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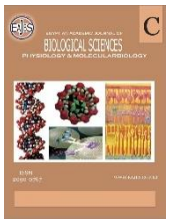
Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 16(2) pp105-112 (2024)*
DOI: 10.21608/EAJBSC.2024.374312

Egypt. Acad. J. Biolog. Sci., 16(2):105-112 (2024)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



Validation and Application of FBPP: Software to Design Foodborne Pathogen PCR Primers And Probes

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ARTICLE INFO

Article History
Received:13/7/2024
Accepted:16/8/2024
Available:20/8/2024

Keywords:
PCR primers and probes design; PCR validation; foodborne pathogen and nucleic acid base detection method.

ABSTRACT

The nucleic acid base method is a widely used method in foodborne pathogen detection as a rapid method and confirmation of traditional methods. This method is simply hybridizing the target region sequence of a foodborne pathogen with a synthetic oligonucleotide (probes or primers), and the prediction of this primer or probe is a critical step that needs more care. Hence Proper validation of the application used for this prediction is important. In this paper, we validate FBPP application by the evaluation of the predicted primers takes place at the three levels: genus level by using the Inv A gene for all *salmonella* species; species level by using the HipO gene for only *Campylobacter jejuni* species; and serotype level through the O antigen flippase gene for *Escherichia coli O157*:*H7*. It includes measures of inclusivity (detection of the target microorganism) and exclusivity (non-detection of non-target microorganisms) as recommended for the performance characteristics of the PCR-based method (ISO 22118). Furthermore, the sequence of the PCR product has been verified to confirm the correct PCR product has been amplified. The results demonstrate the ability of FBPP to create useful primers for foodborne pathogens under the three levels of evaluation.

INTRODUCTION

Foodborne diseases are an important cause of morbidity and mortality, and a significant impediment to socioeconomic development worldwide (Organization, 2017). One strategy to lower the incidence and the costs of food borne illnesses is the detection of foodborne pathogens to provide safe food supply and to prevent foodborne diseases.

The nucleic acid base method is one of the most widely used method in food applications. It is considered a rapid method used in the detection of foodborne pathogens; moreover, recently it has been part of the conformation test of conventional methods, for instance, ISO method for *Bacillus cereus* group (ISO 7932 :2004/Amd1:2020) (Standardization, 2020) and *Vibrio parahaemolyticus* (ISO 21872-1:2017) (Standardization, 2017). it depends on the hybridizing of the target nucleic acid sequence with a synthetic oligonucleotide (probes or primers) that is complementary to the target sequence (Zhao, Lin, Wang, & Oh, 2014).

The process of predicting Primers and Probes Design is essentially important in this method, especially for detection purposes, and it is a preliminary and critical step (Soliman, Azab, Hussein, Roushdy, & Abu el-naga, 2024). This task can be challenging because it requires the identification of highly unique conserved regions of target sequences and the ability to display highly sensitive (i.e., able to amplify its intended target), and specific (i.e., able not to amplify any non-target) (Sachse & Frey, 2003). In addition to computing the biological parameters of the primers, like GC content, melting temperature, and the formation of secondary structures, which include self-dimers, hairpins, and cross-dimers, they are essential for evaluating the efficient amplification of a target sequence (Yuryev, 2008).

There are a number of public software tools available to predict primers / probe design (Abd-Elsalam, 2003). However, these tools often present limitations in their capabilities to define the target region. Also, the users need to evaluate the primer specificity by using additional tools that are not sensitive enough to detect targets that have a significant number of mismatches and consequently not suitable to apply in the nucleic acid detection method. There are several simulation software programs that do not have the ability of primers design but rather determine the amplification targets of user-supplied primer pairs (Ye *et al.*, 2012), and some of them are not easily usable without skill in bioinformatics(Sobhy & Colson, 2012).

FBPP, an open-source Python-based application, could: (i) design primers /probes; (ii) perform PCR and gel electrophoreses Photo simulation. (iii) checks the specificity of primers / probes, to overcome many of the earlier limitations (Soliman *et al.*, 2024). However, it requires careful validation and evaluation before it can be adopted as a primer / probe design tool.

The aim of this paper is to validate and apply the FBPP application as a useful tool for predicting primers and probes in foodborne pathogen nucleic acid base detection methods.

MATERIALS AND METHODS

1-Design of Specific Oligonucleotide Primer: Three primers were designed using the FBPP application (Soliman *et al.*, 2024), and the common published primer (16s rRNA) (Lane, 1991) were synthesized by Invitrogen (Thermo Fisher Scientific), then reconstituted as the below equation to prepare master stock 100µM.

$$100\mu M = X\ nmoles\ lyophilized\ primer + (X \times 10\ \mu l\ molecular\ grade\ H2O)$$

Primers were designed for *Salmonella* species (spp.) based on the InvA gene (Sachse & Frey, 2003), *Campylobacter jejuni* based on the hipO gene (Wu, Hu, & Li, 2022), *Escherichia coli O157*:*H7* based on the O antigen Flippase (Liu *et al.*, 2020), and the 16s rRNA gene. The primer sequences for each gene are illustrated in Table 1.

Table 1: The predicted primer used in validation.

Primer Name	Sequence 5`–3`	Bacterial species	Target gene description	Gene ID	Amplicon Size
InvA F InvA R	CTT GAT TGA AGC CGA TGC CG TCA TCG CAC CGT CAA AGG AA	*Salmonella Sp.*	Invasion protein	1254419	305
hipO F hipO R	GAG GGT TTG GGT GGT GCT AA AGA AGC CAT CAT CGC ACC TT	Campylobacter *jejuni*	HipO	905276	141
O antigen F O antigen R	CAG TCT TGG TGC TGC TCT GA CCA TGT TGC CAG AGT GCC TA	*E. Coli O157*	O antigen flippase	912601	598
16S rRNA F 16S rRNA R	AGA GTT TGA TCM TGG CTC AG CGG TTA CCT TGT TAC GAC TT	All	16S ribosomal RNA	-	~1500

2-Theoretical Evaluation of the Design primer: The three primers predicted by FBPP were evaluated by performing a sequence similarity search against the NCBI GenBank database.

3-Bacterial Strains: 45 bacterial strains bought from Microbiologics (KWIK-STIK), and the Microbiological Resources Centre (Cairo MIRCEN) (slant) and collected from King Abdulaziz University, Al Azhar university, and Egypt Atomic Energy Authority listed in Table (2) were used for the validation of the program. The KWIK-STIK strains are lyophilized microorganism pellets with ampoules of hydrating fluid and inoculating swabs. By cracking ampoules, the fluid flows into a lyophilized bacterial pellet, where the specific bacterial strain is dissolved and ready for transfer to the appropriate agar medium and incubation at a suitable temperature and time (Table 2).

Table 2: List of Reference strains used for the validation of program.

No.	Strain	Source	Media / Atmosphere	Time/ temp. incubation	No.	Strain	Source	Media	Time/ temp. incubation
1	*Clostridium perfringens ATCC 3624*	Microbiologics	Blood Agar / Anaerobic	35°C / 24 H	24	*Staphylococcus aureus subsp. aureus ATCC 12600*	KAU*	Plate Count Agar / Aerobic	35°C / 48 H
2	*Enterococcus fuecalis ATCC 29212*	Microbiologics	Plate Count Agar / Aerobic	35°C / 24 H	25	*Escherichia coli ATCC 15597*	KAU*	Plate Count Agar / Aerobic	35°C / 24 H
3	*Escherichia coli (serotype O157:H7) ATCC 43888*	Microbiologics	Plate Count Agar / Aerobic	35°C / 24 H	26	*Vibrio parahaemolyticus ATCC 17802*	KAU*	Tryptic Soy Agar/ Aerobic	35°C / 48 H
4	*Escherichia coli ATCC 35218*	Microbiologics	Plate Count Agar / Aerobic	35°C / 24 H	27	*Bacillus cereus ATCC 14579*	KAU*	Plate Count Agar / Aerobic	35°C / 24 H
5	*Staphylococcus aureus subsp. aureus ATCC 25923*	Microbiologics	Plate Count Agar / Aerobic	35°C / 48 H	28	*Salmonella enterica ATCC 51741*	KAU*	Plate Count Agar / Aerobic	35°C / 24 H
6	*Bacillus cereus ATCC 10876*	Microbiologics	Plate Count Agar / Aerobic	35°C / 24 H	29	*Campylobacter jejuni NCTC 11322*	KAU*	Chocolate Agar / Microaerophilic	35°C / 72 H
7	*Salmonella enterica subsp. enterica serovar Enteritidis ATCC 13076*	Microbiologics	Plate Count Agar / Aerobic	35°C / 24 H	30	*Listeria monocytogenes ATCC 19115*	KAU*	Plate Count Agar / Aerobic	35°C / 48 H
8	*Listeria monocytogenes (serotype 1) ATCC 19111*	Microbiologics	Plate Count Agar / Aerobic	35°C / 48 H	31	*Escherichia coli (serotype O157:H7) ATCC 51659*	MIRCEN	Plate Count Agar / Aerobic	35°C / 24 H
9	*Cronobacter sakazakii ATCC 29544*	Microbiologics	Plate Count Agar / Aerobic	35°C / 48 H	32	*Campylobacter jejuni subsp. jejuni ATCC 33560*	MIRCEN	Chocolate Agar / Microaerophilic	35°C / 72 H
10	*Campylobacter jejuni subsp. jejuni ATCC 29428*	Microbiologics	Chocolate Agar / Microaerophilic	35°C / 72 H	33	*Staphylococcus aureus ATCC 6538*	AZU**	Plate Count Agar / Aerobic	35°C / 48 H
11	*Listeria monocytogenes (serotype 4b) ATCC 19115*	Microbiologics	Plate Count Agar / Aerobic	35°C / 48 H	34	*Bacillus subtilis ATCC 6633*	AZU**	Plate Count Agar / Aerobic	35°C / 24 H
12	*Clostridium perfringens ATCC 13124*	Microbiologics	Blood Agar / Anaerobic	35°C / 24 H	35	*Escherichia coli ATCC 8739*	AZU**	Plate Count Agar / Aerobic	35°C / 24 H
13	*Staphylococcus aureus subsp. aureus ATCC 11632*	Microbiologics	Plate Count Agar / Aerobic	35°C / 48 H	36	*Pseudomonas aeruginosa ATCC 9022*	AZU**	Plate Count Agar / Aerobic	35°C / 48 H
14	*Escherichia coli ATCC 33876*	Microbiologics	Plate Count Agar / Aerobic	35°C / 24 H	37	*Bacillus dimenuta ATCC.19146*	AZU**	Plate Count Agar / Aerobic	35°C / 48 H
15	*Vibrio parahaemolyticus ATCC 10885*	Microbiologics	Tryptic Soy Agar/ Aerobic	35°C / 48 H	38	*Pseudomonas fluorescens ATCC 25289*	EAEA***	Plate Count Agar / Aerobic	35°C / 48 H
16	*Bacillus cereus ATCC 11778*	Microbiologics	Plate Count Agar / Aerobic	35°C / 24 H	39	*Lactobacillus acidophilus ATCC 4356*	EAEA***	Plate Count Agar / Aerobic	35°C / 48 H
17	*Salmonella enterica subsp. enterica serovar Enteritidis ATCC 51741*	Microbiologics	Plate Count Agar / Aerobic	35°C / 24 H	40	*Streptococcus thermophiles ATCC 19987*	EAEA***	Plate Count Agar / Aerobic	35°C / 48 H
18	*Campylobacter coli ATCC 33559*	Microbiologics	Chocolate Agar / Microaerophilic	35°C / 72 H	41	*klebsiella pneumoniae*	EAEA***	Plate Count Agar / Aerobic	35°C / 48 H
19	*Listeria monocytogenes ATCC 13932*	Microbiologics	Plate Count Agar / Aerobic	35°C / 48 H	42	*Salmonella typhi ATCC 14028*	EAEA***	Plate Count Agar / Aerobic	35°C / 24 H
20	*Pseudomonas aeruginosa ATCC 27853*	Microbiologics	Plate Count Agar / Aerobic	35°C / 48 H	43	*Lactobacillus plantarum*	EAEA***	Plate Count Agar / Aerobic	35°C / 24 H
21	*Enterococcus fuecalis ATCC 13280*	Microbiologics	Plate Count Agar / Aerobic	35°C / 24 H	44	*Lactobacillus casei ATCC 334*	EAEA***	Plate Count Agar / Aerobic	35°C / 24 H
22	*Campylobacter jejuni subsp. jejuni ATCC 33291*	Microbiologics	Chocolate Agar / Microaerophilic	35°C / 72 H	45	*Lactiplantibacillus plantarum strain MOEZ1*	EAEA***	Plate Count Agar / Aerobic	35°C / 48 H
23	*Clostridium perfringens ATCC 3624*	KAU*	Blood Agar / Anaerobic	35°C / 24 H					

4-DNA Extraction and PCR Amplification:

4.1-DNA Extraction: By using loop 10 µl, a colony from bacteria strain plates were picked up and resuspended in 500 µl of Saline Solution (0.85%) in 1.5 mL microcentrifuge tube, then centrifugated for 3 min at 10000 X g. Discard the supernatant.

Total bacterial genomic DNA was extracted by means of the Thermo GeneJET Genomic DNA Purification Kit (ThermoScientific). According to the manufacturer's instructions. The purified DNA was detected by electrophoresis in 1% agarose gel and visualized by ethidium bromide (EB) with the quantitative GeneRuler 1 kb DNA Ladder (ThermoScientific) under a UV transilluminator, and concentration was measured by using a Qubit 4 fluorometer (Invitrogen), then stored at -20 °C until further use.

4.2-Primer and PCR Reaction Optimization*:* Primer, template concentration, and annealing temperature were determined by applying different primer and template volumes, various annealing temperatures and SYBR™ Green PCR Master Mix (Applied Biosystems™) (Table 3) to PCR plate and placing it in QuantStudio™5 (Applied Biosystems™). Then compile the result for ΔRn and Ct.

Table 3: List of PCR components concentration with different annealing Temperature.

Template Concentration (ng of gDNA)	Annealing Temp. ˚C	Reverse primer (nM)	Forward primer (nM)			
			300	500	800	1000
20	55.8	300	300/300	500/300	800/300	1000/300
		500	300 / 500	500 / 500	800 / 500	1000 / 500
		800	300 / 800	500 / 800	800 / 800	1000 / 800
	57.4	300	300/300	500/300	800/300	1000/300
		500	300 / 500	500 / 500	800 / 500	1000 / 500
		800	300 / 800	500 / 800	800 / 800	1000 / 800
	58.8	300	300/300	500/300	800/300	1000/300
		500	300 / 500	500 / 500	800 / 500	1000 / 500
		800	300 / 800	500 / 800	800 / 800	1000 / 800
50	55.8	300	300/300	500/300	800/300	1000/300
		500	300 / 500	500 / 500	800 / 500	1000 / 500
		800	300 / 800	500 / 800	800 / 800	1000 / 800
	57.4	300	300/300	500/300	800/300	1000/300
		500	300 / 500	500 / 500	800 / 500	1000 / 500
		800	300 / 800	500 / 800	800 / 800	1000 / 800
	58.8	300	300/300	500/300	800/300	1000/300
		500	300 / 500	500 / 500	800 / 500	1000 / 500
		800	300 / 800	500 / 800	800 / 800	1000 / 800
80	55.8	300	300/300	500/300	800/300	1000/300
		500	300 / 500	500 / 500	800 / 500	1000 / 500
		800	300 / 800	500 / 800	800 / 800	1000 / 800
	57.4	300	300/300	500/300	800/300	1000/300
		500	300 / 500	500 / 500	800 / 500	1000 / 500
		800	300 / 800	500 / 800	800 / 800	1000 / 800
	58.8	300	300/300	500/300	800/300	1000/300
		500	300 / 500	500 / 500	800 / 500	1000 / 500
		800	300 / 800	500 / 800	800 / 800	1000 / 800

4.3-PCR Reaction*:*

PCR was performed using a thermal cycler (SimpliAmp "Applied Biosystems™"). The genomic DNA from 45 strains representing major foodborne pathogens and closely related strains (Table 2) was used as a templet with four different primers (Table 1) for validation. The PCR reaction mixture performed in 50µL consisted of 5µL 10X Dream Taq Green Buffer (ThermoScientific), 5µL dNTP Mix (2mM), 1µL of MgCl2, optimized value of each primer set (10µM), optimize volume of template DNA, Dream Taq DNA Polymerase (1.25U), and water nuclease-free (filled to a final volume of 50µL).

The PCR program was performed with the following parameters: initial denaturation step at 95°C for 3 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing for 30 s at different grading temperatures according to primers (table 1) , extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The PCR products were purified using the Thermo Scientific GeneJET PCR Purification Kit, quantified by electrophoresis in different concentration s of agarose gel along with the expected amplicon yield, and visualized by ethidium bromide (EB) with the quantitative GeneRuler 100 bp DNA Ladder (ThermoScientific) under a UV transilluminator.

5-DNA Sequencing and Alignment:

Amplicons from the expect band were purified using GeneJET Gel Extraction Kit, and sequencing was performed at Bioneer Company using automated sequence analyzer ABI 3730xl. In order to validate the gene from predicted primers, sequences were

input as a query against the NCBI databases and showed similarity by suing clusatlX.

RESULTS AND DISCUSSION

The DNA from all 45 strains was extracted and examined by electrophoresis (Fig. 1.A). high-molecular -sharp bands with a minimum of fuzzy were noted for all strains. Moreover, the concentration of the extracted DNA was determined. The extracted gDNA was used as a template to evaluate the predicted primers designed by FBPP software.

The three predicted primers designed by FBPP were evaluated theoretically by performing a sequence similarity search against the NCBI GenBank database (Primer-BLAST), and the result confirmed that the complete identity of each primer only matches the expected sequence of the target organism.

The PCR conditions were optimized by establishing the primer, template concentration, and annealing temperature for the three predicted primers, as shown in Table 3. Then, the minimum forward and reverse primer concentrations that yielded the maximum ΔRn values and low Ct values were selected. The optimum conditions for the three primers InvA, hipO, and O antigen were 57.4°C for the annealing temperature, 50 ng of gDNA for the template per reaction and 500/500, 500/800, and 500/500 nM for the forward/reverse primer concentrations, respectively.Four PCR experiments were performed using the optimized conditions for each primer to validate the design. Each primer was evaluated against the 45 extracted DNA templates, which contain sequence complementary to target primers and DNA from closely related species and other foodborne pathogen species as non-target templates.

The PCR experiments showed that the 16s rRNA primers gave a clear band with all strains (Fig. 1.B); four bands were noted from the InvA primer with four different salmonella species (Salmonella enteritidis ATCC 13076, *Salmonella enteritidis ATCC 51741*, *Salmonella enterica ATCC 35664*, and *Salmonella typhi ATCC* 14028) (Fig. 1.C) ; four bands from the hipO primer with four different stains of *Campylobacter jejuni (ATCC 29428, NCTC 11322, ATCC 33560, and ATCC 33291*) (Fig. 1.D); and two bands from the O antigen Flippase primer with *E. coli O157 (ATCC 43888 and ATCC 51659)* (Fig. 1.E) .

In a way to verify the PCR amplicon, one band from each primer was purified and sequenced. The sequence was evaluated by performing a sequence similarity search against the NCBI GenBank database (BLAST), and the results showed a high similarity of each amplicon with the desired organism; moreover, the sequence similarity with the target gene was determined by a cluster tool and highly showed similarity with the desired gene.

The nucleic acid base method is currently widely applied for the detection of foodborne microorganisms, in addition to being part of conformation of the traditional method (ISO 7932:2004/Amd 1:2020) (ISO 21872-1:2017). (Zhao *et al.*, 2014). The design of Primer and probe in this method is considered a fundamental and critical step (Sachse & Frey, 2003). Hence Proper validation of the tools used for design is a preliminary step (Soliman *et al.*, 2024). Therefore, we have validated and applied primers predicted by using the FBPP tool for detecting the foodborne pathogen to careful validation before it can be adopted as an accepted application.

The evaluation of the predicted primers took place at three levels: genus level by using the primer Inv A gene for all *salmonella* species; species level by using the HipO gene for only *Campylobacter jejuni* species; and serotype level through the O antigen flippase gene *for E. coli O157*. It includes measures of inclusivity (detection of the target microorganism) and exclusivity (non-detection of non-target microorganisms) as recommended for the performance characteristics of the PCR-based method (ISO 22118). Furthermore, the sequence of the PCR product has been verified to confirm the correct PCR product has been amplified. The results demonstrate the ability of FBPP to create useful primers for foodborne pathogens under the three levels of evaluation.

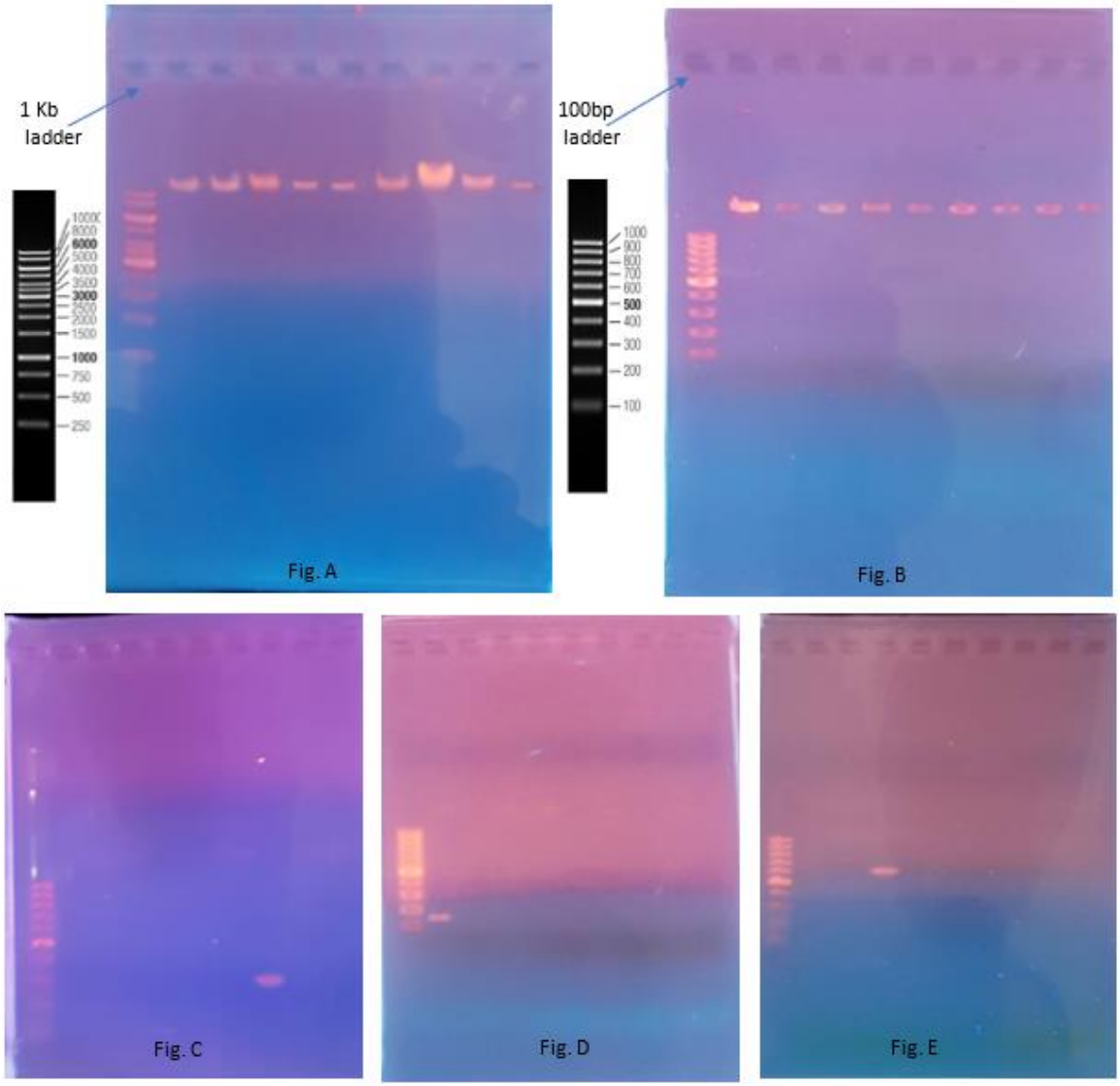

Fig. 1: Agarose gel electrophoresis of **(A):** DNA extracted from different strains. Lane 1 1Kb ladder; lane 2 to lane 9 different bacterial strains. **(B):** PCR amplified product of purified DNA extraction from different strains with 16s rRNA primers. Lane 1 100 bp ladder; lane 2 to lane 9 different bacterial strains. **(C):** Determination of the specificity of InvA primer. Lane 1 100 bp ladder; lane 2 *Clostridium perfringens ATCC 3624;* lane 3 *Enterococcus faecalis ATCC 29212;* lane 4 *Escherichia coli (serotype O157:H7) ATCC 43888;* lane 5 *Escherichia coli ATCC 35218;* lane 6 *Staphylococcus aureus subsp. aureus ATCC 25923;* lane 7 *Bacillus cereus ATCC 10876*; lane8 *Salmonella enterica subsp. enterica serovar Enteritidis ATCC 13076*; lane 9 *Listeria monocytogenes (serotype 1) ATCC 19111;* and lane 10 *Cronobacter sakazakii ATCC 29544.* **(D):** Determination of the specificity of hipO primer . Lane 1 100 bp ladder; lane 2 *Campylobacter jejuni subsp. jejuni ATCC 29428;* lane 3 *Listeria monocytogenes (serotype 4b) ATCC 19115;* lane 4 *Clostridium perfringens ATCC 13124;* lane 5 *Staphylococcus aureus subsp. aureus ATCC 11632;* lane 6 *Escherichia coli ATCC 33876;* lane 7 *Vibrio parahaemolyticus ATCC 10885;* lane 8 *Bacillus cereus ATCC 11778*; lane9 *Salmonella enterica subsp. enterica serovar Enteritidis ATCC 51741*; and lane 10 *Campylobacter coli ATCC 33559.* **(E):** Determination of the specificity of O antigen Flippase primer . Lane 1 100 bp ladder; lane 2 *Clostridium perfringens ATCC 3624;* lane 3 *Enterococcus faecalis ATCC 29212;* lane 4 *Escherichia coli (serotype O157:H7) ATCC 43888;* lane 5 *Escherichia coli ATCC 35218;* lane 6 *Staphylococcus aureus subsp. aureus ATCC 25923;* lane 7 *Bacillus cereus ATCC 10876*; lane8 *Salmonella enterica subsp. enterica serovar Enteritidis ATCC 13076*; lane 9 *Listeria monocytogenes (serotype 1) ATCC 19111;* and lane 10 *Cronobacter sakazakii ATCC 29544.*

Proper validation of the application took place through apply of the ISO standard method (ISO 22118:2011) for the performance characteristics for the detection and quantification of food-borne pathogens by using PCR (Standardization, 2011). According to the last report of the global burden of foodborne diseases from the World Health Organisation (WHO), The most frequent causes of foodborne illness were diarrheal disease agents, particularly norovirus, *Campylobacter spp*,, non-typhoidal *Salmonella enterica*, and enteropathogenic *Escherichia coli* (Organization, 2017)(Organization, 2017)(Organization, 2017)[14]. So, the evolution of application is applied to these organisms. The genes nominated for three levels, in the first level under the genus level by selecting the InvA gene, this gene is widely used in PCR method detection and use for the detection of all *Salmonella* spp. (Sachse & Frey, 2003), because it is found in both *Salmonella species (Salmonella bongori* and *Salmonella enterica)* (Microorganisms & Toxins, 2005). At the species level, the hippuricase gene (hipO) was used for the identification of *C. jejun*. The hipO gene is considered the key gene for *Campylobacter jejuni* other than other *campylobacter species* (Wu et al., 2022). Finally, for the identification of serotype strains of *E. coli* primers based on the O antigen flippase gene were designed. Several *E. coli O157* were examined by using these gene in PCR reactions (Liu et al., 2020).

Many primers and probes designed by the FBPP program for foodborne pathogens such as *Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus*, and *Vibrio parahaemolyticus* have been tested and validated in numerous laboratories; furthermore, some of this work is under publication.

Conclusions:

In this paper, we validate and apply FBPP as a specific-purpose tool for predicting foodborne pathogen primers and probes that use the nucleic acid-based detection method. FBPP has been confirmed on the three levels of foodborne pathogen bacteria: genius, specie, and serotype, by using remarkably similar strains of foodborne pathogen, and it was able to create highly selective primers for each of them. This result proves the ability of the FBPP application to predict and probe for the detection of foodborne pathogens. Further development could be applied to the software that can be used to predict multiplex primers.

Declarations:

Ethical Approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interests: The authors have no relevant financial or non-financial interests to disclose.

Authors Contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed Mohamed A. Soliman, Mohamed S. Azab, Hala A. Hussein, Mohamed N. Abu el-naga. The first draft of the manuscript was written by Mohamed A. Soliman and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding: The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Availability of Data and Materials: The FBPP Design Tools Desktop application is available here: https://github.com/mohamedmoez1983/FBPP**.**

Acknowledgements: We are grateful to the National Centre for Radiation Research and Technology, Atomic Energy Authority for providing the essential equipment and facilities needed to conduct this research. Special thanks to the staff of the microbiology radiation department for their invaluable technical support.

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