

利用RAPD-PCR技術發展特異性聚合[?]鎖反應引子以建立大腸桿菌之快速檢測系統

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摘要

大腸桿菌一直是飲用水及食品中是否遭受糞便污染之微生物指標菌，其檢測是食品衛生檢驗之重要工作。傳統方法因耗時費力且具高假陽性率，而利用核酸技術檢測大腸桿菌卻因DNA探針或聚合[?]鎖反應(PCR)引子(PRIMER)之特異性不佳而正確性堪慮。近年來，利用非特異性單一引子進行PCR所產生的RAPD(RANDOMLY AMPLIFIED POLYMORPHIC DNA)圖譜(PROFILE)已成功地用於選定菌種(SPECIES)，甚至菌株(STRAIN)之特異性DNA探針。本研究利用市售長度為10個鹼基之引子，針對非病原型、數種病原型大腸桿菌、SHIGELLA SPP.及其他腸內菌株，進行RAPD反應。結果在由OPQ5+OPQ4及U69的引子所得之RAPD圖譜中產生大小為240 BP及1675 BP之RAPD片段，具有區分E. COLI及其他非E. COLI菌株之潛力，分別命名為Q54E01及U69E3。而採用OPQ3、U76和U82的引子所得之RAPD圖譜中分別產生大小為456 BP，404 BP及506 BP針對EHEC特有之RAPD片段，這些片段分別命名為Q3H01，U76H15及U82H01。以墨點雜交反應進行對E. COLI特異性評估，其結果Q54E01片段對E. COLI DNA雜交檢出率為58.8%，而SHIGELLA SPP.中只有S. SONNEI(SHI20)呈現干擾之雜交訊號；U69E3片段對E. COLI之檢出率為100%，但仍無法區別SHIGELLA SPP.。而Q3H01片段對血清型O157:H7及O157:NM則具有特異性；U76H15及U82H01對E. COLI之墨點雜交結果與U69E3類似，其中仍有部分腸內菌株會造成干擾。將上述RAPD片段經定序分析後，針對Q54E01設計兩組聚合[?]鎖反應引子ECP1/ECP2和ECP1/ECP3，以檢測所有大腸桿菌，其結果顯示ECP1/ECP2及ECP1/ECP3對大腸桿菌檢出率分別只有35%及31.4%。由Q3H01片段序列中設計一組聚合[?]鎖反應引子AF1/AR1，以檢測所有E. COLI O157:H7，其結果顯示AF1/AR1引子組對所測試之O157:H7以及O157:NM血清型特異性佳。而AF1/AR1對目標菌之檢測靈敏度可達每一反應(25 mL)內含有100 CFU的菌量。該引子組應用於生牛肉中E. COLI O157:H7之檢測，其檢測靈敏度在每克生牛肉中原含有100 CFU之E. COLI O157:H7細胞，經LB預培養8小時後即可被檢測出。

關鍵詞：大腸桿菌，O157:H7血清型，隨機擴增多型性去氧核糖核酸，墨點雜交，聚合[?]鎖反應

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參考文獻

1.簡良兆。1995。腸出血性大腸桿菌類志賀型毒素I、II基因之PCR引子及EHEC、ETEC多套PCR技術之發展。國立中興大學食品科學研究所碩士論文。2.食品衛生檢驗手冊。1987。行政院衛生署印行。3.ARGENTON, F., DE MAS, S., MALOCCO, C., DALLA VALLE, L., GIORGETTI, G. AND COLOMBO, L.19-96.USE OF RANDOM DNA AMPLIFICATION TO GENERATE SPECIFIC MOLECULAR PROBES FOR HYBRIDIZATION TESTS AND PCR-BASED DIAGNOSIS OF YERSINIA RUCKERI. DIS. AQU. ORGANISMS.24:121-127. 4.BEJ, A. K., DICESARE, J. C., HAFT, L. AND ATLAS, R. M. 1991. DETECTION OF ESCHERICHIA COLI AND SHIGELLA SPP. IN WATER BY USING THE POLYMERASE CHAIN REACTION AND GENE PROBES FOR UID. APPL. ENVIRON. MICROBIOL. 57:1013-1017. 5.BENNETT, A. R., MACPHEE, S. AND BETTS, R. P. 1996. THE ISOLATION AND DETECTION OF ESCHERICHIA COLI O157 BY USE OF IMMUNOMAGNETIC SEPARATION AND IMMUNOASSAY PROCEDURES. LETT. APPL. MICROBIOL. 22:237-243. 6.BETTELHEIM, K. A. 1997. ESCHERICHIA COLI O157 OUTBREAK IN

JAPAN: LESSONS FOR AUSTRALIA. AUST. VET. J. 75:108. 7. BEUTIN, L., BODE, L., OZEL, M. AND STEPHAN, R. 1990. ENTEROHEMOLYSIN PRODUCTION IS ASSOCIATED WITH A TEMPERATE BACTERIOPHAGE IN ESCHERICHIA COLI SERO- GROUP O26 STRAINS. J. BACTERIOL. 172:6469-6475. 8. BOLTON, F. J., CROZIER, L. AND WILLIAMSON, J. K. 1996. ISOLATION OF ESCHERICHIA COLI O157 FROM RAW MEAT PRODUCTS. LETT. APPL. MICROBIOL. 23:317-321. 9. BRANDT, M. E., PADHYE, A. A., MAYER, L. W. AND HOLLO WAY, B. P. 1998. UTILITY OF RANDOM AMPLIFIED POLYMORPHIC DNA PCR AND TAQMAN AUTOMATED DETECTION IN MOLECULAR IDENTIFICATION OF ASPERGILLUS FUMIGATUS. J. CLIN. MICROBIOL. 36:2057-2062. 10. BRAKSTAD, O. G., ASBAKK, K. AND MAELAND, J. A. 1992. DETECTION OF STAPHYLOCOCCUS AUREUS BY POLYMERASE CHAIN REACTION AMPLIFICATION OF THE NUC GENE. J. CLIN. MICROBIOL. 30:1654-1660. 11. CARSON, C. A., SHEAR, B. L., ELLERSIECK, M. R. AND ASFAW, A. 2001. IDENTIFICATION OF FECAL ESCHERIC -HIA COLI FROM HUMANS AND ANIMALS BY RIBOTYPING. APPL. ENVIRON. MICROBIOL. 67:1503-1507. 12. CANDRIAN, U., FURRER, B., HOFELIN, C., MEYER, R., JERMINI, M. AND LUTHY, J. 1991. DETECTION OF ESCH -ERICHIA COLI AND IDENTIFICATION OF ENTEROTOXIGENIC STRAINS BY PRIMER-DIRECTED ENZYMATI -C AMPLIFICATION OF SPECIFIC DNA SEQUENCES. INT. J. FOOD MICROBIOL. 12:339-351. 13. CDC. 1993. FOODBORNE OUTBREAK OF ESCHERICHIA COLI O157:H7 INFECTIONS FROM HAMBURGERS-WEST -ERN UNITED STATES, 1993. MMWR PRELIMINARY REPORT. 42:85-86. 14. EBULA, T. A., PAYNE, W. L. AND FENG, P. 1995. SIMULTANEOUS IDENTIFICATION OF STRAINS OF ESCHERIC -HIA COLI SEROTYPE O157:H7 AND THEIR SHIGA-LIKE TOXIN TYPE BY MISMATCH AMPLIFICATION MUTATION ASSAY-MULTIPLEX PCR. J. CLIN. MICROBIOL. 33:248-250. 15. CHAPMAN, P. A., SIDONS, C. A., ZADIK, P. M. AND JEWES, L. 1991. AN IMPROVED SELECTIVE MEDIUM FOR THE ISOLATION OF ESCHERICHIA COLI O157. J. MED. MICROBIOL. 35:107-110. 16. CHAPMAN, P. A., WRIGHT, D. J. AND SIDONS, C. A. 1994. A COMPARISON OF IMMUNOMAGNETIC SEPARATION AND DIRECT CULTURE FOR THE ISOLATION OF VEROCYTOTOXIN- PRODUCING ESCHERICHIA COLI O157 FROM BOVINE FAECES. J. MED. MICROBIOL. 40:424-427. 17. CHUNG, C. T. AND MILLER, R. H. 1988. A RAPID AND CONVENIENT METHOD FOR THE PREPARATION AND STORAGE OF COMPETENT BACTERIAL CELLS. NUCL. ACIDS. RES. 16: 3580. 18. COCOLIN, L., ASTORI, G., MANZANO, M., CANTONI, C. AND COMI, G. 2000. DEVELOPMENT AND EVALUATION OF PCR-MICROPLATE CAPTURE HYBRIDIZATION METHOD FOR DIRECT DETECTION OF VEROTOXIGENIC ESCHERIC -RICHIA COLI STRAINS IN ARTIFICIALLY CONTAMINATED FOOD SAMPLES. INTER. J. FOOD MICROBIOL. 54:1-8. 19. CUBBON, M. D., COIA, J. E. AND HANSON, M. F. 1996. A COMPARISON OF IMMUNOMAGNETIC SEPARATION, DIRECT CULTURE, AND POLYMERASE CHAIN REACTION FOR THE DETECTION OF VEROCYTOTOXIN-PRODUC -ING ESCHERICHIA COLI O157 IN HUMAN FAECES. J. MED. MICROBIOL. 44:219-222. 20. DOYLE, M. P. AND SCHOENI, J. L. 1984. SURVIVAL AND GROWTH CHARACTERISTICS OF ESCHERICHIA COLI ASSOCIATED WITH HEMORRHAGIC COLITIS. APPL. ENVIRON. MICROBIOL. 48:855-856. 21. DOYLE, M. P. AND SCHOENI, J. L. 1989. ISOLATION OF ESCHERICHIA COLI O157:H7 FROM RETAIL FRESH MEATS AND POULTRY. APPL. ENVIRON. MICROBIOL. 53:2394-2396. 22. FANI, R., DAMIANI, G., DI SERIO, C., GALLORI, E., GRIFONI, A. AND BAZZICALUPO, M. 1993. USE OF RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) FOR GENERATING SPECIFIC DNA PROBES FOR MICROORGANISMS. MOL. ECO. 2: 243-250. 23. FARBER, J. M. AND ADDISON, C. J. 1994. RAPD TYPING FOR DISTINGUISHING SPECIES AND STRAINS IN THE GENUS LISTERIA. J. APPL. BACTERIOL. 77:242-250. 24. FENG, P. C. S. AND HARTMAN, P. A. 1982. FLUOROGENIC ASSAY FOR IMMEDIATE CONFIRMATION OF ESCHERICHIA COLI. APPL. ENVIRON. MICROBIOL. 43:1320-1329. 25. FENG, P. AND LAMPEL, K. A. 1994. GENETIC ANALYSIS OF uidA GENE EXPRESSION IN ENTEROHEM -ORRHAGIC ESCHERICHIA COLI O157:H7. MICROBIOL. 140:2101-2107. 26. FENG, P., WEAGANT, S. D. AND MONDAY, S. R. 2001. GENETIC ANALYSIS FOR VIRULENCE FACTORS IN ESCHERICHIA COLI O104:H21 THAT WAS IMPLICATED IN AN OUTBREAK OF HEMORRHAGIC COLITIS. J. CLIN. MICROBIOL. 39:24-28. 27. FORTIN, N. Y., MULCHANDANI, A. AND CHEN, W. 2001. USE OF REAL- TIME POLYMERASE CHAIN REACTION AND MOLECULAR BEACONS FOR THE DETECTION OF ESCHERICHIA COLI O157:H7. ANALY. BIOCHEM. 289:281-288. 28. FRATAMICO, P. M., SCHULTZ, F. J. AND BUCHANAN, R. L. 1992. RAPID ISOLATION OF ESCHERICHIA COLI O157:H7 FROM ENRICHMENT CULTURES OF FOODS USING AN IMMUNO- MAGNETIC SEPARATION METHOD. FOOD MICROBIOL. 9:105-113. 29. FRATAMICO, P. M., SACKITEY, S. K., WIEDMANN, M. AND DENG, M. Y. 1995. DETECTION OF ESCHERICHIA COLI O157:H7 BY MULTIPLEX PCR. J. CLIN. MICROBIOL. 33:2188-2191. 30. FRATAMICO, P. M. AND STROBAUGH, T. P. 1998. SIMULTANEOUS DETECTION OF SALMONELLA SPP. AND ESCHERICHIA COLI O157:H7 BY MULTIPLEX PCR. J. INDUS. MICROBIOL. BIOTECH. 21:92-98. 31. GICQUELAIS, K. G., BALDINI, M. M., MARTINEZ, J., MARTIN, W. C., PRADO, V., KAPER, J. B. AND LEVINE, M. M. 1990. PRACTICAL AND ECONOMICAL METHOD FOR USING BIOTINYLATED DNA PROBES WITH BACTERIAL COLONY BLOTS TO IDENTIFY DIARRHEA-CAUSING ESCHERICHIA COLI. J. CLIN. MICROBIOL. 28:2485-2490. 32. GILLILAND, G., PERRIN, S., BLANCHARD, K. AND BUNN, H. F. 1990. ANALYSIS OF CYTOKINE MRNA AND DNA: DETECTION AND QUANTITATION BY COMPETITIVE POLYMERASE CHAIN REACTION. PROC. NATL. ACAD. SCI. USA. 87:2725-2729. 33. GONZALEZ, I., GARCIA, T., FERNANDEZ, A., SANZ, B., HERNANDEZ, P. E. AND MARTIN, R. 1999. RAPID ENUMERATION OF ESCHERICHIA COLI OYSTERS BY A QUANTITATIVE PCR-ELISA. J. APPL. MICROBIOL.

-OBIOL. 86:231- 236. 34.GOODRIGE, L., CHEN, J. AND GRIFFITHS,M. 1999. THE USE OF A FLUORESCENT BACTERIOPHAGE AS -SAY FOR DETECTION OF ESCHERICHIA COLI O157:H7 IN INOCULATED GROUND BEEF AND RAW MILK. INT. J. FOOD MICROBIOL.47:43-50. 35.GRUNSTEIN, M. AND HOGNESS, D. S. 1975. COLONY HYBRIDI- ZATION : A METHOD FOR THE ISOLAT -ION OF CLONED DNAs THAT CONTAIN A SPECIFIC GENES. PROC. NATL.ACAD SCI.USA.72:3961-3965 36.HAHN, G. AND WITTRUCK, E. 1991. COMPARISON OF CHROMOGENIC AND FLUROGENIC SUBSTRATES FOR DIFFERENTIATION OF COLIFORM AND E. COLI IN SOFE CHEESES. ACTA. MICROBIOL. HUNG. 38: 265-271. 37.HILL, C. W., SANDT, C. H. AND VLAZNY, D. A. 1994. RHS ELEMENTS OF ESCHERICHIA COLI : A FAMILY OF GENETIC COMPOSITES EACH ENCODING A LARGE MOSAIC PROTEIN. MOL. MICROBIOL.12:86 5-871. 38.HILL, C. W., FEULNER, G., BRODY, M. S., ZHAO, S., SADOSKY, A. B. AND SANDT, C. H. 1995. CORRELATION OF RHS ELEMENTS WITH ESCHERICHIA COLI POPULATION STRUCTURE. GENETICS 141:15 -24. 39.HORNES, E., WASTESON, Y. AND OLSVIK, O. 1991. DE- TECCTION OF ESCHERICHIA COLI HEAT-STA -BLE ENTEROTOXIN GENES IN PIG STOOL SPECIMENS BY AN IMMOBILIZED, COLORIMETRIC NESTED PO -LYMERASE CHAIN REACTION. J. CLIN. MICROBIOL. 29:2375-2379. 40.ISLAM, D.AND LINDBERG, A. A. 1992.DETECTION OF SHIGELLA DYSENTERIAE TYPE I AND SHIGELLA FLEXNERI IN FECES BY IMMUNOMAGNETIC ISOLATION AND PLOYMERASE CHAIN REACTION.J. CLIN.MIC -ROBIOL. 30:2801-2806. 41.JOHNE,B.,JARP,J.AND HAAHEIM,L.R.1989.STAPHLOCOCCUS AUREUS EXOPOLYSACCHARIDE IN VIVO DEM -ONSTRATED BY IMMUNOMAGNETIC SEPARATION AND ELECTRON MICROSCOPY. J. CLIN. MICROBIOL. 27 :1613- 1615 42.JONES, D. 1988. COMPOSITION AND PROPERTIES OF THE FAMILY ENTEROBACTERIACEAE.J.APP.BACTE -RIOL. SYMPO. SUPPLE. 1-19. 43.KADO,C.I.AND LIU, S. T. 1981. RAPID PROCEDURE FOR DETECTION AND ISOLATION OF LARGE AND SMALL PLASMIDS. J. BACTERIOL. 145:1365-1373. 44.KARCH, H. AND MEYER, T. 1989. EVALUTION OF OLIGO- NUCLEOTIDE PROBES FOR IDENTIFICATION ON SHIGA-LIKE TOXIN PRODUCING ESCHERICHIA COLI. J. CLIN. MICROBIOL. 27:1180-1186. 45.KARCH,H.,JANETZKI-MITTMANN, C. ALEKSIC,S. AND DATZ,M.1996.ISOLATION OF ENTEROHEMORRHAG -IC SYNDROME BY USING IMMUNOMAGNETIC SEPARATION, DNA-BASED METHODS, AND DIRECT CULTURE. J. CLIN. MICROBIOL. 34:516-519. 46.KARMALI, M. A., PETRIC, M., LIM, C., FLEMING, P. C., ARBUS, G. S. AND LIOR, H. 1985. THE ASSOCIATION BETWEEN THE IDIOPATHIC HEMOLYTIC UREMIC SYNDROME AND INFECTION BY VEROT -OTOXIN PRODUCING ESCHERICHIA COLI. J. INFECT. DISE. 151:775-782. 47.KATZ, E. D. AND DONG, M. W. 1990. RAPID ANALYSIS AND PURIFICATION OF POLYMERASE CHAIN REACTION PRODUCTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPH. BIOTECH- NIQUES 8:546-555. 48.KAWAI, S., MAEKAWAJIRI, S. AND YAMANE, A. 1993. A SIMPLE METHOD OF DETECTING AMPLIFIED DNA WITH IMMOBILIZED PROBES ON MICROTITER WELLS. ANAL. BIOCHEM. 209:63-69. 49.KILLAN,M.AND BULLOW,P.1976.RAPID DIAGNOSIS OF ENTEROBACTERIACEAE.ACTA PATHOL.MICROBIOL. SCAND. SECT. B84: 245-251. 50.LE MINOR,L.1979.TETRATHIONATE-REDUCTASE, b-GLUCURO- NIDASE AND ONPG-TEST IN THE GENUS SALMONELLA. ZENTRALBL. BAKTERIOL. PARASITENKD. INFEKTIONSKR. HYG. ABT. 1 ORIG. REICHE A 243: 321-325. 51.LEVINE, M. M. 1987. ESCHERICHIA COLI THAT CAUSE DIARRHEA: ENTEROTOXIGENIC, ENTEROPATHO -GENIC, ENTEROINVASIVE, ENTEROHEMORRHAGIC, AND ENTEROADHERENT ESCHERICHIA COLI. J.INFE -CT. DIS. 155: 377-389. 52.LI,W.AND DRAKE,M.A.2001. DEVELOPMENT OF A QUANTITATIVE COMPETITIVE PCR ASSAY FOR DETECT -ION AND QUANTIFICATION OF ESCHERICHIA COLI O157:H7 CELLS. APPL. ENVIRON. MICROBIOL.67: 3291-3294. 53.LIN, C. K. AND TSEN, H. Y. 1999. COMPARISON OF THE PARTIAL 16S RRNA GENE SEQUENCES AND DEVELOPMENT OF OLIGONUCLEOTIDE PROBES FOR THE DETECTION OF ESCHERICHIA COLI CELLS IN WA -TER AND MILK. FOOD MICROBIOL. 16:551-562 54.LIU, H. W., GOODWIN, P. H. AND KUSKE, C. R. 1994. QUANTIFICATION OF DNA FROM THE ASTER YELLOWS MYCOPLASMALIKE ORGANISIN IN ASTER LEAFHOPPERS (MACROSTELAS FASCIFRONSSTAL) BY A COMPETITIVE POLYMERASE CHAIN REACTION. SYSTEM. APPL. MICROBIOL. 17:274-280. 55.LUND, A., WASTESON, Y. AND OLSVIK, O. 1991. IMMUNO- MAGNETIC SEPARATION AND DNA HYBRIDI -ZATION FOR DETECTION OF ENTEROTOXIGENIC ESCHERICHIA COLI IN A PIGLET MODEL. J. CLIN. MICROBIOL. 29:2259-2262. 56.MARTINEZ-MURCIA, A. J. AND RODRIQUEZ-VALERA, F. 1994. THE USE OF ARBITRARILY PRIMED PCR (AP-PCR) TO DEVELOP TAXA SPECIFIC DNA PROBE OF KNOWN SEQUENCE. FEMS MICROBIOL. LETT. 124:265-270. 57.MANAFI, M., KNEIFEL, W. AND BASCON, S. 1989. FLURO- GENIC AND CHROMOGENIC SUBSTRATES USED IN BACTERIAL DIAGNOSIS. MICROBIOL. REV. 55: 335-348. 58.MAZURIER, S. I., VAN DE GIESSEN, A., HEUVELMAN, K. AND WERNARS, K. 1992. RAPD ANALYSIS OF CAMPYLOBACTER ISOLATES : DNA FINGERPRINTING WITHOUT THE NEEDS TO PURIFY DNA. LETT. APPL. MICROBIOL. 14:260-262. 59.MENG, J., DOYLE, M. P., ZHAO, T. AND ZHAO, S.1995. DETECTION AND CONTROL OF ESCHERICHIA COLI O157:H7 IN FOOD. TRENDS FOOD SCI. TECHNOL. 5:179-185. 60.MERCK. 1996. REVIEW OF CHROMOCULT-DEHYDRATED CULTURE MEDIA IN "MICROBIOLOGY MANUAL MERCK ".310-311. MERCK KGAA, DARMSTADT, DEUTSCHLAND. 61.MIYAMOTO, T., TIAN,H.Z.,OKABE,T.,TREVANICH,S.,ASOH,K.,TOMODA,S.,HONJOH,K.I.AND HATANO,S. 1998.APPLICATION OF RANDOM AMPLIFIED POLYMORPHIC DNA ANALYSIS FOR DETECTION OF SALMONEL -LA SPP. IN FOODS. J. FOOD PROT. 61: 785-791. 62.MOBERG, L. J. 1985. FLUROGENIC ASSAY FOR RAPID DE- TECCTION OF ESCHERICHIA COLI IN FOOD .APPL. ENVIRON.

MICROBIOL. 50: 1383-1387. 63. MORTLOCK, S. 1994. RECOVERY OF ESCHERICHIA COLI O157:H7 FROM MIXED SUSPENSIONS: EVALUATION AND COMPARISON OF PRE-COATED IMMUNOMAGNETIC BEADS AND DIRECT PLATING. BR. J. BIOMED. SCI. 51:207-214. 64. MULLIS, K. AND FALOONA, F. A. 1987. SPECIFIC SYNTHESIS OF DNA IN VITRO VIA A POLYMERASE CATALYZED CHAIN REACTION. IN METHODS ENZYMOLOGY. 155:335-350. 65. NATARO, J. P. AND KAPER, J. B. 1998. DIARRHEAGENIC ESCHERICHIA COLI. CLIN. MICROBIOL. REV. 11: 142-201. 66. NEILL, M. A. 1989. E. COLI O157:H7 CURRENT CONCEPTS AND FUTURE PROSPECTS. J. FOOD SAFETY. 10:99-106. 67. NEWLAND, J. W. AND NEILL, R. J. 1988. DNA PROBES FOR SHIGA-LIKE TOXIN I AND II AND FOR TOXIN-CONVERTING BACTERIOPHAGES. J. CLIN. MICROBIOL. 26:1292-1297. 68. OAKEY, H. J., ELLIS, J. T. AND GIBSON, L. F. 1998. THE DEVELOPMENT OF RANDOM DNA PROBES SPECIFIC FOR AEROMONAS SALMONICIDA. J. APPL. MICROBIOL. 84: 37-46. 69. O'BRIEN, A. D., LEVECK, G. D., THOMPSON, M. R. AND FORMAL, S. B. 1982. PURIFICATION OF SHIGELLA DYSENTERIAE 1-LIKE CYTOTOXIN BY ESCHERICHIA COLI. J. INFECT. DIS. 146:763-769. 70. O'BRIEN, A. D. AND LEVECK, G. D. 1983. PURIFICATION AND CHARACTERIZATION OF SHIGELLA DYSENTERIAE 1-LIKE TOXIN PRODUCED BY ESCHERICHIA COLI. INFECT. IMMUN. 40:675-683. 71. OKREND, A. J. G., ROSE, B. E. AND LATTUADA, C. P. 1992. ISOLATION OF ESCHERICHIA COLI O157:H7 USING O157 SPECIFIC ANTIBODY COATED MAGNETIC BEADS. J. FOOD PROT. 55:214-217. 72. OLSVIK, O., POPOVIC, T., SKJERVE, E., CUDJOE, K. S., HORNES, E., UGELSTAD, J. AND UHLEN, M. 1994. MAGNETIC SEPARATION TECHNIQUES IN DIAGNOSTIC MICROBIOLOGY. CLIN. MICROBIOL. REV. 7:43-54. 73. PADHYE, N. V. AND DOYLE, M. P. 1991. PRODUCTION AND CHARACTERIZATION OF A MONOCLONAL ANTIBODY SPECIFIC FOR ENTEROHEMORRHAGIC ESCHERICHIA COLI OF SEROTYPE O157:H7 AND O26:H11. J. CLIN. MICROBIOL. 29:99-103. 74. PANNETIER, C., DELASSUS, S., DARCHE, S., SAUCIER, C. AND KOURILSKY, P. 1993. QUANTITATIVE TITRATION OF NUCLEIC ACIDS BY ENZYMATIC AMPLIFICATION REACTION RUN TO SATURATION. NUCL. ACIDS RE-S. 21:577-583. 75. PATON, A. W., PATON, J. C., GOLDWATER, P. N. AND MANNING, P. A. 1993. DIRECT DETECTION OF ESCHERICHIA COLI O157 DIRECTLY FROM STOOL SPECIMENS. J. CLIN. MICROBIOL. 34:988-990. 76. PIATAK, M. JR., LUK, K. C., WILLIAMS, B. AND LIFSON, J. D. 1993. QUANTITATIVE COMPETITIVE POLYMERASE CHAIN REACTION FOR ACCURATE QUANTITATION OF HIV DNA AND RNA SPECIES. BIOTECHNIQUES 14:70-81. 77. PYLE, B. H., BROADAWAY, S. C. AND MCFETERS, G. A. 1999. SENSITIVE DETECTION OF ESCHERICHIA COLI O157:H7 IN FOOD AND WATER BY IMMUNOMAGNETIC SEPARATION AND SOLID-PHASE LASER CYTOMETRY. APPL. ENVIRON. MICROBIOL. 65:1966-1972. 78. QUERE, F., DESCHAMPS, A. AND URDADI, M. C. 1997. DNA PROBE AND PCR-SPECIFIC REACTION FOR LACTOBACILLUS PLANTARUM. J. APPL. MICROBIOL. 82: 783-790. 79. RAMOTAR, K., WALDHART, B., CHRUCH, D., SZUMSKI, R. AND LOUIE, T. 1995. DIRECT DETECTION OF VEROTOXIN-PRODUCING ESCHERICHIA COLI IN STOOL SAMPLES BY PCR. J. CLIN. MICROBIOL. 33:519-524. 80. RILEY, L. W., REMIS, R., HELGERSON, S. D., JOHNSON, H. B., HARGRETT, N. T., BLAKE, P. A. AND COHEN, M. L. 1983. HEMORRHAGIC COLITIS ASSOCIATED WITH A RARE ESCHERICHIA COLI SEROTYPE, N. ENGL. J. MED. 308:681-685. 81. RIPPEY, S. R., CHANDLER, L. A. AND WATKINS, W. D. 1987. FLUOROMETRIC METHOD FOR THE ENUMERATION OF ESCHERICHIA COLI IN MOLLUSCAN SHELLFISH. J. FOOD PROT. 50: 685-690. 82. ROBINSON, B. J. 1984. FLUOROGENIC ASSAY FOR DETECTION OF E. COLI IN FOODS. APPL. ENVIRON. MICROBIOL. 48: 285-288. 83. RONNER, A. B. AND CLIVER, D. O. 1990. ISOLATION AND CHARACTERIZATION OF COLIPHAGE SPECIFIC FOR ESCHERICHIA COLI O157:H7. J. FOOD PROT. 53:944-947. 84. SAMADPOUR, M., LISTON, J., ONGERTH, J. E. AND TARR, P. I. 1990. EVALUATION OF DNA PROBES FOR DETECTION OF SHIGA LIKE TOXIN-PRODUCING ESCHERICHIA COLI IN FOOD AND CALF FECAL SAMPLES. APPL. ENVIRON. MICROBIOL. 56:1212-1215. 85. SANDERSON, M. W., GAY, J. M., HANCOCK, D. D., GAY, C. C., FOX, L. K. AND BESSER, T. E. 1995. SENSITIVITY OF BACTERIOLOGIC CULTURE FOR DETECTION OF ESCHERICHIA COLI O157:H7 IN BOVINE FECES. J. CLIN. MICROBIOL. 33:2616-2619. 86. SCHMIDT, H., RUSSMANN, H. AND KARCH, H. 1993. VIRULENCE DETERMINANTS IN NONTOXINOGENIC ESCHERICHIA COLI O157 STRAINS THAT CAUSE INFANTILE DIARRHEA. INFECT. IMMUN. 61:4849-4898. 87. SIEBERT, P. D. AND LARRICK, J. W. 1993. PCR MIMIC: COMPETITIVE DNA FRAGMENTS FOR USE AS INTERNAL STANDARDS IN QUANTITATIVE PCR. BIOTECHNIQUES 14:244-249. 88. SKJERVE, E., RORVIK, L. M. AND OLSVIK, O. 1990. DETECTION OF LISTERIA MONOCYTOGENES IN FOODS BY IMMUNOMAGNETIC SEPARATION. APPL. ENVIRON. MICROBIOL. 56:3478-3481. 89. SOUTHERN, E. M. 1975. DETECTION OF SPECIFIC SEQUENCES AMONG DNA FRAGMENTS SEPARATED BY GEL ELECTROPHORESIS. J. MOL. BIOL. 98:503-517. 90. STEPHEN, R. 1996. RANDOMLY AMPLIFIED POLYMORPHIC DNA (RAPD) ASSAY FOR GENOMIC FINGERPRINTING OF BACILLUS CEREUS. INT. J. FOOD MICROBIOL. 31: 311-316. 91. STROCKBINE, N. A., JACKSON, M. P., SUNG, L. M., HOLMES, R. K. AND O'BRIEN, A. D. 1988. CLONING AND SEQUENCING OF THE GENES FOR SHIGA TOXIN FROM SHIGELLA DYSENTERIAE TYPE I. J. BACTERIOL. 170:1116-1122. 92. TAKAO, T., TANABE, T., HONG, Y. M., SHIMONISHI, Y., KURAZONO, H., YUTSUDO, T., SASAKAWA, C., YOSHIKAWA, M. AND TAKEDA, Y. 1988. IDENTIFICATION OF MOLECULAR STRUCTURE OF SHIGA-LIKE TOXIN I (VT I) FROM ESCHERICHIA COLI O157:H7: OVERVIEW OF CLINICAL AND EPIDEMIOLOGICAL ISSUES. J. FOOD PROT. 57:632-636. 93. THOMAS, S., SMITH, H. R., WILLSHAW, G. A. AND ROWE, B. 1991. NON-RADIOACTIVELY LABELED POLYNUCLEOTIDE AND OLIGONUCLEOTIDE DNA PROBES, FOR SELECTIVELY

DETECTING ESCHERICHIA COLI STRAINS PRODUCING VEROCYTOTOXINS VT1, VT2 AND VT2 VARIANT. MOL. CELL. PROBES.5:129-135. 94.TILSALA-TIMISJARVI, A. AND ALATOSSAVA, T. 1998. STRAIN-SPECIFIC IDENTIFICATION OF PROBIOTIC LACTOBACILLUS RHAMNOSUS WITH RANDOMLY AMPLIFIED POLYMORPHIC DNA-DERIVED PCR PRIMERS. APPL. ENVIRON. MICROBIOL. 64:4816-4819. 95.TOMOYASU, T. 1992. DEVELOPMENT OF THE IMMUNOMAGNETIC ENRICHMENT METHOD SELECTIVE FOR VIBRIO PARAHAEMOLYTICUS SEROTYPE-K AND ITS APPLICATION FOR FOOD-POISONING STUDY. APPL. ENVIRON. MICROBIOL. 58:2679-2682. 96.TOMOYASU, T. 1998. IMPROVEMENT OF THE IMMUNOMAGNETIC SEPARATION METHOD SELECTIVE FOR ESCHERICHIA COLI O157 STRAINS. APPL. ENVIRON. MICROBIOL. 64:376-382. 97.TORTORELLO, M. L., REINEKE, K. F., STEWART, D. S. AND RAYBOURNE, R. B. 1998. COMPARISON OF METHODS FOR DETERMINING THE PRESENCE OF ESCHERICHIA COLI O157:H7 IN APPLE JUICE. J. FOOD PROT. 61:1425-1430. 98.TOTH, I., BARRETT, T. J., COHEN, M. L., RUMSCHLAG, H. S., GREEN, J. H. AND WACHSMUTH, I. K. 1991. ENZYME-LINKED IMMUNOSORBENT ASSAY FOR PRODUCTS OF THE 60-MEGADALTON PLASMID OF ESCHERICHIA COLI SEROTYPE O157:H7. J. CLIN. MICROBIOL. 29:1016-1019. 99.TRIPORI, S., KARCH, H. AND WACHSMUTH, I. K. 1987. ROLE OF A 60-MEGADALTON PLASMID AND SHIGA-LIKE TOXINS IN PATHOGENESIS OF INFECTION CAUSED BY ENTEROHEMORRHAGIC ESCHERICHIA COLI O157:H7 IN GNOTOBIOTIC PIGLETS. INFECT. IMMUN. 55:3117-3125. 100.TSEN, H. Y., LIOU, J. W. AND LIN, C. K. 1994. POSSIBLE USE OF A POLYMERASE CHAIN REACTION METHOD FOR SPECIFIC DETECTION OF SALMONELLA IN BEEF. J. FERMENT. BIOENG.77:137-143. 101.TSEN, H. Y., LIN, C. K. AND CHI, W. R. 1998. DEVELOPMENT AND USE OF 16S RRNA GENE TARGETED PCR PRIMERS FOR THE IDENTIFICATION OF ESCHERICHIA COLI CELLS IN WATER. J. APPL. MICROBIOL. 85:554-560. 102.VENKATESWARAN, K., KAMIJOH, Y., OHASHI, E. AND NAKANISHI, H. 1997. A SIMPLE FILTRATION TECHNIQUE TO DETECT IN BEEF BY MULTIPLEX PCR. APPL. ENVIRON. MICROBIOL. 63:4127-4131. 103.WANG, A. M., DOYLE, M. V. AND MARK, D. F. 1989. QUANTITATION OF MRNA BY THE POLYMERASE CHAIN REACTION. PROC. ACAD. SCI. USA. 86:9717-9721. 104.WANG, Y. D., ZHAO, S. AND HILL, C. W. 1998. RHS ELEMENTS COMPRISE THREE SUBFAMILIES WHICH DIVERGED PRIOR TO ACQUISITION BY ESCHERICHIA COLI. J. BACTERIOL. 180:4102-4110. 105.WERNARS, K., HEUVELMAN, C. J., CHAKRABORTY, T. AND NOTERMANS, S. H. W. 1991. USE OF THE POLYMERASE CHAIN REACTION FOR DIRECT DETECTION OF LISTERIA MONOCYTOGENES IN SOFT CHEESE. J. APPL. BACTERIOL. 70:121-126. 106.WILLIAMS, J. G., KUBELIK, A. R., LIVAK, K. J., RAFALSKI, J. A. AND TINGEY, S. V. 1990. DNA POLYMORPHISMS AMPLIFIED BY ARBITRARY PRIMERS ARE USEFUL AS GENETIC MARKERS. NUCLEIC ACIDS RES. 18: 6531-6535. 107.WITHAM, P. K., YAMASHIRO, C. T., LIVAK, K. J. AND BATT, C. A. 1996. A PCR-BASED ASSAY FOR THE DETECTION OF ESCHERICHIA COLI SHIGA-LIKE TOXIN GENES IN GROUND BEEF. APPL. ENVIRON. MICROBIOL. 62:1347-1353.