

พิมพ์ต้นฉบับบทคัดย่อวิทยานิพนธ์ภายในกรอบสี่เหลี่ยมนี้เพียงแผ่นเดียว

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SUPAKIJ SORNPAJAK : GENOMIC DNA FINGERPRINTING OF *Lactobacillus pentosus* and *Lactobacillus plantarum* FROM TRADITIONAL FERMENTED FOOD USING RANDOM AMPLIFIED POLYMORPHIC DNA. THESIS ADVISOR : ASSIS. PROF. SUWIMON KEERATIPIBUL, Ph. D.
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58 strains of lactic acid bacteria isolated from traditional fermented foods eg. *nham*, *pla-som* and *phak-dong* are classified using arbitrary primer polymerase chain reaction. A method of extraction for the total genomic DNA was developed. The bacterial cells were treated with lysozyme (100 µg/ ml Tris-EDTA-Sodium chloride; TES) and mutanolysin (1 U/ µl TES) followed by proteinase K (1 mg/ ml TES). Cells were lysed by sodium dodecyl sulfate (20 % in TES). The cell wall and cell membrane were removed by CTAB/NaCl (10 % in water) and chloroform / isoamyl alcohol (24 : 1) extraction. The total yield of DNA of 3 µg were obtained from 2 ml culture grown to OD₆₀₀ = 3.5. The chromosomal DNAs of the three type strains of *L. pentosus* DSM 20314, *L. plantarum* DSM 20174 and *P. pentosaceus* DSM 20336 were amplified using 100 different arbitrary primers (10 mers oligonucleotide). The four oligonucleotide primers of 5'-AGTCAGCCAC-3', 5'-CAATCGCCGT-3', 5'-GATGACGCC-3' and 5'-ACTTCGCCAC-3' were found to differentiate between the species of *L. pentosus* and *L. plantarum*. The optimal amplification conditions were; denaturation at 94 °C for 1 minute, annealing at 35 °C for 1 minute and nucleotide extension at 72 °C for 2 minute. The 10 µl reaction composed of 10 mM Tris-HCl, 2 mM MgCl₂, 50 mM KCl, 0.001% gelatin, 100 mM deoxynucleoside triphosphate, 0.2 µM arbitrary primer, 1.0 unit Taq DNA Polymerase and 3.0 ng of DNA template. The similarity indice of different strains of bacteria were calculated using :

$$\text{Similarity Index} = 2n_{xy} / n_x + n_y$$

where n_x and n_y are the number of bands in the lanes X and Y

n_{xy} is the number of bands common to the lanes X and Y

The similarity indice were used to construct a dendrogram. The 58 strains could be divided into 4 groups. The first group consisted of 21 different strains of *L. pentosus* which had the similarity indice between 85 to 40% to the type strain. The second group consisted of 25 different strains of *L. plantarum* which had the similarity indice between 92 to 23% to the type strain. The third group of bacteria consisted of 7 different strains which had the similarity indice between 94 to 58% to the type strain of *P. pentosaceus* DSM 20336. And the fourth group which were 4 different bacterial strains 1145, FN 12-1, P 322-1 and P 46-1 with the similarity indice of 7%, 7%, 10% and 13% to both type strains of *L. pentosus* DSM 20314 and *L. plantarum* DSM 20174 and 4% to the the type strain of *P. pentosaceus* DSM 20336. These strains were unidentified because of their low values of similarity indices to the type strains. These results obtained from DNA fingerprints were in good agreement with biochemical results.

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