CHAPTER V

CONCLUSIONS

Fourty soil samples collected in Tadan district, Nakhon Nayok province; Nasuan district, Suratthani province; Hatyai district, Songkla province; Puak district, Nan province and 10 biofertilizers were screened for cellulase-producing bacteria at 40 $^{\circ}$ C by enrichment culture technique. Twenty seven isolates were cellulase producers as shown by clear zone surround colonies grown on carboxymethyl cellulose-basal medium after flooding by Congo red solution.

The twenty seven isolates were divided into 6 groups based on their cell morphologies, cultural, physiological and biochemical characteristics. Representative strains of each groups were selected and characterized by chemotaxonomic and molecular techniques.

- Group 1, PA4-1: Gram-positive, spore forming, rod-shaped. Colony was round with raised margin, umbonate, translucent, off-white color. The bacteria contained meso-diaminopimelic acid as a diagnostic diamino in cell wall peptidoglycan. It had MK-7 as a major menaquinone. The 16S rRNA gene sequence (1080 bp) was 97.2 % similar to *Cohnella thermotolerans* CCUG 47242^{T}.
- Group 2, PBS5: Gram-positive, spore forming bacilli. It showed circular, umbonate, entire margins, opaque, cream color colony, and contained meso-diaminopimelic acid as a diagnostic diamino in cell wall peptidoglycan. It had MK-7 as a major menaquinone. The 16S rRNA gene sequence (1493 bp) was 98.8% similar to *Bacillus drentensis* LMG 21831^{T}.
- Group 3, T3-3: Gram-positive, spore forming bacilli. Colony was circular, concentric, umbonate, opaque, white color. The bacteria contained meso-diaminopimelic acid as a diagnostic diamino in the cell wall peptidoglycan, had MK-7 as a major menaquinone. The 16S rRNA gene sequence (1204 bp) was 88.0% similar to *Bacillus megaterium* IAM 13418^{T}.
- Group 4, T3-2: Gram-positive, spore forming bacilli. It showed irregular and spreading, erose margins, flat, opaque, cream color colony, and contained meso-diaminopimelic acid as a diagnostic diamino in cell wall peptidoglycan. It had MK-7

as a major menaquinone. The 16S rRNA gene sequence (1167 bp) was 88.7% similar to *Bacillus cereus* IAM 12605^{T}.

- Group 5, N14-2: Gram-negative rods. It showed circular with flat and rough margins, umbonate, translucent, white cream color colony, and did not contain *meso*-diaminopimelic acid as a diagnostic diamino in cell wall peptidoglycan. The 16S rRNA gene sequence (803 bp) was 93.0% similar to *Pseudomonas pseudoalcaligenes* JCM 5968^{T}.
- Group 6, T6-4: Gram-negative rods. Colony was circular, convex or drop-like, entire margins, translucent, no pigment. The bacteria did not contain *meso*-diaminopimelic acid as a diagnostic diamino in cell wall peptidoglycan. The 16S rRNA gene sequence (800 bp) was 92.7% similar to *Pseudomonas nitroreducens* DSM 14399^{T}.

The 27 cellulase producing bacteria isolated exhibited a cellulolytic clearance zone with diameter range 0-2.1 cm. Strain PB11 produced maximum endoglucanase (0.015 units/ml) and strain PA4-3 produced maximum ß-glucosidase (0.005 units/ml). Strain PA4-1 identified as *Cohnella* showed highest hydrolysis capacity (HC value) at 10. Five strains which produced highest endoglucanase (PB11), highest ß-glucosidase (PA4-3), high for both endoglucanase and ß-glucosidase (PA3-3), highest HC value (PA4-1) and clear zone on cellulose powder medium (PD1-2), were selected to verify an effect of pH and incubation temperature on cellulase activity. Optimal pH and temperature for endoglucanase activity of strain PB11 were 7.5 and 60° C. At the optimal conditions, the endoglucanase activity of strain PB11 was 0.11 units/ml. Optimal pH and temperature of ß-glucosidase activity of strain PA4-3 was 7 and 60° C, respectively. The activity at the optimal conditions was 0.0091 units/ml. Cocultivation of the above five strains resulted in highest filter paper degradation, dry weight lost of the filter paper was 5%. Maximum reducing sugar occurred when strain PB11 was cocultivated with strain PA4-3. This confirmed an important of endoglucanase and ß-glucosidase ratio in cellulase system.

Species of cellulase producing bacteria in soil samples collected was diverse. Two *Bacillus* and one *Pseudomonas* in Tadan district, Nakhon Nayok province, one *Bacillus* in Nasuan district, Suratthani province, one *Pseudomonas* in Puak district, Nan province were found to be novel species. *Cohnella* was isolated from biofertilizer samples and it was a novel species. However, taxonomic status of some isolates should be confirmed by DNA-DNA hybridization.

Xylanase producing bacteria were screened from fourty five soil samples collected in Nangrong and Tadan district, Nakhon Nayok province and Puak district, Nan province at 40 ° C by enrichment culture technique. Twenty four isolates were identified as xylanase producers from showing clear zone surround colonies grown on xylan medium after flooding by Congo red solution.

The twenty four isolates were divided into 11 groups based on their cell morphologies and cultural, physiological and biochemical characteristics. Representative strains of each groups were selected and characterized by chemotaxonomic and molecular techniques.

- Group 1, PT4-2: Gram-positive, spore forming, rods-shaped. Colony was round with raised and entire margin, translucent, without pigment. The bacteria contained meso-diaminopimelic acid as a diagnostic diamino in cell wall peptidoglycan, had MK-7 as a major menaquinone, and DNA G+C content 56.4 mol%. The 16S rRNA gene sequence (1510 bp) was 92.4 % similar to *Cohnella panacarvi* KCTC 13060^T.
- Group 2, PN8-3: Gram-positive, spore forming bacilli. Colony was round with raised and entire margin, opaque, cream color. The bacteria did not contained meso-diaminopimelic acid as a diagnostic diamino in cell wall peptidoglycan, had MK-7 as a major menaquinone, and DNA G+C content 64.9 mol%. The 16S rRNA gene sequence (1491 bp) was 91.4% similar to *Cohnella thermotolerans* CCUG 47242^T .
- Group 3, PN12-3: Gram-positive, spore forming, rods-shaped. It showed circular, raised or low convex, entire margin, translucent, cream color colony, and contained meso-diaminopimelic acid as a diagnostic diamino in cell wall peptidoglycan. It had MK-7 as a major menaquinone, and DNA G+C content 60.1 mol%. The 16S rRNA gene sequence (1505 bp) was 91.2 % similar to *Cohnella panacarvi* KCTC 13060^T.
- Group 4, PT6-2: Gram-positive, spore forming bacilli. It showed circular, umbonate, entire margin, opaque, white color colony, and did not contained meso-diaminopimelic acid as a diagnostic diamino in cell wall peptidoglycan. MK-7 was a major menaquinone. The 16S rRNA gene sequence (1508 bp) was 96.4 % similar to *Cohnella thermotolerans* CCUG 47242^T.
- Group 5, PN13-1:Gram-positive rods. The bacteria showed circular, umbonate, entire margin, opaque, yellowish color colony, and contained meso-diaminopimelic acid as a diagnostic diamino in cell wall peptidoglycan. It had MK-7 as a major

menaquinone. The 16S rRNA gene sequence (1468 bp) was 87.4% similar to *Paenibacillus agarexedens* DSM 1327^{T}.

- Group 6, T3-2X: Gram-positive rods. Colony was circular, umbonate, entire margin, opaque, light pink color. It contained meso-diaminopimelic acid as a diagnostic diamino in cell wall peptidoglycan. It had MK-7 as a major menaquinone. The 16S rRNA gene sequence (1509 bp) was 95.3% similar to *Paenibacillus agarexedens* DSM 1327^{T}.
- Group 7, PT2-3: Gram-positive rods. Colony was circular, umbonate, undulate margin, translucent, without pigment. It contained meso-diaminopimelic acid as a diagnostic diamino in cell wall peptidoglycan. MK-7 was a major menaquinone. The 16S rRNA gene sequence (1331 bp) was 91.8% similar to *Paenibacillus popilliae* ATCC 14706^{T}.
- Group 8, PN8-2: Gram-positive rods. Colony was irregular and spreading, rough, opaque, cream color. It contained meso-diaminopimelic acid as a diagnostic diamino in cell wall peptidoglycan. MK-7 was a major menaquinone. The 16S rRNA gene sequence (1524 bp) was 98.8% similar to *Bacillus subtilis* KCTC 3135^{T}.
- Group 9, PN1-2: Gram-negative rods. It showed round with lobate margin, translucent, green brown color colony, and did not contain *meso*-diaminopimelic acid as a diagnostic diamino in cell wall peptidoglycan. The 16S rRNA gene sequence (1083 bp) was 97.0% similar to *Pseudomonas aeruginosa* MML 2212^{T}.
- Group 10, PN9-3: Gram-negative rods. It showed circular, convex, entire margin, opaque or translucent, cream color colony, and did not contain *meso*-diaminopimelic acid as a diagnostic diamino in cell wall peptidoglycan. The 16S rRNA gene sequence (1563 bp) was 98.4% similar to *Acinetobacter baumannii* ATCC 19606^{T}.
- Group 11, N9-2: Gram-negative rods. Colony was round, entire margin, translucent, cream color. It did not contain *meso*-diaminopimelic acid as a diagnostic diamino in cell wall peptidoglycan. The 16S rRNA gene sequence (691 bp) was 97.6% similar to *Escherichia coli* KCTC 2441^{T}.

Xylanolytic clearance zone diameter ranged of the 24 xylanase producing bacteria isolated 0.4-2.8 cm. Strain PN12-2 produced maximum xylanase at 0.5 units/ml. Strain PN13-1 identified as *Paenibacillus* showed highest hydrolysis capacity (HC value) at 4.8. Top five highest xylanase producing strains (PN12-2, PT4-2, PT6-2, PN12-3, PN8-3) which identified as

Colnella and one isolate, PN13-1, which showed highest hydrolysis capacity (HC) value were selected to verify an effect of pH and incubation temperature on xylanase activity. Optimal pH and temperature for xylanase activity of strain PN12-2 were 8.0 and 65° C. The activity at the optimal conditions was 0.51 units/ml.

The xylanase producing bacteria isolated from soil samples collected were diverse in species. Two *Colnella* and two *Paenibacillus* from Tadan district, Nakhon Nayok province, two *Colnella*, one each of *Paenibacillus*, *Bacillus*, *Pseudomonas*, and *Acinetobacter* from Nangrong district, Nakhon Nayok province, and one *Escherichia* from Puak district, Nan province were found to be novel species. However, their taxonomic status of some isolates should be confirmed by DNA-DNA hybridization.