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MOLECULAR BASED DETECTION OF *Salmonella* sp. IN SELECTED FISH SPECIES VIA MULTIPLEX POLYMERASE CHAIN REACTION ASSAY

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledge as referenced work. This thesis has not been submitted to any other institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Undergraduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Multiplex polymerase chain reaction (multiplex PCR) assay offers a very specific multiple detections of target bacteria by amplifying more than one gene in a single assay, which aids directly in reducing cost, energy and time. The target bacteria in this study is *Salmonella* sp. which comes from family of Enterobacteriaceae. This study aims to implement the molecular-based detection of *Salmonella* sp. by using specific *invA* gene and 16S rRNA as the internal amplification control gene through multiplex PCR. The assay used to detect bacteria isolated from fish samples specifically on *Salmonella* sp. The *invA* gene was chosen in this study as a specific fragment of *Salmonella* sp. since it gives this bacteria the ability to invades the intestinal cell of its host. BLAST was performed in both target genes. The specific primer sequence of *invA* gene was selected since this gene expressed in almost all *Salmonella* spp., and 16S rRNA was chosen since it presents in almost all gram negative bacteria. The genomic DNA was extracted from bacteria culture of 5 *Salmonella* sp., 3 other bacteria strains and selected fish species. The optimized condition of multiplex PCR was performed with Ta = 58ºC, $MgCl_2$ = 1.5 mM, dNTPs = 0.25 mM, both *invA* primers = 0.8µM, both 16S rRNA primers = 0.3 µM and *Taq* polymerase = 2U. Both 16S rRNA and *invA* genes were successfully amplified by giving two fragments with size of 478 bp and 254 bp respectively. The specificity test showed 2 bands of 16S rRNA and *invA* gene were formed for all target bacteria of *Salmonella* sp., but only single band was formed for all other bacteria strains as non-targeted samples. As a proof-of-concept, bacteria culture isolated from seven fish samples showed none *Salmonella* sp. as no fragment of *invA* gene produced. This study was done to provide benefits in term of the detection of pathogenic *Salmonella* sp. in the food industry by focusing on fish samples.

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