A STUDY OF TWO VARIABLE REGIONS (V2 AND V6) IN THE 16S RIBOSOMAL RNA GENE OF *Salmonella* SPECIES

by

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ABSTRACT

Two variable regions, V2 and V6, of the 16S rRNA genes from a total of 15 *Salmonella* serovars were amplified by the polymerase chain reaction (PCR). Using the conserved sequences that flank the regions of interest, V2 and V6, as primers, the V2 region was amplified using TP1/TP2 and the V6 region using TP3/TP4.

Due to the apparent specificity of the V2 region, PCR optimization was concentrated on this region to obtain a *S. typhi*-specific product but after much optimization did not yield such a product. However the optimized PCR conditions when tested on serum samples gave positive results. Using the PCR amplified V2 region of *S. typhi* as probe, a series of hybridization experiments were conducted. The slot-blot hybridization studies under high stringency conditions appeared to be more specific as compared to Southern hybridization.

PCR amplified V2 regions of *S. typhi* and *S. typhimurium* were also sequenced using direct sequencing and results showed a high degree of sequence similarity between the two species. The results of the study showed that, due to the low heterogeneity in the variable regions of V2 and V6, in particular the V2 region of 16S rRNA gene of *Salmonella* species, it was difficult to generate a suitable species-specific probe for *S. typhi* based on these variable regions.

ABSTRAK

Dua kawasan terubah, V2 dan V6 dari gen-gen 16S rRNA dari 15 serovar *Salmonella* telah diamplifikasikan dengan menggunakan kaedah amplifikasi DNA in vitro iaitu, tindakbalas berantai polimerase (PCR) melibatkan dua primer oligonukleotid, TP1-TP2 dan TP3-TP4 yang diguna untuk mengamplifikasikan V2 dan V6 masing-masing.

Oleh kerana kawasan terubah V2 kelihatan lebih spesifik maka kerja pengoptimaan PCR untuk mendapat produk spesifik untuk *S. typhi* telah dikonsentrasikan pada kawasan ini. Tetapi walaupun selepas pengoptimaan PCR tiada produk spesifik untuk *S. typhi* didapati. Namun begitu, keadaaan optima PCR yang diperolehi,apabila diperlakukan terhadap sampel serum telah memberi keputusan positif. Dengan menggunakan produk PCR dari kawasan terubah V2, *S. typhi*, sebagai prob, satu siri kerja hybrid telah dijalankan dan didapati bahawa hybrid slot-blot yang menggunakan keadaaan yang lebih "stringent" adalah lebih spesifik berbanding dengan hybrid Southern.

Produk PCR dari kawasan terubah V2 dari *S. typhi* dan *S. typhimurium* juga dibaca turutannya dan didapati bahawa turutan nukleotid untuk kedua spesis itu amat serupa. Sebagai rumusannya keputusan penyelidikan ini menunjukkan bahawa turutan nukleotida yang amat serupa antara spesis bagi kawasan terubah V2 dan V6 menyukarkan kebolehan untuk mendapat prob yang sesuai untuk *S. typhi*.

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ABBREVIATIONS

Most of the abbreviations used are standard. However, attention is drawn to the following:

bp	:	base pair
BPB	:	bromophenol blue
°C	:	degree celsius
ccc	:	covalently closed circular
cDNA	:	complementary deoxyribonucleic acid
DNA	:	deoxyribonucleic acid
ds	:	double stranded
dATP	:	deoxyadenosine triphosphate
dCTP	:	deoxycytidine triphosphate
dGTP	:	deoxyguanosine triphosphate
dTTP	:	deoxythymidine triphosphate
e.g	:	exempli gratia/for example
EtBr	:	ethidium bromide
etc	:	*et cetera*
g	:	gramme
i.e.	:	that is
IPTG	:	isopropylthiogalactoside
kb	:	kilobase pair
LB	:	Luria-Bertani
M	:	molar
Met	:	Methionine
mg	:	milligramme
ml	:	millilitre
mm	:	millimetre
mM	:	millimolar
mRNA	:	messenger ribonucleic acid
MW	:	molecular weight
N	:	normal
no.	:	number
µg	:	microgramme
µl	:	microlitre
µM	:	micromolar
OD	:	optical density
pg	:	picogram

psi	:	pounds per square inch
PEG	:	polyethylene glycol
PCR	:	polymerase chain reaction
RNA	:	ribonucleic acid
rRNA	:	ribosomal ribonucleic acid
RNase	:	ribonuclease
rpm	:	revolution per minute
SDS	:	sodium dodecyl sulphate
TBE	:	Tris-borate EDTA
tRNA	:	transfer ribonucleic acid
Trp	:	Tryptophane
Tris	:	tris (hydroxymethyl) methylamine
UV	:	ultraviolet
V	:	volt
Vol	:	volume
(v/v)	:	volume per volume
(w/v)	:	weight per volume
%	:	percentage

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