# 16S rDNA SEQUENCE ANALYSES AND ANTIMICROBIAL ACTIVITIES OF *STREPTOMYCES* STRAINS FROM THAI SOILS

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**ABSTRACT:** 16S rDNA sequences and phylogenetic analyses of 12 actinomycete strains from soils were carried out including S1-2 and S3-1 from Chiangrai; SB12-1 and S33-3 from Nan; S38-2 from Phatthalung; S49-1 from Songkhla; S55-4 from Chaiyaphum; and S71-1, S72-10, S75-3, S75-5, and S76-1 from in Trat. The percentage of 16S rDNA sequence similarity revealed that S72-10 and S76-1 were closed to *Streptomyces termitum* NBRC 13087$^T$ (99.6 and 99.8%, respectively), S49-1 to *S. aureoversilis* NBRC 13021$^T$ (99.4%), S1-2 and S75-5 to *S. hygroscopicus* DSM 41599$^T$ (99.8%), S38-2 to *S. aureofaciens* NBRC 12483$^T$ (99.4%), S33-3 to *S. xanthocidicus* NBRC 13469$^T$ (99.8%), S55-4 to *S. roseocinereus* NBRC 13829$^T$ (99.9%), S71-1 to *S. mycarofaciens* NBRC 13792$^T$ (99.4%), S75-3 to *S. albospinus* NBRC 13846$^T$ (99.4%), and S3-1 and SB12-1 to *S. spectabilis* NBRC 13424$^T$ (99.6 and 99.7%), respectively. In addition, their phenotypic and chemotaxonomic characteristics were determined for supporting the identification. In the course of screening for antimicrobial activities, most of them exhibited antimicrobial activities against *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 9341 but few strains did against *Escherichia coli* ATCC 25922, *Pseudomonas. aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231. The strain S3-1 was selected for secondary metabolite fermentation in YM broth due to its crude extract showing good antimicrobial activity. The ethyl acetate extract was fractionated by chromatographic method and the fractions were tested for antimicrobial activity by agar disc diffusion and bioautographic methods. The active spot found at $R_f$ value 0.8 (Silica gel TLC, solvent system 15% MeOH in $CH_2Cl_2$) was active against tested bacteria.

**Key words:** *Streptomyces*, Antimicrobial activity, 16S rDNA sequence, soils

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**INTRODUCTION:** The actinomycetes especially the strains of *Streptomyces* are the most common antibiotic - producing microorganisms found in soils. Among antibiotic produced from actinomycetes, *Streptomyces* strains could produce approximately 6,550 antibiotics(1). *Streptomyces* are prokaryotic gram-positive, aerobic bacteria which produce an extensive branch mycelia that rarely fragment(2). Several antibiotics such as actinomycin, amphotericin, chloramphenicol, geldanamycin, nystatin, streptomycin, and streptothricin are produced by *Streptomyces*(3,4). Recently, the genus *Streptomyces* was reported to include about 527 species(5) based on 16S-ITS RFLP (Internal Transcribes Spacer region Restriction Fragment Length Polymorphisms) fingerprinting(5). In Thailand, the identification, antimicrobial activity and antibiotics from of *Streptomyces* strains isolated from soils have been reported(6-10). This study deals with the analysis of 16S rDNA sequences and antimicrobial activity of *Streptomyces* strains isolated from Thai soils.

## MATERIALS AND METHODS:

### Screening and Identification

The actinomycete strains were isolated from soil samples collected from Chiangrai, Nan, Phatthalung, Songkhla, Chaiyaphum, and Trat provinces, Thailand (Table 1) using starch-casein nitrate agar(11). The phenotypic characteristics were determined by the methods described by Shirling and Gottlieb(12) and Arai(13). Primary screening of antimicrobial activities was performed on Yeast extract-Malt extract agar (YMA)

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plates[9]. Secondary screening of the strains was examined by the agar disc diffusion method[14] against *S. aureus* ATCC 6538, *B. subtilis* ATCC 6633, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *M. luteus* ATCC 9341 and *C. albicans* ATCC 10231. All tested microorganisms were cultivated on Mueller-Hinton agar slants at 37 °C for 24 h, except for the yeast strain that was cultivated on Sabouraud's dextrose agar slant at 30 °C for 24 h. Scanning electron microscope was used for determining the morphology of strains grew on YMA. Cell wall diaminopimelic acid (DAP) isomers were determined as described by Kutzner[15]. Menaquinone system was analysed as described by Komagata and Suzuki[16].

### 16S rDNA Sequence and Phylogenetic Analyses

DNA of the strains was isolated from cells grown in Yeast extract-Malt extract broth (YMB) with 0.2% of glycine reported by Yamada and Komagata[17] and purified as described by Saito and Miura[18]. DNA base composition analysis was analysed by the method of Tamaoka[19]. The complete 16S rDNA was amplified by PCR using primers, 8-27f and 1492r. The amplified 16S rDNA was used as templates for sequencing with Big Dye Terminator sequencing Kit (Perkin Elmer) and analyzed by AB1377 automated DNA sequencer (Perkin Elmer). The sequencing reaction for each sample was performed in DNA Thermal Cycler (Gene Amp PCR System 2400; Perkin Elmer) by using primers, 8-27f 5'-AGAGTTTGATC(A/C) TGGCTCAG-3'), 530f (5'-GTCCAGC (A/C) GCCGCGG-3') and 1114f (5'-GCAACGAGCGCAACCC-3'). Homology search was performed using the standard BLAST sequence similarity searching program version 2.2.1 from the web server. http://www.ncbi.nlm.nih.gov/BLAST/ against previously reported sequence at the GenBank/EMBL/DDBJ database. The sequence was multiply aligned with selected sequences obtained from Genbank/EMBL/DDBJ by using the CLUSTAL_X[20]. The alignment was manually verified and adjusted prior to the construction of phylogenetic tree. The phylogenetic tree was constructed by using neighbor-joining[21] in the MEGA program version 2.1[22]. The confidence values of branches of the phylogenetic tree were determined using the bootstrap analyses[23] based on 1000 resamplings. 16S rDNA sequence of *Micromonospora chalcea* JCM 3082$^T$ was used as an out group. The values for sequences similarity among the closest strains were calculated manually after pairwise alignments obtained using the CLUSTAL_X program[20]. Gaps and ambiguous nucleotides were eliminated from calculations. The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strains S72-10, S76-1, S49-1, S1-2, S75-5, S38-2, S33-3, S55-4, S71-1, S75-3, SB12-1, S3-1, are EU521702, EU521703, EU521697, EU521693, EU521700, EU521696, EU521695, EU521698, EU521701, EU521699, EU521694, and EU521692, respectively.

### Fermentation and Extraction of Strains

A loopful of each selected strain (total 12 strains) was inoculated into a 500-ml Erlenmeyer flask containing 250 ml of YM broth and incubated on a rotary shaker at 200 rpm, 30°C for 11 days. The culture broth was extracted with ethyl acetate (EtOAc) and concentrated under reduced pressure to yield the crude extract.

### Extraction and Fractionation of the Extract of Strain S3-1

The YM fermentation broth (20L) of strain S3-1 was filtered through a glass funnel packed with Whatman No. 1. The filtrate was partitioned with ethyl acetate 3 times. The ethyl acetate layer was collected and concentrated under reduced pressure and was fractionated by quick column chromatography using silica gel as the adsorbent (column, 4 x 4 cm.). The gradient of EtOAc: hexane was used as the mobile phase and methanol was finally used to elute the extract. Twenty-five milliliters of subfractions were collected. Combination of the subfractions with similar chromato-graphic patterns (TLC Si Gel, 30%hexane in EtOAc) gave 8 fractions (S001, S002, S003, S004, S005, S006, S007, S008). Fraction S007 was further fractionated by flash column chromatography using silica gel as the adsorbent (column, 2 x 16 cm.). MeOH (2%) in $CH_2Cl_2$ was used as the mobile phase and methanol was finally used to elute the extract. Fifteen milliliters of subfractions were collected. Combination of the subfractions with similar chromatographic patterns (TLC Si Gel, 5%MeOH in $CH_2Cl_2$) gave 5 subfractions (S009, S010, S011, S012, S013). All subfractions were monitored for antimicrobial activity by agar disc diffusion method[14)].

## RESULTS AND DISCUSSION:

Total of 12 selected actinomycete strains were isolated from soil samples collected from Chiangrai, Nan, Phatthalung, Songkhla, Chaiyaphum, and Trat provinces, Thailand based on their morphological and cultural characteristics (Table 1). On primary screening, most of these strains showed the antimicrobial activities against *S. aureus* ATCC 6538, *B. subtilis* ATCC 6633 and *M. luteus* ATCC 9341, while few strains showed activities against *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *C. albicans* ATCC 10231. On the secondary screening of the strains, 11 strains exhibited antimicrobial activity against *S. aureus* ATCC 6538 and *B. subtilis* ATCC 6633, 4 strains against *E. coli* ATCC 25922, 7 strains against *M. luteus* ATCC 9341, 8 strains against *P. aeruginosa* ATCC 27853, and 5 strains against *C. albicans* ATCC 10231 (Table 1). Six strains against methicillin resistant (MRSA) *S. aureus* 266 and 5 strains against both MRSA 269 and 643 (Table 2).

On the basis of morphological, cultural, physiological, biochemical and chemotaxonomic characteristics including phylogenetic analyses of 16S rDNA sequences, strains, S1-2 and S3-1 from soil in Chiangrai, SB12-1 and S33-3 from soil in Nan, S38-2 from soil in Phatthalung, S49-1 from soil in Songkhla, S55-4 from soil in Chaiyaphum, S71-1, S72-10, S75-3, S75-5, and S76-1 from soil in Trat were identified as *Streptomyces*. Spore chain morphology of 12 strains were rectiflexibiles or spiral. Scanning electron micrograph of strain S3-1, S38-2, S72-10 and S75-5 were shown in Fig 1. Spores were non-motile. Colonies on agar media were powdery, granular, or velvety. All strains contained MK-9 ($H_6$) and MK-9 ($H_8$) as major menaquinones and LL-diaminopimelic acid in cell wall peptidoglycan. The DNA G+C contents ranged from 69.0-75.4 mol%. They showed the same chemotaxonomic pattern which were similar to the member of the genus *Streptomyces* [24)].

Phylogenetic analysis of S1-2, S3-1, SB12-1, S33-3, S38-2, S49-1, S55-4, S71-1, S72-10, S75-3, S75-5, S76-1 strains revealed that they were belonged to the genus *Streptomyces* comparing with some of the type strains validly described, and *Micromonospora chalcea* JCM 3082$^T$ was selected as an outgroup (Figs. 2,3 and Table 3). They were closed to the type strains of *S. termitum* NBRC 13087$^T$, *S. aureoversilis* NBRC 13021$^T$, *S. hygroscopicus* DSM 41599$^T$, *S. xanthocidicus* NBRC 13469$^T$, *S. roseocinereus* NBRC 13829$^T$, *S. mycarofaciens* NBRC 13792$^T$, *S. albo-spinus* NBRC 13846$^T$, *S. spectabilis* NBRC 13424$^T$ and *S. aureofaciens* NBRC 12483$^T$ with sequence similarity ranged from 99.4-99.9% as shown in Table 3. Therefore, strain S72-10

**Table 1** Location, strain number and antimicrobial activity of *Streptomyces* strains

| Province/ Strain no. | Inhibition zone (mm) | | | | | |
|---|---|---|---|---|---|---|
| | ***S. aureus*** **ATCC 6538** | ***B. subtilis*** **ATCC 6633** | ***E. coli*** **ATCC 25922** | ***M. luteus*** **ATCC 9341** | ***Ps. aeruginosa*** **ATCC 27853** | ***C. albicans*** **ATCC10231** |
| Chiangrai/ S1-2 | 27 | 20 | 11 | 31 | 8 | 11 |
| Chiangrai/ S3-1 | 26 | 22 | - | 31 | 9 | - |
| Nan/ SB12-1 | 23 | 22 | - | 31 | 8 | - |
| Nan/ S33-3 | 17 | 15 | - | - | - | - |
| Phatthalung/ S38-2 | 8 | 16 | - | 21 | 10 | - |
| Songkhla/ S49-1 | 12 | 19 | 11 | 30 | 8 | - |
| Chaiyaphum/ S55-4 | 17 | 12 | - | - | - | 9 |
| Trat/ S71-1 | 18 | - | - | 12 | - | 15 |
| Trat/ S72-10 | 20 | 20 | - | - | 9 | - |
| Trat/ S75-3 | - | 15 | 9 | - | - | 10 |
| Trat/ S75-5 | 27 | 21 | 12 | 31 | 9 | 11 |
| Trat/ S76-1 | 19 | 18 | - | - | 8 | - |

and S76-1 should be identified as *S. termitum*, strain S49-1 as *S. aureoversilis*, S1-2 and S75-5 as *S. hygroscopicus*, S38-2 as *S. aureofaciens*, and strain S33-3 as *S. xanthocidicus*. Strain S55-4 should be identified as *S. roseocinereus*, S71-1 as *S. mycarofaciens*, strains S75-3 as *S. albospinus*, and S3-1 and SB12-1 were identified as *S. spectabilis*. Our strains mentioned above closed to the strains such as *S. hygroscopicus* strains that were reported to produce geldanamycin and 17-0-demethylgeldanamycin(3). *S. aureofaciens* produced tetracycline reported by Tortora and colleage(4) The strains of *S. xanthocidicus* produced respinomycins(25). Strains of *S. mycarofaciens* were reported to produce midecamycin(26) and strains of *S. albospinus* which produced spinamycin(27).

Strain S3-1 which was identified as *S. spectabilis* (Tables 3 and 4) was selected for secondary metabolite fermentation study because this strain exhibited good antimicrobial activity against *S. aureus* ATCC 6538, *B. subtilis*ATCC6633, *M.luteus* ATCC 9341, *P.aeruginosa* ATCC 27853 and MRSA *S. aureus* 266, 269 and 643 (Tables 1 and 2). Moreover, there were no reports on antimicrobial substances study from *S. spectabilis* in Thailand. While S1-2 and S75-5 identified as *S. hygroscopicus* were similar in antimicrobial activity as the strain produced geldanamycin the component was reported by Jongrungruanchok *et al* (8).

The crude ethyl acetate extract from the fermentation broth of S3-1 was determined for active spot by bioautography technique and showed an active spot with $R_f$ value 0.8 (solvent system 15%MeOH in $CH_2Cl_2$). Crude extract of S3-1 was purified by quick column chromatography and flash column chromatography. The fractions from the ethyl acetate extract were tested by agar disc diffusion method using *S. aureus* ATCC 6538, MRSA *S. aureus* 266, 269 and 643, *B. subtilis* ATCC 6633, *M. luteus* ATCC 9341 and *P. aeruginosa* ATCC 27853 as tested microorganisms. The active fractions (fractions S005, S006, S007, S010, S011, and S012) against tested microorganisms were shown in Table 5. The fraction S010 which contained a yellow spot at $R_f$ value 0.8 (solvent system 15% MeOH in $CH_2Cl_2$) showing the highest antimicrobial activity was submitted to measure proton NMR spectrum (data not shown). The proton MNR spectrum of S010 revealed that there were a mixture of several components containing the characteristics of olefinic protons in the region of chemical shifts at 5.5-8.5 ppm, the chelated hydroxyls at about 12 ppm, the methoxy signals at 3.5-4.0 ppm, the heteroatom-connected methine or methylene protons at 3.0-5.5 ppm and the methyl protons at 0.9-2.5 ppm. The strains of *S. spectabilis* were previously reported to produce several antimicrobial agents such as spectinabilin(28), spectinomycin(29), desertomycin(30), spectomycin(31), and streptovaricin(32). The active fractions of strain S3-1 are still interesting for further purification and structure elucidation of the active compounds.

**Table 2** Antimicrobial activity of strains against methicillin resistant (MRSA) *S. aureus*

| **Strain no.** | **Inhibition zone (mm)** | | |
|---|---|---|---|
| | **MRSA 266** | **MRSA 269** | **MRSA 643** |
| S1-2 | 18 | 17 | 17 |
| S3-1 | 15 | 16 | 15 |
| SB12-1 | 14 | 15 | 14 |
| S33-3 | 12 | 11 | 11 |
| S49-1 | 9 | - | - |
| S75-5 | 18 | 18 | 17 |

Strains S38-2, S55-4, S71-1, S72-10 S75-3, and S76-1 showed no activity

**Table 3** Similarity (%) of 16S rDNA sequences and identification of *Streptomyces* strains

| **Strain** | **Closest species** | **% Similarity of 16S rDNA** | **Identification** |
|---|---|---|---|
| S1-2 | *S. hygroscopicus* DSM 41599$^T$ | 99.8 | *S. hygroscopicus* |
| S3-1 | *S. spectabilis* NBRC 13424$^T$ | 99.6 | *S. spectabilis* |
| SB12-1 | *S. spectabilis* NBRC 13424$^T$ | 99.7 | *S. spectabilis* |
| S33-3 | *S. xanthocidicus* NBRC 13469$^T$ | 99.8 | *S. xanthocidicus* |
| S38-2 | *S. aureofaciens* NBRC 12483$^T$ | 99.4 | *S. aureofaciens* |
| S49-1 | *S. aureoversilis* NBRC 13021$^T$ | 99.4 | *S. aureoversilis* |
| S55-4 | *S. roseocinereus* NBRC 13829$^T$ | 99.9 | *S. roseocinereus* |
| S75-3 | *S. albospinus* NBRC 13846$^T$ | 99.4 | *S. albospinus* |
| S75-5 | *S. hygroscopicus* DSM 41599$^T$ | 99.8 | *S. hygroscopicus* |
| S71-1 | *S. mycarofaciens* NBRC 13792$^T$ | 99.4 | *S. mycarofaciens* |
| S72-10 | *S. termitum* NBRC 13087$^T$ | 99.6 | *S. termitum* |
| S76-1 | *S. termitum* NBRC 13087$^T$ | 99.8 | *S. termitum* |

**Table 4** Characteristics of *Streptomyces* sp. S3-1

| Characteristics | Result | Characteristics | Result |
|---|---|---|---|
| Spore chain | Rectiflexibiles | Growth in NaCl (2-6%) | Positive |
| Spore color | Vivid red | Decomposition of Strach, Esculin, Gelatin | Positive |
| Colonial appearance | Velvety | Production of melanin | Negative |
| Upper colony color on YMA | Vivid red | Reduction of nitrate | Negative |
| Lower colony color on YMA | Vivid red | Coagulation of skim milk | Positive |
| Growth on agar medium: | | Growth on sole carbon source (1%, w/v) | |
| Yeast Extract-Malt extract, Tyrosine, Oatmeal, Asparagine and Inorganic salt starch | Positive | Arabinose, fructose, glucose, glycerol, D-mannitol, melibiose, sucrose, rhamnose, raffinose, D-xylose, | Positive |
| Growth at: | | Cell wall | LL- diamino-pimelic acid |
| 10°C | Negative | | |
| 28°C | Positive | Major menaquinone | MK-9($H_6$) and MK-9($H_8$) |
| 45°C | Negative | | |
| pH 4-10 | Positive | G+C content (mol%) | 74.1 |

**Table 5** Antimicrobial activity from the extract of *Streptomyces* sp. S3-1

| Fraction no. | Inhibition zone (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | S. *aureus* ATCC 6538 | MRSA 266 | MRSA 269 | MRSA 643 | *B. subtilis* ATCC 6633 | *M. luteus* ATCC 9341 | *Ps. aeruginosa* ATCC 27853 |
| S005 | 24 | 12 | 11 | 12 | 18 | 31 | 8 |
| S006 | 26 | 13 | 14 | 14 | 19 | 31 | 9 |
| S007 | 26 | 15 | 15 | 15 | 22 | 31 | 9 |
| S010 | 26 | 15 | 15 | 15 | 21 | 31 | 9 |
| S011 | 20 | 11 | 11 | 10 | 14 | 30 | - |
| S012 | 12 | 8 | 8 | 8 | 11 | 15 | - |

Fractions S001, S002, S003 S004, S008, S009, and S0013 showed no activity

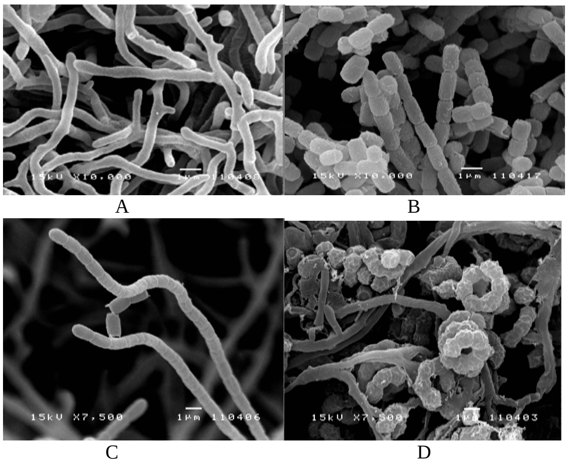

**Fig.1** Scanning electron micrograph of *Streptomyces* sp. S3-1, mycelium (A); S38-2, rectiflexibiles spore chain (B); S72-10, rectiflexibiles spore chain (C); and S75-5, spiral spore chain (D) on YMA medium (14 days).

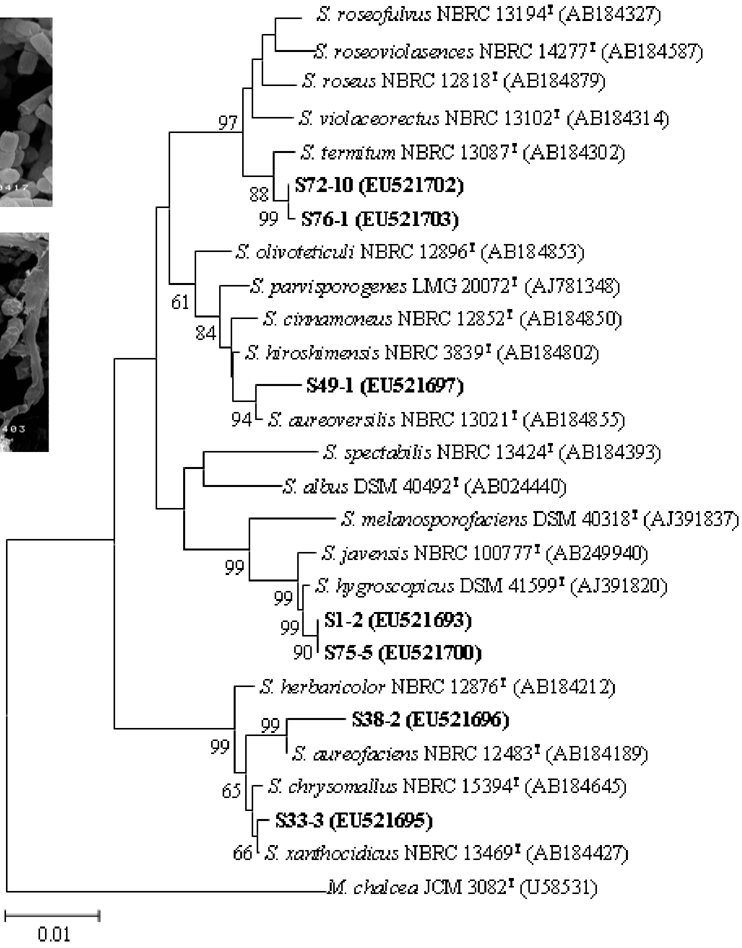

**Fig. 2.** A neighbour-joining tree based on 16S rDNA sequences, showing the position of S72-10, S76-1, S49-1, S1-2, S75-5, S38-2, and S33-3. The tree validated by a bootstrap analysis (1000 replications) and values greater than 61% are indicated at the nodes. Bar, 0.01 substitutions per nucleotide position.

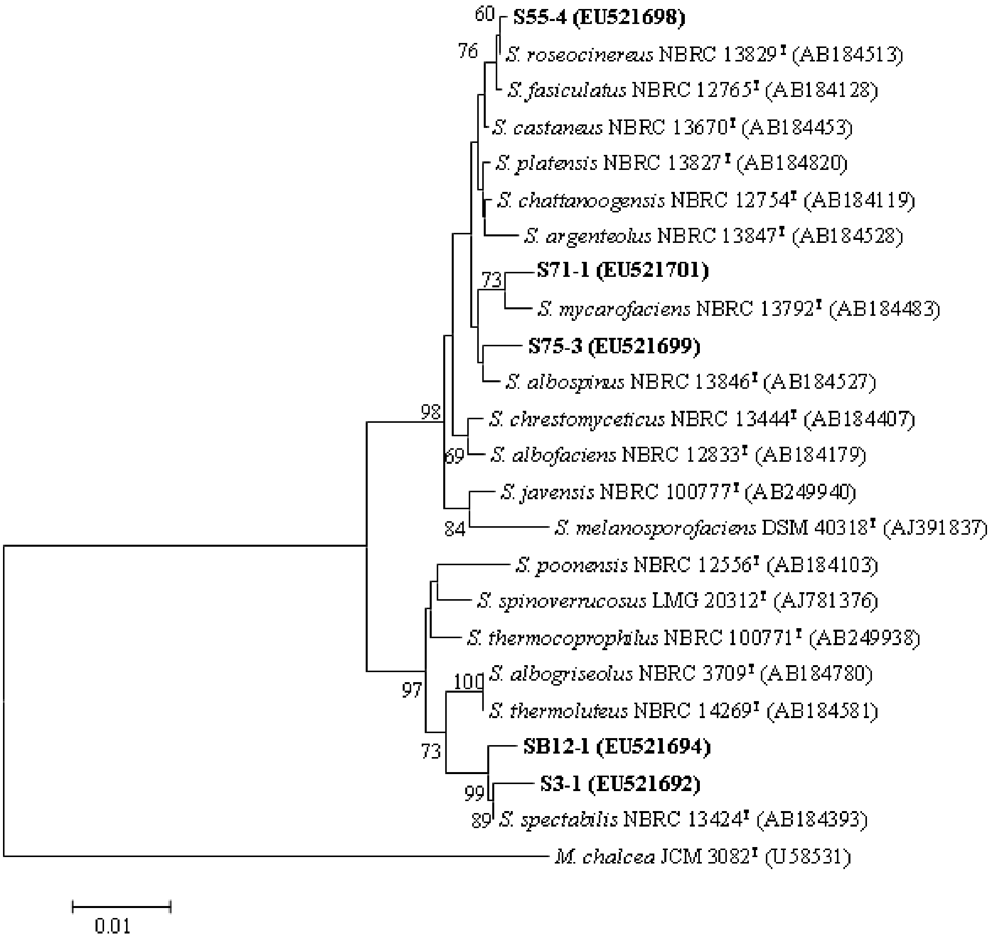

**Fig. 3** A neighbour-joining tree based on 16S rDNA sequences, showing the position of S55-4, S71-1, S75-3, SB12-1, and S3-1. The tree validated by a bootstrap analysis (1000 replications) and values greater than 60% are indicated at the nodes. Bar, 0.01 substitutions per nucleotide position.

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# การวิเคราะห์ลำดับเบสในช่วง 16S rDNA และฤทธิ์ต้านจุลชีพของสายพันธุ์แบคทีเรียสเตรปโตมัยซีสจากดินไทย

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**บทคัดย่อ:** การวิเคราะห์ลำดับเบสในช่วง 16S rDNAและสายวิวัฒนาการของแอคติโนมัยซีทส์ 12 สายพันธุ์ ได้แก่ S1-2 และ S3-1 ซึ่งแยกได้จากดินในจังหวัดเชียงราย SB12-1 และ S33-3 จากดินในจังหวัดน่าน S38-2 จากดินในจังหวัดพัทลุง S49-1 จากดินในจังหวัดสงขลา S55-4 จากดินในจังหวัดชัยภูมิ S71-1, S72-10, S75-3, S75-5 และ S76-1 จากดินในจังหวัดตราด พบว่าผลความคล้ายคลึง (%) จากการวิเคราะห์ลำดับเบสในช่วง 16S rDNA แสดงให้เห็นว่า สายพันธุ์ S72-10 และ S76-1 มีลำดับเบสใกล้เคียงกับ *Streptomyces termitum* NBRC 13087$^T$ (99.6 และ 99.8% ตามลำดับ) ในทำนองเดียวกันมีความใกล้เคียงกันของลำดับเบสระหว่างสายพันธุ์ S49-1 กับ *S. aureoversilis* NBRC 13021$^T$ (99.4%) สายพันธุ์ S1-2 และ S75-5 กับ *S. hygroscopicus* DSM 41599$^T$ (99.8%) สายพันธุ์ S38-2 กับ *S. aureofaciens* NBRC 12483$^T$ (99.4%) สายพันธุ์ S33-3 กับ *S. xanthocidicus* NBRC 13469$^T$ (99.8%) สายพันธุ์ S55-4 กับ *S. roseocinereus* NBRC 13829$^T$ (99.9%) สายพันธุ์ S71-1 กับ *S. mycarofaciens* NBRC 13792$^T$ (99.4%) สายพันธุ์ S75-3 กับ *S. albospinus* NBRC 13846$^T$ (99.4%) สายพันธุ์ S3-1 และ SB12-1 กับ *S. spectabilis* NBRC 13424$^T$ (99.6 และ 99.7% ตามลำดับ) นอกจากนี้ได้ตรวจสอบลักษณะทางฟีโนไทป์และลักษณะอนุกรมวิธานทางเคมีของสายพันธุ์เพื่อช่วยในการพิสูจน์เอกลักษณ์ด้วย ผลการศึกษาฤทธิ์การต้านจุลชีพพบว่าส่วนใหญ่แสดงฤทธิ์ต้าน *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 9341 แต่มีเพียงบางสายพันธุ์แสดงฤทธิ์ต้าน *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231 ได้คัดเลือกสายพันธุ์ *S. spectabilis* S3-1 ซึ่งแสดงฤทธิ์ดีในการต้านจุลชีพ เพื่อทำการหมักสารทุติยภูมิในอาหารเหลว YM เมื่อนำสิ่งสกัดด้วยเอทิลอะซิเตทจากน้ำหมักของสายพันธุ์นี้มาสกัดแยกโดยวิธีทางโครมาโทกราฟีพร้อมทั้งทดสอบฤทธิ์ต้านจุลชีพโดยวิธี agar disc diffusion และวิธี bioautographic (Silica gel TLC ระบบตัวทำละลายคือ 15% MeOH ใน $CH_2Cl_2$) พบว่าส่วนที่แยกได้ที่มีค่า $R_f$ เท่ากับ 0.8 แสดงฤทธิ์ในการต้านแบคทีเรียทดสอบ

**คำสำคัญ:** สเตรปโตมัยซีส ฤทธิ์ต้านจุลชีพ การวิเคราะห์ลำดับเบสในช่วง 16S rDNA ดิน

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