嘉南藥理科技大學專題研究計畫成果報告

利用 PCR 檢測食品中 *Salmonella* Enteritidis

計畫類別：□個別型計畫　　□整合型計畫

計畫編號： 90 - FH - 02

執行期間：90 年 1 月 1 日至 90 年 12 月 31 日

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摘　　要

本實驗以 PCR(polymerase chain reaction) 檢測食品中 *Salmonella*, 選用的 primer 爲 Sef127L-Sef661R)對 *Salmonella* Enteritiidis 具有特異性，其 PCR 產物爲 535bp。 由結果顯示對 27 株 *S.* Enteritidis 具有很高的專一性，對於與 Salmonella 血緣相近及一些腸內菌，皆無假性正反應產生。

緒　　言

Salmonella 爲引起人畜中毒之重要病原菌，傳統檢測需經增菌、分離培養、純化、血清試驗及生化試驗，需 6-7 天。進年來 polymerase chain reaction (PCR)技術以應用於病原菌之檢測(1,2)。對於 *S.* Enteritidis 之檢測，有使用對 virulence plasmid 具特異性的 primer 來檢測 for *S.* Enteritidis (3,4). 以多套式 PCR(multiplex PCR) 來檢測 *S.* Enteritidis，有檢測所有血清型 *Salmonella* 及 確認 *S.* Enteritidis 及 *S.* Typhimurium(5,6)。而本研究所使用的PCR primer與其他學者所發表之 PCR primers 不同。PCR primer (Sef127L-Sef661R)爲 *Salmonella* 鞭毛蛋白的一段基因，對 *S.* Enteritidis 具有特異性。

材料與方法

1. PCR primer：Sef127L-Sef661R
2. DNA 製備：培養後的菌液，經煮沸作粗萃取液。
3. PCR 增幅作用：94℃ 50sec、60℃ 50sec、72℃ 40sec，共 35cycles，最後於 72℃ 2min。
4. *Salmonella* 及相關細菌之檢測:
5. *Salmonella* Enteritidis 之靈敏度：
6. 南方吸漬法：

結果與討論

Salmonella Entertidis 特異性：

由實驗結果顯示，primer Sef127L-Sef661RPCR 與 27 株 *Salmonella* Enteritidis 有正反應，而其他非 *S.* enteritidis 之 *Salmonella* 及血緣相近之腸內菌皆無反應 (Table 1, Table 2)。爲了確認 PCR 產物，我們合成一段 checking probe 進行 Southern blot hybridization，結果如 Fig 1 所示，顯示 primer Sef127L-Sef661RPCR 增幅之 PCR 產正確性。

檢測靈敏度：

檢測結果顯示，經稀釋加熱萃取 DNA，每次只要 104CFU，就可檢測出來 (Fig2)。若要提高檢測靈敏度，可採預培養及選擇性培養來提高結果靈敏度。

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Table 1. PCR results for *Salmonella* isolates

Species	No. of isolates	positive results SefB127L-661R	Species	No. of isolates	Positive results SefB127L-661R
S. aberdeen	1	0	*S. kuru*	1	0
S. adelaide	1	0	*S. lagos*	1	0
S. agona	1	0	*S. lanka*	1	0
S. albany	1	0	*S. limete*	1	0
S. amager	1	0	*S. litchfield*	1	0
S. anatum	2	0	*S. london*	1	0
S. allandale	1	0	*S. meleagridis*	1	0
S. azteca	1	0	*S. manhattan*	1	0
S. bareilly	1	0	*S. minnesota*	1	0
S. berta	3	0	*S. miami*	1	0
S. bonn	1	0	*S. montevideo*	2	0
S. boussol	1	0	*S. muenchen*	1	0
S. bovismorbificans	1	0	*S. munster*	2	0
S. bredeny	1	0	*S. nchanga*	1	0
S. braenderup	2	0	*S. newbrunswick*	1	0
S. cairo	1	0	*S. newington*	1	0
S. california	1	0	*S. newport*	2	0
S. cerro	1	0	*S. ngor*	1	0
S. chailey	1	0	*S. nigeria*	1	0
S. chester	1	0	*S. ohio*	1	0
S. colorado	1	0	*S. oranienburg*	1	0
S. choleraesusis	1	0	*S. panama*	2	0
S. coleypark	1	0	*S. portsmouth*	1	0
S. cubana	1	0	*S. richmond*	1	0
S. derby	2	0	*S. rubislaw*	1	0
S. djakarta	1	0	*S. sandiego*	1	0
S. drypool	1	0	*S. senftenberg*	3	0
S. enteritidis	27	27	*S. seremban*	1	0
S. eppendorf	1	0	*S. schwarzengrund*	1	0
S. essen	1	0	*S. sinstorf*	1	0
S. gera	1	0	*S. stanley*	1	0
S. goerlitz	1	0	*S. tananarive*	1	0
S. hadar	3	0	*S. tennessee*	2	0
S. halmstad	1	0	*S. thomasville*	1	0
S. hartford	1	0	*S. thompson*	1	0
S. havana	2	0	*S. trachau*	1	0
S. heidelbrg	1	0	*S. typhimurium*	4	0
S. hvittingfoss	1	0	*S. vejle*	1	0
S. infantis	3	0	*S. victoria*	1	0
S. johannesburg	2	0	*S. weltevreden*	1	0
S. kentucky	1	0	*S. worthington*	1	0
S. kinshasa	1	0			

Table2. PCR results for non-*Salmonella* isolates

Species	No. of Isolates	positive results SefB 127L-SefB 661R
Escherichia coli	23	0
Escherichia coli (ETEC)	9	0
Staphylococcus aureus	7	0
Acinetobacter calcoaceticus	1	0
Alcaligenes faecalis	1	0
Bacillus stearothermophilus	1	0
Bacillus sutilis	1	0
Brevibacterium linens	1	0
Citrobacter freundii	2	0
Enterobacter aerogenes	2	0
Enterobacter cloacae	1	0
Erwinia carotovora	1	0
Hafnia alvei	1	0
Proteus vulgaris	2	0
Salmonella arizonae	1	0
Serratia marcescens	1	0
Shigella flexneri	1	0
Shigella sonnei	1	0

ATCC: American Type Culture Collection, Maryland, U.S.A.
CDC: Center for Disease Control, Georiga, U.S.A.
PT : National Ping Tung Institute of Agriculture.
US: The City of New York Department of Health. U. S. A.
CCRC: Culture Collection and Research Center Taiwan R.O.C.
WHO: World Health of Organization.
FDB: Food Drug Bureau, Department of Health Executive Yuan, Taiwan. R. 0. C.
VSE: Strains of *S. enteritidis* from Veterinary Service Laboratory of USDA.
ISE: Strains of *S. enteritidis* from National Institute of preventive Medicine, Department of health, executive Yuan, Taipei, Taiwan, R.O.C.

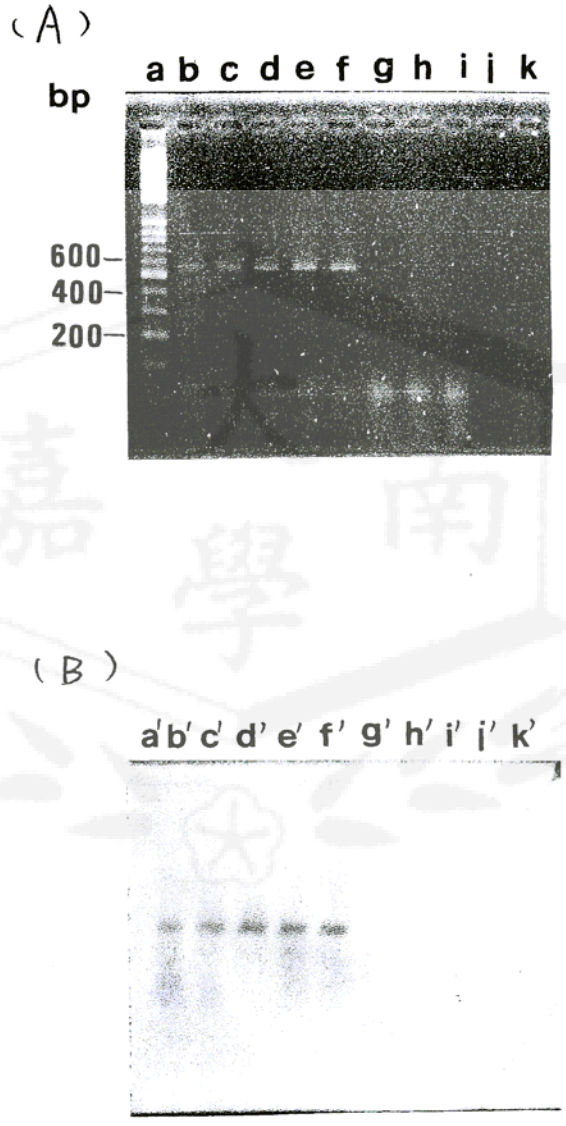

Fig.1 Agorose gel electrophoresis of the PCR products amplified from *Salmonella enteritidis* with PCR primer SefB127L-661R (A) southern blot hybridization of the PCR products with digoxigenin-labeled oligonucleotide (B) Lane a :marker ; b~f : *Salmonella enteritidis* ; g : *Salmonella anatum* ; h : *Salmonella raenderup* ; i : *Escherichia coli* ; j : *Escherichia coli*(EIEC03) ; k : *Bacillus sutilis* .Lane b'~k': southern blot hybridization results for Lane b~k in Fig.1(A)

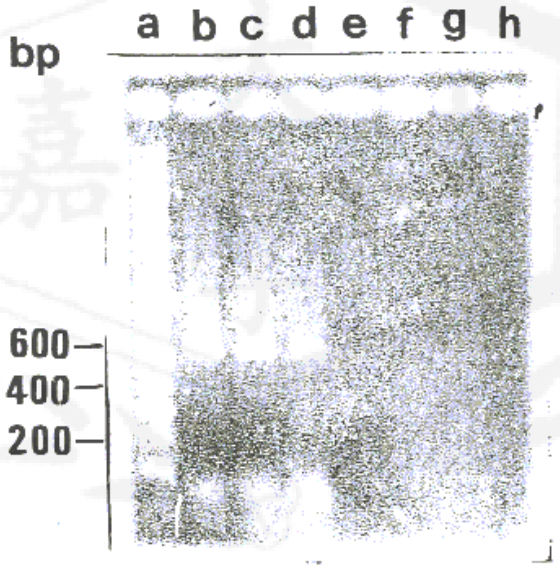

Fig2. Detection sensitivity for *Salmonella enteritidis* using PCR primer SefB127L-661R，Lane a：marker ；b~d：*Salmonella enteritidis* were spiked 10^9，10^8 10^7CFU/ml respectively.