DEVELOPMENT OF A SPECFIC PCR-BASED ASSAY FOR THE DETECTION OF *ESCHERICHIA COLI* O157: H7

By

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A total of nine *Escherichia coli* O157:H7 isolates were originally isolated from imported Indian beef purchased from local retail markets. These nine *Escherichia coli* O157:H7 isolates were confirmed as *Escherichia coli* O157:H7 by their positive growth characteristics on Rainbow agar O157 and PCR assay for the detection of the presence of the H7 (*fliC*) gene unique to *Escherichia coli* O157:H7. Published specific primers *FLICH* (625 bp) of the *fliC* gene encoding the H7 antigen were utilized in the specific PCR assay. Three of the *Escherichia coli* O157:H7 isolates were selected for the amplification of their H7 (*fliC*) gene. The amplicon of the PCR assay were successfully cloned into pGEM-T vector and sequenced. The sequence of the *Escherichia coli*

O157:H7 that was obtained from sequencing was analyzed. This sequence was identified using Basic Local Alignment Search Tools (Blast) program. Based on the sequence obtained, primer pairs were designed to detect the *Escherichia coli* O157:H7, but only Sk7 and Sk8 were found to be specific for detection of locally isolated *Escherichia coli* O157:H7. Sk7 and Sk8 produced an amplicon size of 520 bp and 603 bp respectively against all tested locally isolated *Escherichia coli* O157:H7. Both new primers were specific against *Escherichia coli* O157:H7, as they did not produce any amplicons from the 32 isolats representing 20 different bacterial species. DNA hybridization analysis of 10 other bacterial species, including *E. coli, Salmonella* and other isolates showed that the probe (SkP) reacted only with *Escherichia coli* O157:H7 isolates.

Abstrak tesis yang dikemukakan kepada senat Univetsiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGHASILAN PENGGUNAAN TEKNIK SPECIFIK RANTAIAN POLIMERASE (PCR)- MELALUI TEKNIK PENGESANAN *ESCHERICHIA COLI* O157:H7

Oleh

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Pencilan *E.coli* yang digunakan dalam kajian inin adalah berasal daripada daging lembu yang diperolehi dari pasaraya tempatan. Sembilan pencilan *E.coli* disahkakeputusan positif cirri-ciri pertumbuhan koloni atas Rainbow agar O157 dan analysia teknik rantaian polimerase (PCR) dalam pengenalpastian kehadiran gen "*fliC"* yang unik kepada *E.coli* O157: H7. Primer yang digunakan dalam teknik rantaian polimerase khas merupakan primer penerbitan khas iaitu *FLICH* (625 bp) yang mengekodkan antigen H7. Tiga pencilan *E.coli* O157: H7 telah dipilih untuk mengamplifikasikan gen H7 (*fliC*)nya. Hasil amplifikasi (amplikon) dari PCR telah berjaya diklonkan kedalam ke dalam vector PGEM-T dan jujukan.

Analisis jujukan *E.coli* O157: H7 dari teknik jujukan gen dilakukan. Jujukan ini dikenalpasti melalui program Basic Local Alignment Search Tools (BLAST) yang disediakan oleh 'National Center for Biotechnology Information' (**http://www.ncbi.nlm.nih.gov**). Berdasarkan jujukan yang diperolehi, beberapa primer baru telah direka untuk mengesan *E.coli* O157: H7. Akan tetapi, hanya SK7 dan SK8 di dapati spesifik atas pengesanan *E.coli* O157: H7. SK 7 memberi saiz amplifikasi 520 bp manakala SK8 menghasilkan 603bp terhadap semua kultur *E.coli* O157: H7 yang dikaji. Kedua-dua primer baru adalah spesifik terhadap *E.coli* O157: H7 disebabkan ia tidak memberikan sebarang hasil amplifikasi daripada 32 strain bacteria yang mewakili 20 spesies bacteria yang lain. Analisis DNA hibridasi untuk 10 spesies bacteria lain termasuk *E.coli*, Salmonella dan isolat bacteria lain memaparkan bahawa probe SKP hanya memberi reaksi terhadap *Escherichia coli* O157:H7.

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I certify that an Examination Committee met onto conduct the final examination of Sushi Kauri on her Master of Science thesis entitled DETECTION OF *ESCHERICHIA COLI* O157: H7 USING POLYMERASE CHAIN REACTION in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The member of the Supervisory Comitee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknoeledged. I also declare that it has not been previously or currently submitted for any other degree at UPM or other institutions.

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SUSHIL KAUR D/O GURNAM SINGH

Date:

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