Thesis Title Detection of *Salmonella spp.* by Genus Specific DNA Probe

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ABSTRACT

DNA probe is one of powerful tools for organisms detection. The technique is rapid, does not require complicate instrument and gives reliable results. In this study, DNA probes for *Salmonella* detection was studied. The DNA fragments to be used as probe were cloned by selection on differential media, Rambach agar. The principle of selection depends on a major component of Rambach agar, 1,2 propanediol, to be metabolized to propionic acid by propanediol dehydratase enzyme of *Salmonella spp.* and produced crimson-growth of their colonies on the agar plates (except *S.* Typhi, *S.* Paratyphi A). The chromosomal DNA from *S.* Typhimurium 23566 was cut by *Eco*RI and ligated to *Eco*RI linearized pGEM7 and transformed into *E. coli*. The clones harbored the gene for propanediol utilization (*pdu*) was selected by screening for pink colonies on Rambach agar. Plasmids from these clones were isolated and was found to contain a 10 kb *Eco*RI insert. A recombinant plasmid (designated as pDU1) was subcloned for restriction map analysis and different colony color development was observed. A 3.2 kb *Cla*I, a 1.8 kb

*Cla*I-*Eco*RI and a 2 kb *Xho*I-*Kpn*I from the insert were tested for their specificity by hybridization with 85 *Salmonella* serotypes, *Enterobacteriaceae* and some gram positive bacteria. A 2 kb *Xho*I-*Kpn*I fragment was selected to be used for further analysis such as sensitivity test and sequencing. It was found that at least 10^5 bacteria/dot was required for positive hybridization to the 2 kb of *Xho*I-*Kpn*I (probe PDU-XK2). Nucleotide sequences of this probe revealed that the fragment was 2,142 bp and open reading frame encoding a 15 kDa and 14.7 kDa proteins were found. The function of protein is not yet identified. It may function as regulatory protein since deletion of DNA fragment encoded these proteins resulted in red colony formation. The sequence information obtained will be useful in primer synthesis for increasing sensitivity of detection by other techniques such as PCR.