherichia coli*, *Lactobacillus*, *Bacillus cereus*

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INTRODUCTION

Food technologists are currently showing their interest in molecular methods as a powerful tool for identifying and detecting contaminants in food specimens.[1] Colony PCR Analysis is one of the widely used techniques that utilizes the principles of polymerase chain reaction (PCR) to observe contaminants at the molecular level.[2,3] Powerful screening techniques are necessary as demand for the safety and integrity of food products has shot up due to the expansion of the food business globally.[4] Colony PCR is cost-effective for detecting bacterial contamination because it directly amplifies DNA from colonies, avoiding expensive steps like DNA extraction and purification required in traditional methods. This reduces overall expenses while maintaining accuracy in identifying contaminants.[5] One of the main important concerns for food manufacturers, agencies, and customers is quality and safety.[6] Bacteria, fungi, viruses, toxins, and chemical residues pose hazardous health risks and economic implications.[7] Conventional detection methods consume a lot of time and as well as money with hard labor work, which may lack sensitivity, specificity, or the capacity to effectively detect emerging pathogens.[8,9] Furthermore, these techniques may not support rapid detection, identification, and delay in responses which can increase risk factors in public health.[10] In response to these challenges, molecular techniques have transformed food safety by offering sensitive, specific, and rapid means of detecting and identifying contaminants.[11] Analysis by colony PCR in food safety involves a broad spectrum of contaminants and food pathogens such as *Salmonella*, *Escherichia coli*, *Listeria*, *Lactobacillus, Bacillus cereus* and *Staphylococcus* due to their prevalence in foodborne infections.[12,13] Fungal contaminants also exhibit health effects such as *Aspergillus*, *Penicillium*, and *Fusarium* species, which produce mycotoxins, are also investigated.[14,15] Colony PCR offers rapid, sensitive and diverse solutions for detecting and identifying various contaminants in foods.[16,17] With the advent increase in use of molecular approach methods in the field of molecular biology and recombinant technology, screening of contaminants in colony is possible with the use of primers in bacterial culture.[18] This method offers quick identification of pathogens and the results are promising among the other methods in sensitivity and specificity.[19]

MATERIALS AND METHODS

Preparation of food samples

Food samples were collected from the canteen and supermarkets of the KL University campus, Andhra Pradesh, India. The food specimens were collected in 5 ml screw cap tubes. Sample is collected carefully and sealed with parafilm until it is spread over agar plates. The list of food samples is shown in Table 1.

Identification of food contaminants

Samples were collected in vials, and double-distilled water was added to dilute them. Following dilution, the samples were streaked onto LB agar plates, which were then incubated for 24 hours. After incubation, bacterial growth was observed, and colonies were collected into PCR tubes, to which master mix was added.[20] Bacterial identification is accomplished using primers designed for specific genes at specific temperatures 56°C for *Escherichia coli*, 55.4°C for *Lactobacillus* and 54°C for *Bacillus cereus* which facilitate DNA amplification.

	Forward Primer	Backward Primer
E. coli	AACTGCAGATGCCCAAATCCAACGATG	GGTACCTCAATCGTGATTTACTGAGAGA
Lactobacillus	ATGATTGATCTAACGAGTCGATTG	TTATTTGGCGGTGTAGGTGG
B. cereus	CGGCGGCAACTACGAGAC	CCGGTGATGCTGTCGCTCTCC

Colony PCR process

PCR Cocktail- 2X Master mix- 5 µl Forward Primer- 0.5 µl Backward Primer- 0.5 Nuclease Free Water- 4. PCR Program- Initial Denaturation (94° for 5 min), Final Denaturation (94° for 1 min), Annealing Temperature (55.4°, 54°, 56° for),

Extension (72° for 1 min), Final Extension (72° for 5 min). The PCR was performed for 35 cycles.

Characterization of contaminant by AGE

The samples after PCR were taken out and loaded into 1% agarose gel after the electrophoresis the gel was observed under gel doc.[21]

RESULTS AND DISCUSSION

The assessment of specific bacterial strains in food samples was conducted by observing bands in gel electrophoresis. The presence of bands serves as an indicator of successful primer annealing to bacterial genomic DNA, resulting in amplification. This process allows for the identification of specific bacteria targeted by each primer set. Conversely, the

Table 1. List of food samples collected from various sources

1-Chicken	2-Biryani	3-Noodles	4-Pongal	5-Tamrid Rice
6-Jaggery	7-Corn Flakes	8- White Bread	9-Palak Curry	10-Corn
11-Biscuit Cream	12-Kaju Katle	13-Canta Loupe	14-Pineapple	15-Mousambi
16-Raw Milk	17- Muskmelon	18-Sapodilla	19-Butter	20-Brinjal Curry
21-Potato Curry	22-Capsicum Curry	23-Tomato	24-Ivy Gourd	25-Sweet Neem Leaf
26-Carrot Curry				

Table 2. The dataset comprises information regarding food samples and their corresponding band areas

Sample No.	*E. Coli*	*Lacto-bacillus*	*Bacillus cereus*
1	166	0	0
2	0	0	0
3	560	260	336
4	0	0	0
5	0	0	0
6	0	0	0
7	138	112	177
8	309	235	194
9	543	280	225
10	0	0	0
11	58	166	84
12	248	120	208
13	228	0	0
14	151	244	186
15	133	92	160
16	0	0	0
17	78	396	0
18	0	128	216
19	1123	336	898
20	164	0	0
21	0	0	0
22	0	136	247
23	130	79	210
24	0	0	0
25	199	0	0
26	64	112	70

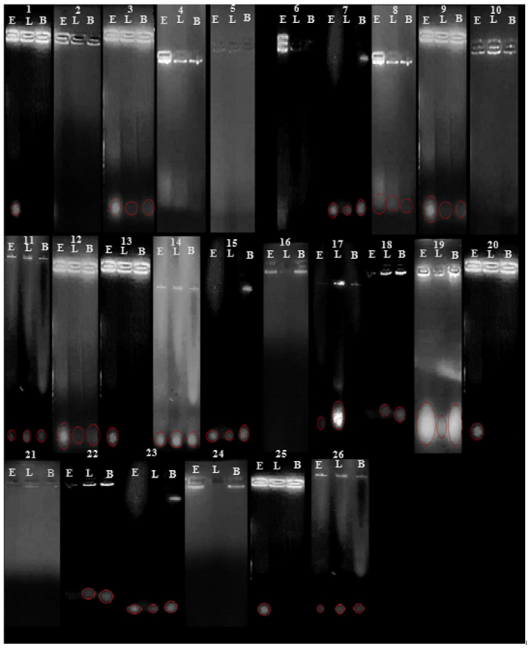

Figure 1. Amplicon bands were observed for 26 contaminated food samples. (Chicken Curry, Biryani, Noodles, Pongal, Tamrid Rice, Jaggery, Corn Flakes, Bread, Palak Curry, Sweet Corn, Cream Biscuit, Kaju Katle, Canta Loupe, Pineapple, Mousambi, Raw Milk, Muskmelon, Sapodilla, Butter, Brinjal Curry, Potato Curry, Capsicum Curry, Tomato, Ivy Gourd, Sweet Neem Leaf, Carrot Curry). Here E- *Escherichia coli*, L- *Lactobacillus*, B- *Bacillus cereus*

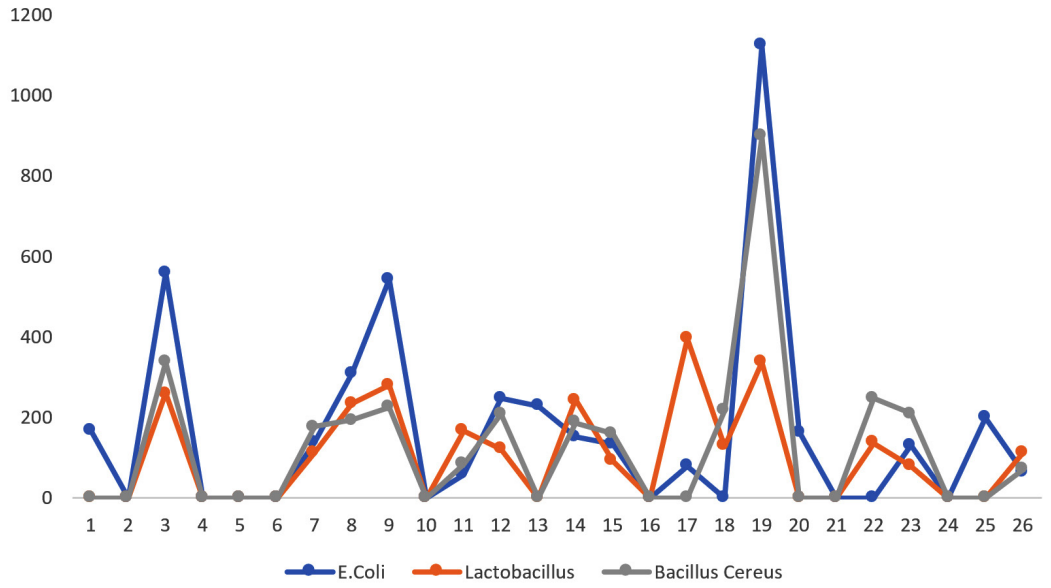

Figure 2. Graphical representation of food samples with bacteria vs area of the bands obtained. Blue line represents *E. coli*, orange line represents *Lactobacillus* and grey line represents *Bacillus cereus*

absence of bands in certain gels suggests the non-existence of the targeted bacteria in those food samples. This absence might be attributed to the presence of other bacterial strains not covered by the three different primers designed for the specific bacteria. The gel electrophoresis results are visually represented in Figure 1. For quantitative analysis of the amplified bands, ImageJ software was utilized to measure the band areas, and the corresponding values are presented in Table 2. A graphical representation of the relationship between food sample and the area of the bands is shown in Figure 2. This graphical analysis offers a comprehensive insight into the bacterial composition across various food samples, emphasizing differences in band areas and facilitating the identification of specific bacterial strains. The graph indicates that *E. coli* exhibits higher intensity compared to *Lactobacillus* and *Bacillus cereus*. *Bacillus cereus* demonstrates the lowest intensity among the three bacterial strains.

CONCLUSION

In this research endeavour, colony PCR was conducted on a total of 26 food samples suspected of contamination. The DNA extraction and amplification process were conducted using PCR for 35 cycles. To verify the presence of PCR amplicons, 12 µl of the PCR product was loaded onto a 1% agarose gel. This investigation introduces a rapid method for detecting contaminants in food samples. However, for enhanced accuracy and reliability, it is advisable to optimize further and validate the colony PCR technique, particularly when dealing with larger sample sets. Additional efforts towards optimization and validation can contribute to the robustness and effectiveness of the colony PCR method in identifying contaminants, thereby strengthening its utility in food safety and quality assurance protocols.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not Applicable.

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