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Evaluation of Primer Sequence targeting *inv A* Gene of *Salmonella* sp. by *in silico* PCR

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ABSTRACT

In silico PCR via algorithm and computer simulation aim to provide an easy way to analyse and obtain the theoretical PCR results we may expect from DNA, by using up to date bacterial genomes sequences. In this study, primer set *invA*-F and *invA-R* targeting *Salmonella* invasion gene, *invA*, was evaluated by *in-silico* PCR amplification against prokaryotic genome of major foodborne pathogens. A total of 127 strains of bacterial genome sequences from *Salmonella* sp. (27), *Escherichia* sp. (59), *Listeria* sp.(26) and *Campylobacter* sp. (15) were used as DNA templates in the PCR simulation analysis. The primer set simulatively amplified a single band of 285 bp PCR product with all 25 strains of *Salmonella enterica* subsp. *enterica*, whereas no amplification is produced with *Salmonella bongori* and *Salmonella enterica* subsp. *arizonae*. There was no cross-reaction obtained with other bacterial genomes indicated that the primer set is specific to *Salmonella enterica* subsp. *enterica* only. PCR experiments using *invA*-F and *invA*-R that was carried out in the laboratory had successfully amplified the 285 bp amplicons using DNA from *S.* Typhimurium, *S.* Enteritidis, *S.* Polarum and *S.* Gallinarum.

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INTRODUCTION

In silico Polymerase Chain Reaction (PCR) amplification via computer simulation aims to provide an easy way to analyse and obtain the theoretical PCR results we may expect from DNA, by using up-to-date bacterial genomes sequences based on deposited genome database (Bikandi *et al*., 2004). PCR allows amplification of specific DNA sequences by the use of primers, DNA template, PCR reagents in an optimized cycling condition. *In silico* PCR technique predicts the theoretical PCR products based on the input primers sequence against the chosen bacterial genome that we would like to test (Canene-Adams, 2013). *Salmonella* spp. has been documented as a key and vital foodborne pathogen for humans and animals over more than a century, causing human foodborne illness as well as incurring burden cost to human and animal health (Lee *et al*., 2015). *Salmonella* bacteria is implicated as the most common cause of food poisoning in many countries all over the worldd for more than over 100 years (Alakomi & Saarela, 2009).

PCR has been used for identification of *Salmonella* species and detection of its virulence and unique genes (Ziemer & Steadham, 2003; Thong, Hoe, Puthucheary, & Yasin, 2005 and Kaur & Jain, 2012). Here, we report the use of *in silico* PCR program available at http://insilico.ehu.es to analyse primer set for *invA* which had previously published by Rahn *et al*., (1992) to demonstrate the usefulness of the program in obtaining theoretical PCR products and later tested in actual PCR. In this study, primer set *invA*-F and *invA*-R targeting *Salmonella* invasion gene, *invA* was evaluated by *in-silico* PCR against prokaryotic genome of major foodborne pathogens. A total of 127 strains of bacterial genome sequences from *Salmonella* sp. (27), *Escherichia* sp. (59), *Listeria* sp. (26) and *Campylobacter* sp. (15) were used as bacterial genome templates in the *in silico* PCR amplification analysis. *invA* primers targeting this gene fragment was used in this study and tested in PCR with selected important *Salmonella* serovar to determine its amplifiability and specificity.

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MATERIALS AND METHODS

i) in silico PCR & BLAST Analysis:

Primers, invA-F (5'-GTGAAATTATCGCCACGTTCGGGCAA-3') and invA-R (5'-TCATCGCACCGTCAAAGGAACC-3') for PCR was chosen to be used in this study. They were evaluated using *in silico* PCR, web-based program (http://insilico.ehu.es) against genome database of *Salmonella* sp., *Escherichia* sp., *Listeria* sp. and *Campylobacter* sp. by BLAST program (www.ncbi.nlm.nih.gov) to predict their specificity and product's sequence and size.

ii) Bacterial strains and culture conditions:

Bacterial cultures used in this study were obtained from ATCC. *S.* Typhimurium ATTC™ 53648, *S.* Pullorum ATTC™ 10398, *S.* Gallinarum ATTC™ 9184 and local isolate *S.* Enteritidis. They were used as reference serovars for primers specificity evaluation. All *Salmonella* serovars were cultured overnight in tryptone soy broth (TSB, Oxoid) at 37^{o}C, 150 rpm.

iii) Crude DNA extraction:

Crude DNA extraction was performed on each strain of pure culture. A 1 mL portion of each broth culture was centrifuged at 15,000 g for 4 min. The pellet was resuspended in 500 µL sterile distilled water and vortexed vigorously. The cell suspension was boiled for 10 min, immediately chilled on ice for 10 min and centrifuged again at 15,000 g for 4 min. The supernatant containing crude DNA was transferred into a new tube and 5 uL was used as DNA template in PCR. The remaining crude DNA were stored in -20^{o}C.

iv) PCR amplification & analysis of PCR products:

A reaction volume of 25µL of PCR mixture using 1X PCR master mix (containing 2 mM $MgCl_2$, 0.025U/µL *Taq* DNA polymerase and 0.2 mM of each dNTP), 0.5µM of each *invA* primer, 5µl of crude DNA extract, and nuclease-free water adjusted to a total volume of 25 µl. PCR reaction was performed in a thermocycler (DNA Dyad, BioRad). The thermocyler was programmed as stated by Rahn *et al*., (1992) by preheated at 95^{o}C for 2 min, followed by 30 cycles of 95^{o}C for 30s, 57^{o}C for 30s, 72^{o}C for 30s and final extension at 72^{o}C for 4 min. A 5µL of PCR product was analysed by electrophoresis on 2% agarose gel and stained with ethidium bromide to visualize the amplicons under UV light.

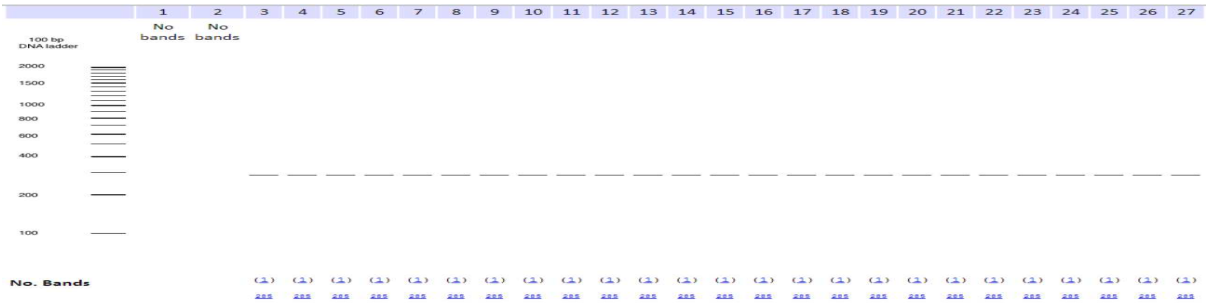

Fig. 1: *In silico* PCR result of *invA* primers against all *Salmonella* serovar (Primer mismatch = 0)

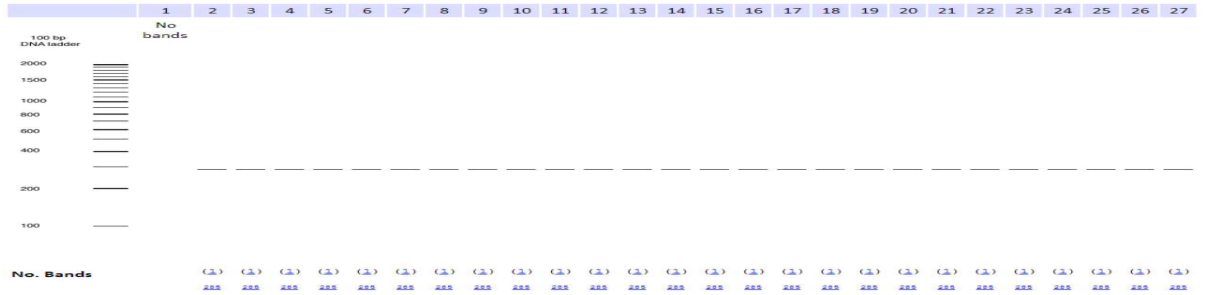

Fig. 2: *In silico* PCR result of *invA* primers against all *Salmonella* serovar (Primer mismatch = 1)

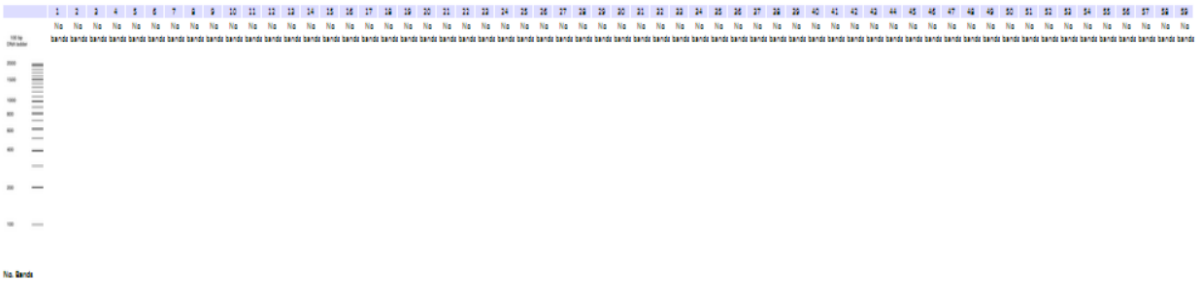

Fig. 3: *In silico* PCR result of *invA* primers against *E. coli* (Primer mismatch = 0)

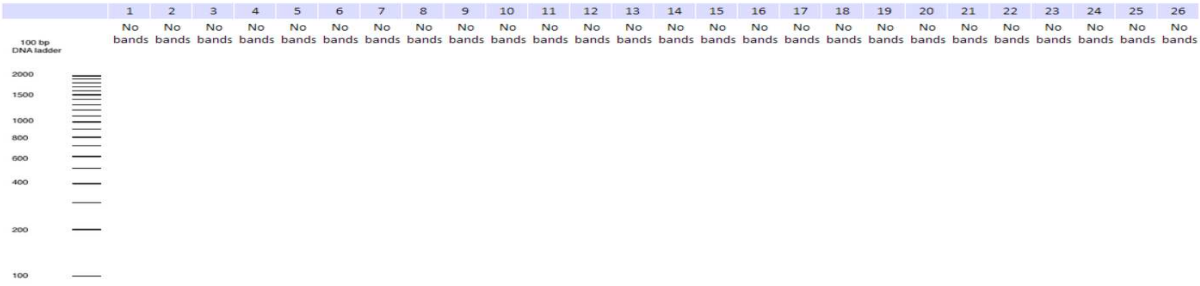

Fig. 4: *In silico* PCR result of *invA* primers against *Listeria* sp. (Primer mismatch = 0)

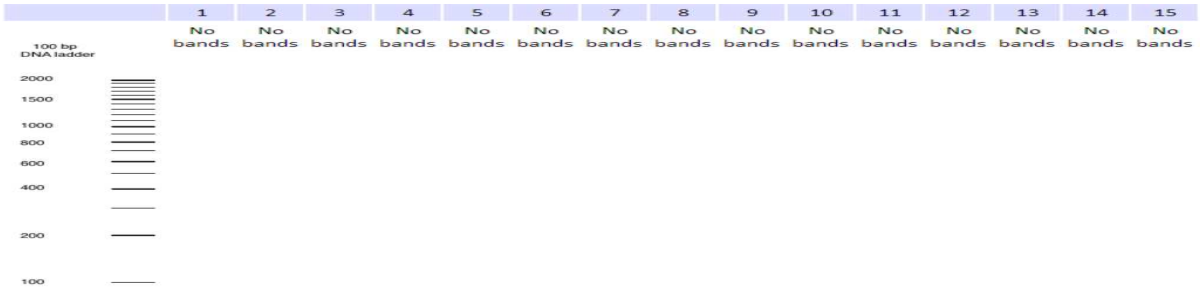

Fig. 5: *In silico* PCR result of *invA* primers against *Campylobacter* sp. (Primer mismatch = 0)

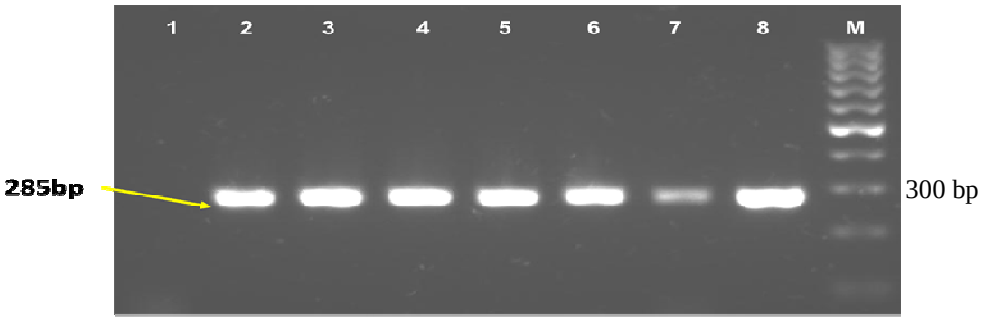

Fig. 6: PCR with *invA* primers using crude DNA from selected *Salmonella* serovars
Lane 1: Negative control (ddH_2O), 2: *S.* Typhimurium, 3: *S.* Enteritidis, 4: *S.* Pollarum, 5: *S.* Gallinarum, 6 & 8: *S.* Typhimurium (pure DNA),7 : Diluted *S.* Typhimurium (pure DNA), Lane M: 100 bp DNA ladder

RESULTS AND DISCUSSION

The *invA* primers amplified *in silico* a single band of 285 bp PCR product with all strains of *Salmonella* sp. except *S.* bongori and *Salmonella enterica* subsp. *arizonae* when the mismatch of the primers was set to zero (Figure 1) whereby mismatch is the recognition errors between primers and DNA template (Ishii & Fukui, 2001 and Sipos *et al*., 2007). Zero mismatch make theoretical experiment very stringent and primers were very specific to the intended template (Wu, Hong, & Liu, 2009). However, when less stringent theoretical experiment was used by setting the mismatch to 1, an additional

band of identical size (Figure 2, Lane 2) was amplified *in silico* from *Salmonella enterica* subsp. arizonae which demonstrated that the *invA* primers were capable to amplify DNA form all *Salmonella enterica* strains including the subspecies arizonae. In addition, BLAST result showed that both invA-F and invA-R primers have 100% sequence similarity to *Salmonella enterica* subsp. *enterica* which imply the primers ability to amplify all *Salmonella enterica* strains which was in agreement with the *in silico* PCR results (results not shown). No cross-reaction was obtained with other bacterial genomes indicated that the primer set was specific to *Salmonella enterica* only (Figure 3 to 5). PCR using *invA-F* and *invA-R* that were carried out in the laboratory had successfully amplified the expected 285 bp PCR products with crude DNA from *S.* Typhimurium, *S.* Enteritidis, *S.* Polarum and *S.* Gallinarum (Figure 6). Based on the *in silico* PCR and BLAST results, it is presumed that these primers are able to amplify PCR products from the tested serovar strain as well as other *Salmonella enterica* subsp. *enterica* serovars as shown in the *in silico* PCR result but excluded in our study, which in agreement to findings by Fach *et al*., (1999).

Conclusions:

In silico PCR is very useful to test primers specificity against targeted bacterial genome and have the ability to predict the theoretical PCR products size and its sequence prior to actual PCR works. Our study had showed that the *in silico* PCR have agreement with BLAST results as demonstrated by our evaluation of *invA* primers by *in-silico* PCR carried out against prokaryotic genome of major foodborne pathogens. The *invA* primers had successfully amplified the expected 258 bp *invA* gene fragment with the selected important *Salmonella* serovar verifying the usefulness of this molecular technique in detection of microorganisms. At present, PCR is a potent technology that serves as an essential tool in a detection of wide range of organisms due to its specificity and sensitivity. The application of *in silico* PCR is seen to work in tandem with actual PCR that could facilitate researchers to fully utilize the deposited bacterial genome sequences in evaluating the specificity of primer sequences.

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