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RESEARCH ARTICLE
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Detection of *OmpA* gene by PCR for specific detection of *Salmonella* serovars

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Abstract

Aim: The study was carried out to determine the sensitivity and specificity of *OmpA* gene in *Salmonella* serovars through PCR.

Materials and Methods: A set of primers were designed targeting the *OmpA* gene specific for the *Salmonella* and polymerase chain reaction was standardized using *Salomonella* Typhimurium as a positive control and as a negative control 4 non *salmonella* cultures such as *Campylobacter coli, Arcobacter butzleri, Brucella abortus and E. coli*. Sensitivity of the test was determined by serial dilution of genomic DNA of standard *S*. Typhimurium. The PCR standardized was used for screening 68 strains of different serovars of *Salmonella*.

Results: The PCR developed targeting *OmpA* specific for *Salmonella* was highly specific in detection of the *salmonella* serovar alone and sensitivity was upto 68.8 fg. A total of 68 virulent/ natural strains of different serovars of *salmonella* taken up for the study were positive by *OmpA* based PCR.

Conclusions: This study reports that, *OmpA* gene which is conserved among *Salmonella* serovars can be used for the detection of *Salmonella* in food or clinical samples in further studies, with high sensitivity and specificity.

Key words: OmpA, PCR, *Salmonella*

Introduction

Salmonella is a food-borne pathogen that is typically acquired through consumption of contaminated food and water [1]. Salmonellosis continues to be a major public health problem worldwide. It also contributes to negative economic impacts due to the cost of surveillance investigation, treatment and prevention of illness [2]. The global burden of human gastroenteritis due to Salmonella has been estimated 93.8 million cases, resulting in 155,000 deaths each year [3]. Poultry is considered a major reservoir for many non-host specific motile serovars of Salmonella, and often human infection is attributed to consumption of contaminated poultry products, such as eggs and meats [4].

The outer membrane protein (OMPs) of Gram-negative bacteria plays a major role in the adaptation of the bacterium to its various external environments, by passively and/ or selectively controlling influx and efflux of important solutes, peptides or proteins, nucleic acids, and other organic compounds such as lipids and polysaccharides [5]. Most OMPs are surface exposed and, therefore, are potentially important in interfacing bacteria with the mammalian host and its defenses, bacteriophages, and other bacteria or micro-organisms [6]. The outer membrane proteins comprise almost 50% of the bacterial membranes of Gram negative bacteria [7].

OmpA super family is one of the most abundant outer membrane proteins in prokaryotes and is most widely studied [8]. Its main role is to provide integrity to the membrane by ensuring physical linkages between the outer membrane and the underlying peptidoglycan layer as well as having importance in bacterial conjugation [9,10]. It also serves as a receptor to some of the bacteriophages [11] and colicins [12]. Of all the OMPs, *OmpA* appears to be a major antigenic protein in *Salmonella* induced ReA/uSpA, as it is common to most of the stimulatory fractions [13].

Salmonella *OmpA* is immunostimulatory as demonstrated by stimulation of IFN-g production and enhanced expression of MHC and costimulatory molecules in dendritic cells and/or T cells and may play a role in modulation of the immune response against salmonellosis [14,15].

Traditional culture-based methods for detecting *Salmonella* are reliable but labor-intensive and time-consuming, demanding several days for a definitive result. Immunoassays such as enzyme-linked immuno-sorbent assay (ELISA) have been developed for

Table-1. PCR results for different *Salmonella* serotypes targeting *OmpA* gene

Sr.No	Species	Culture No.	Results
1-17	*S.*Typhimurium	E-4630, E-73, E-4629, E-275, E-366, E-159, E-2622, E-456, E-2375, E-4741, E-270, E-277, E-364, E-2416, E-2393, E-4810, E-2395	All +
18-29	*S.* Enteritidis	E-74, E-79, E-161, E-2475, E-2473, E-2465, E-20, E-2477, E-4252, E-2478, E-2477, E-2472	All +
30-32	*S.* Pullorum	E-154, E-153, E-152	All +
33-43	*S.* Gallinarum	E-85, E-4668, E-4661, E-2634, E-77, E-75, E-4045, E-76, E-4685, E-76	All +
44	*S.* Anatum	E-907	+
45	*S.* Alachera	E-65	+
46	*S.* 6,7:Y	E-62	+
47	*S.* Bredeney	E-85	+
48	*S.* Abortus equi	E-01	+
49	*S.* Italiana	E-104	+
50	*S.* Aviatum	E-1059	+
51	*S.* Virginia	E-60c	+
52	*S.* Rough	E-1101	+
53	*S.* Paratyphi B	E-801	+
54	*S.* Shipley	E-150	+
55	*S.* Rubislav	E-47	+
56	*S.* Bonariensis	E-84	+
57	*S.* Tennessee	E-56a	+
58	*S.* Derby	E-68	+
59	*S.* Weslaeo	E-64	+
60	*S.* Dahlem	E-712	+
61	*S.* Canada	E-3540	+
62	*S.* Heidelberg	E-3716	+
63	*S.* Lindenburg	E-3772	+
64	*S.* Poona	E-1565	+
65	*S.* Shipley	E-150	+
66	*S.* Bareilly	E-189	+
67	*S.* Wein	E-3636	+
68	*S.* Mons	E-3695	+
69	*C. jejuni*	--	
70	*B. abortus*	--	
71	*A. butzleri*	--	
72	*E. coli*	--	

Salmonella detection, however, low specificity has limited their use [16].

PCR technology represents a rapid procedure with high sensitivity and high specificity to detect *Salmonella* in a wide variety of food. Several PCR assays have been developed by targeting various *Salmonella* genes, such as 16S rRNA , *agfA* , and *viaB* and virulence-associated plasmids [17].

The study was thus, focused on polymerase chain reaction based amplification of *OmpA* gene to prove its conserved nature in *Salmonella* serovars and to evaluate its specificity and sensitivity in detection.

Materials and Methods

A total of 68 *Salmonella* strains and 4 strains of non- *Salmonella* cultures (Table-1) were used in this study. *Salmonella* and non-*Salmonella* cultures were obtained from repositories of National *Salmonella* Centre, Division of Bacteriology and Mycology and Viral Zoonoses Laboratory, Division of Veterinary Public Health, I.V.R.I. All the cultures mentioned in Table-1 were revived, tested for their purity, morphology and biochemical characteristics as per standard protocol [18].

PCR amplification and detection: The sequence of the primers used is as follows: Forward: 5'-AGT CGA GC TCATGAAAAAGACAGCTATCGC-3' Reverse: 5'-AGTCAAGCTTTTAAG CCTGCG GCTGAGTTA-3'.

DNA extraction and quantification: Oligonucleotide primers were designed targeting the *OmpA* gene of *Salmonella* serovar based on the nucleotide sequence available in the Genbank and the primers were got synthesized commercially.

The genomic DNA from the reference strain *S.* Typhimurium was extracted by phenol chloroform method [19] and checked for quality and quantity spectrophotometerically, while, the genomic DNA from the other *salmonella* and non- *salmonella* isolates were extracted by snap chill method. Briefly, 1 mL of the overnight incubated cultures was boiled in boiling water for 10 minutes and immediately transferred onto ice. For PCR assay, 2 µL of this heated broth was taken as template.

Detection of *OmpA* gene by PCR: The PCR mixture of 25µl contained 2.5 µL of Dream taq buffer, 2.5 µL of dNTP(2mM), 10 pM each of forward and reverse primer for *OmpA* gene and 1.0 U of Dream taq DNA polymerase and 2.0µl of template DNA. The PCR reaction was performed in a thermal cycler (BIOER, USA) using the following standard cycling procedure: an initial denaturation at 95°C for 5 min, followed by 34 cyclesof denaturation at 94°C for 1 minute, primer annealing at 55°C for 1 minute and extension at 72°C for1 minute and a final extension at 72°C for 10 min.Amplified products were analysed by agarose gel (1% in 1X TBE) electrophoresis at 5v/cm for 2 h and finally documented using gel documentation system (UVP).

PCR specificity and sensitivity: PCR specificity and sensitivity was determined using all the pure cultures lysate (Table-1). The specificity of *Omp*A primers was determined by PCR and observed for any product generated from other bacteria. For determination of sensitivity of the PCR assay, the DNA was diluted ten fold and the ability of this PCR assay to detect the minimum concentration of DNA was determined and

assay was repeated thrice to confirm its repeatability.

Results

PCR was standardised for the amplification of *OmpA* gene by optimizing reaction mixture and cycling conditions using the genomic DNA from standard *S.* Typhimurium extracted and purified by phenol: chloroform method. Standardized PCR yielded the expected single band of 1053 bp on agarose gel electrophoresis. The size for PCR product was as per the expected size of the primers which were designed to amplify the *OmpA* gene.

A total of 68 isolates were screened for the presence of *OmpA* gene and it was found that all the *Salmonella* isolates were positive for the presence of *OmpA* gene and non of the non *Salmonella* cultures were positive for O*mpA* gene (Table-1).

The concentration of the genomic DNA from the reference *S.* Typhimurium was 3240 ng/µL and the minimum detection limit (sensitivity) of the PCR was found that upto 64.8 fg DNA template which produced a clearly visible PCR band of 1053 bp. The band of 1053 bp in the lane 8 was clearly visible whereas the band in the lane 9 was faint and was difficult to appreciate (Fig-1).

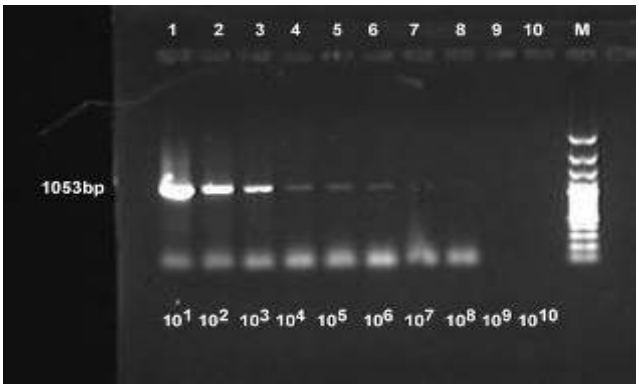

Figure-1. Sensitivity of OmpA PCR after tenfold dilution of genomic DNA of *S.*Typhimurium.

Discussion

Salmonella are widely distributed in nature and they survive well in a variety of foods. Poultry, eggs and dairy products are the most common vehicle of salmonellosis. In recent years, fresh produce like fruits and vegetables have gained concern as vehicles of transmission where contamination can occur at multiple steps along the food chain [20].

OmpA functions in the bacterial structure, physiology, and adaptation to environmental stresses, whereas in disease they can serve as virulence factors causing adhesion, invasion and damage of host tissue or evasion of host defences resulting in clinical disease or death [5].

The development of molecular methods for diagnosis of infectious diseases has improved the sensitivity, specificity, quality and availability of diagnosis and treatment. Several polymerase chain reaction (PCR) assays for detection of *Salmonella* have been developed, and different targets DNAs for amplification have been applied [21].

A total of 68 isolates were screened for the presence of *OmpA* gene and it was found that all the *Salmonella* isolates were positive for the presence of *OmpA* gene. Our results were in confirmation with Freudl and Cole [22] who reported that *OmpA* regulatory region of *S.* Typhimurium is highly conserved and has an overlapping twin-promoter arrangement. In addition, Okamura et al [23] based on bioinformatic analysis also reported that OmpA is well conserved among the various *Salmonella* serovars.

Conclusion

From the overall study, it can be concluded that *OmpA* gene is conserved among *Salmonella* serovars. Since our preliminary study proved the conserved nature of gene in different *Salmonella* serovars, this may be used for the detection of *Salmonella* in food or clinical samples in further studies.

Author's contributions

JLK, SR and JL planned and designed the study. RKA provided the *salmonella* serovars and AK provided the non *Salmonella* cultures. The experiment was performed by JLK and JL. JLK and SR drafted and revised the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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