CHAPTER V

CONCLUSIONS

One hundred and twenty soil samples from Pua and Santisuk districts, Nan province were screened for cellulase-producing bacteria at 40° C by enrichment culture technique. Seventy five isolates were cellulase producer as shown by clearance zone surround colonies grown on carboxymethyl cellulose-basal medium after flooding by Congo red solution.

Seventy-five isolates were divided into 9 groups based on their cell morphology and cultural characteristics, physiological and biochemical characteristics The representative strains of each groups were selected and characterized by their chemotaxonomic and molecular techniques.

- Group 1, P5-5: Gram-positive, spore-forming, rod-shaped. They showed circular, raised, entire margins, smooth, translucent, white colour colonies, and contained *meso*-diaminopimelic acid as a diagnostic diamino in the cell wall peptidoglycan. It had MK-7 as a major menaquinone, and had G+C content 54.2 mol%. The 16S rDNA sequence was 97.2 % similar to *Brevibacillus* .
- Group2, P2-1: Gram-positive, spore-forming, rod-shaped . They showed circular, raised, entire margins, smooth, dull, white cream colour colonies, and contained *meso*-diaminopimelic acid as a diagnostic diamino in the cell wall peptidoglycan. It had MK-7 as a major menaquinone, and had G+C content 52.7 mol%. The 16S rDNA sequence was 96.1% similar to *Paenibacillus cineris* KCTC 3998^T.
- Group 3, S10-4: Gram-positive, spore-forming, rod-shaped. They showed circular, raised, entire margins, smooth, dull, white colour colonies, and contained *meso*-diaminopimelic acid as a diagnostic diamino in the cell

wall peptidoglycan. It had MK-7 as a major menaquinone, and had G+C content 53.5 mol%. The 16S rDNA sequence was 99.1 % similar to to *Paenibacillus cineris* KCTC 3998^T.

- Group 4, P2-3 and P4-7: Gram-positive, spore-forming rods . They showed irregular, raised, undulate margins, smooth, dull, white colour colonies, and contained *meso*-diaminopimelic acid in cell wall peptidoglycan. It had MK-7 as a major menaquinone, and had G+C content 46.3 and 47.8 mol %. The 16S rDNA sequence were 95.5and 97.8 % similar to *Bacillus subtilis* KCTC 3135^T.
- Group 5, S9-2: Gram-positive, spore-forming rods. They showed circular, flat, undulate margins, smooth, dull, white colour colonies, and contained *meso*-diaminopimelic acid in cell wall peptidoglycan. It had MK-7 as a major menaquinone and had G+C content 46.5 mol %. The 16S rDNA sequence was 95.6 % similar to *Bacillus subtilis* KCTC 3135^T.
- Group 6, P6-8: Gram-positive, spore-forming rods. They showed circular, raised, entire margins, smooth, dull, viscid, white colour colonies, and contained *meso*-diaminopimelic acid in cell wall peptidoglycan. It had MK-7 as a major menaquinone and had G+C content 42.4 mol %. The 16S rDNA sequence was 94.3% similar to *Bacillus subtilis* KCTC 3135^T .
- Group 7, P4-8, P5-2, P7-1 and P7-3: Gram-positive, spore-forming rods . They showed irregula, rough, dull, no pigmentation, raised, lobate margins yellowish colour colonies, and contained *meso*-diaminopimelic acid in cell wall peptidoglycan. It had MK-7 as a major menaquinone and had G+C content 42.3, 41.6, 44.6, and 43.2 mol %. The 16S rDNA sequence were 96.1, 96.8, 94.5, 98.3% similar to *Bacillus subtilis* KCTC 3135^T .
- Group 8, P1-5: Gram-positive, spore-forming rods. They showed circular, smooth, dull, pigmentation, raised, entire margin, yellow colour colonies, and contained *meso*-diaminopimelic acid in cell wall peptidoglycan. It had

MK-7 as a major menaquinone and had G+C content 41.5 mol %. The 16S rDNA sequence was 99.3 % similar to *Bacillus subtilis* KCTC 3135^{T}.

- Group 9, P6-7: Gram-positive, spore-forming rods. They showed circular, dull white, wrinkled, dull, no pigmentation, raised, undulate margin, and contained *meso*-diaminopimelic acid in cell wall peptidoglycan. It had MK-7 as a major menaquinone and had G+C content 42.7 mol %. The 16S rDNA sequence was 99.9 % similar to *Bacillus subtilis* KCTC 3135^{T}.

Cellulase producing bacteria, 75 isolates exhibited a cellulolytic clearance zone diameter ranged 1.22-8.5 cm. Strain P4-6 identified as *Bacillus* produced maximum endoglucanase of 0.015 units/ml. Strain P2-1 identified as *Paenibacillus* showed highest hydrolysis capacity (HC value) of 8.5 cm. Two most highest endoglucanase producing strains (P4-6, P3-1) and representatives of a high hydrolysis capacity value strains; P2-1, P2-3, P7-7; were selected to verify an effect of pH and incubation temperature on their cellulase production. Optimal pH and temperature for cellulase production of all the isolates tested were 7.0 and 50^{0}C. Optimal pH and temperature for cellulase activity of P3-1 and P4-6 were 7.0 and 50^{0}C.

The cellulase producing bacteria were found to be diverse species in soil samples collected in Nan province. One strain of *Brevibacillus*, 1 *Paenibacillus*, and 7 *Bacillus* strains isolated from Pua district and one strain of *Bacillus* from Santisuk district were the novel species. One *Paenibacillus* strain should be identified as *P. cineris* from Santisuk district and 2 *Bacillus* strains from Pua district should be identified as *Bacillus subtilis*. However, their taxonomic status should be confirmed by the DNA-DNA hybridization experiments.