# การคัดกรองและพิสูจน์เอกลักษณ์ของ actinomycetes ผลิตสารต้านจุลชีพจากดินป่าชายเลนบริเวณอ่าวไทยตอนใน

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# SCREENING AND IDENTIFICATION OF ANTIMICROBIAL PRODUCING ACTINOMYCETES FROM MANGROVE SOIL OF THE INNER GULF OF THAILAND

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Department of Microbiology
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สิริกานต์ หุณฑนามระ : การคัดกรองและพิสูจน์เอกลักษณ์ของ actinomycetes ผลิตสารต้านจุลชีพจากดินป่าชายเลนบริเวณอ่าวไทยตอนใน (Screening and identification of antimicrobial producing actinomycetes from mangrove soil of the inner gulf of Thailand) อาจารย์ที่ปรึกษา: รศ.ดร. อัญชริดา อัครจรัลญา, อาจารย์ที่ปรึกษาร่วม: รศ.ดร. สมบูรณ์ ธนาศุภวัฒน์; 115 หน้า.

ในการศึกษาเพื่อหาสายพันธุ์แอคติโนมัยซีทส์จากดินป่าชายเลนบริเวณอ่าวไทยตอนในของประเทศไทยที่เก็บจากจังหวัดสมุทรปราการ จังหวัดสมุทรสาคร จังหวัดสมุทรสงคราม และจังหวัดเพชรบุรี พบว่าสามารถแยกแบคทีเรียแอคติโนมัยซีทส์ได้จำนวน 50 ไอโซเลต ซึ่งสามารถพิสูจน์เอกลักษณ์แบคทีเรียที่สร้างสปอร์บนเส้นใยอากาศได้เป็นสเตรปโตมัยซีส จำนวน 38 ไอโซเลต และแบคทีเรียที่สร้างสปอร์เดียวบนเส้นใยเป็นไมโครโมโนสปอรา จำนวน 12 ไอโซเลตโดยอาศัยผลจากการศึกษาลักษณะทางฟีโนไทป์ จากการคัดกรองฤทธิ์ต้านจุลชีพขั้นต้น พบว่า มีฤทธิ์ยับยั้งแบคทีเรียแกรมบวก *Staphylococcus aureus* ATCC 6538P, *Micrococcus luteus* ATCC 9341 และ *Bacillus subtilis* ATCC 6633 จำนวน 28 เชื้อ ยับยั้งแบคทีเรียแกรมลบ *Escherichia coli* ATCC 25922 และ *Pseudomonas aeruginosa* ATCC 27853 จำนวน 6 เชื้อ และยับยั้งยีสต์ *Candida albicans* ATCC 10231 จำนวน 5 เชื้อ เลือกตัวแทน 7 เชื้อ ไปศึกษาความคล้ายคลึงทางลำดับเบสในช่วง 16S rDNA พบว่า SAM2-1, B15-4, D10-1, J17-2, J8-1, SMP 3-1 และ D10-5 มีความคล้ายคลึงกับ *Streptomyces badius* NRRL B-2567$^T$, *S. albolongus* NBRC13465$^T$, *S. parvulus* NBRC13193$^T$, *S. cinnamocastaneus* NBRC14278$^T$, *S.vayuensis* N2$^T$, *S. caesius* NBRC13376$^T$ และ *S. caesius* NBRC13376$^T$ เป็น 98.4%, 98.3%, 94.7%, 93.5%, 98.5%, 97.4% และ 95.9% ตามลำดับ ผลจากค่าความคล้ายคลึงของ16S rDNA ที่ต่ำกว่า 97.0% จัดได้เป็นสเตรปโตมัยซีสสปีชีส์ใหม่ จากผลการศึกษาลักษณะทางอนุกรมวิธานเคมี พบว่าเชื้อสเตรปโตมัยซีสจะมี L-diaminopimelic ในผนังเซลล์ และเชื้อไมโครโมโนสปอราที่แยกได้มีกรด *meso*-diaminopimelic ในผนังเซลล์ เชื้อสเตรปโตมัยซีสสายพันธุ์ที่ทดสอบมี menaquinones ชนิด MK-9($H_6$) และ MK-9($H_8$) เป็นองค์ประกอบหลัก นอกจากนี้พบว่าปริมาณ G+C ของสาย DNA อยู่ในช่วง 69-74 โมล%

นำแอคติโนมัยซีทส์จำนวน 8 สายพันธุ์ที่มีฤทธิ์ต้านจุลชีพดี ได้แก่ SAM2-1, SMP3-1, C10-6, B10-3-2, B10-3-4, C10-2, B10-3-1 และ J15-1 ไปทดสอบคัดกรองขั้นที่สอง และวิเคราะห์ข้อมูลด้วยวิธี NMR spectroscopy นอกจากนี้การศึกษาสารสกัดหยาบด้วยเอทิลอะซิเตตของน้ำหมักปริมาณ 10 ลิตร ของสายพันธุ์ C10-6 ที่คัดเลือกได้ พบว่าสามารถแยกสิ่งสกัดด้วยวิธีการทางโครมาโตกราฟีได้ 10 fraction สารใน fraction ที่ 8 แสดงฤทธิ์ต้านเชื้อจุลินทรีย์ทดสอบทั้งแบคทีเรียแกรมบวก และแบคทีเรียแกรมลบได้มากที่สุด (ความเข้มข้น 1มิลลิกรัมต่อ 20 ไมโครลิตรต่อดิสก์)

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ลายมือชื่อนิสิต..............................................
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SIRIKAN HUNADANAMRA: SCREENING AND IDENTIFICATION OF ANTIMICROBIAL PRODUCING ACTINOMYCETES FROM MANGROVE SOIL OF THE INNER GULF OF THAILAND. THESIS ADVISOR: ASSOC. PROF. ANCHARIDA AKARAJARUNYA, Ph. D., THESIS CO-ADVISOR: ASSOC. PROF. SOMBOON TANASUPAWAT, Ph. D., 115 pp.

In the course of our investigation for actinomycetes strains from mangrove soils collected in Samut prakarn, Samut sakorn, Samut songkram and Phetchaburi provinces, the inner gulf of Thailand. Fifty isolates of actinomycetes were isolated and 38 isolates which produced spores on aerial mycelium were identified as *Streptomyces* and 12 isolates which produced single non-motile spores were *Micrmonospora* based on their phenotypic characteristics. On the primary screening of antimicrobial activity, 28 isolates could inhibit Gram-positive bacteria, *Staphylococcus aureus* ATCC 6538P, *Micrococcus luteus* ATCC 9341 and *Bacillus subtilis* ATCC 6633. Six isolates could inhibit Gram-negative bacteria, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 and 5 isolates could inhibit yeast, *Candida albicans* ATCC 10231. Seven of the active isolates as a representative strains were selected for analysis of 16S rDNA sequences, strains SAM2-1, B15-4, D10-1, J17-2, J8-1, SMP3-1 and D10-5 were closely related to *Streptomyces badius* NRRL B-2567$^T$, *S. caesius* NBRC13376$^T$, *S. albolongus* NBRC13465$^T$, *S. parvulus* NBRC13193$^T$, *S. cinnamocastaneus* NBRC14278$^T$, *S. vayuensis* N2$^T$, and *S. caesius* NBRC13376$^T$ with 98.4, 97.4, 98.3, 94.7, 93.5, 98.5, และ 95.9% 16S rDNA sequence similarity, respectively. The lower 16S rDNA similarity than 97.0% indicated that they should be recognized as the novel *Streptomyces* species. The tested strains of *Streptomyces* contained L–diaminopimelic acid in cell wall (cell wall type I) and *Micromonospora* strains contained *meso*–diaminopimelic acid in cell wall (cell wall type II). Major menaquinone of *Streptomyces* strains tested were MK-9($H_6$) and MK-9($H_8$). The range of G+C contents of the DNA was 69-74 mol%.

Eight isolates that showed good antimicrobial activity SAM2-1, SMP3-1, C10-6, B10-3-2, B 10-3-4, C10-2, B10-3-1 and J15-1 were selected for secondary antibiotic screening and NMR spectroscopy analysis. In addition, *Streptomyces* sp. C10-6 was selected for antibiotic fermentation in Yeast extract-Malt extract broth (10 litres), and ethylacetate crude extract was partially purified by chromatographic technique to give 10 fractions. Fraction 8 showed the most significant antimicrobial activity to Gram positive and Gram negative bacteria at the concentration 1mg per 20 µL per disk.

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# LIST OF ABBREVATIONS AND SYMBOLS

| | | |
|---|---|---|
| ATCC | = | American Type Culture Collection, Maryland, U.S.A. |
| °C | = | Degree Celsius |
| $CDCl_3$ | = | Deuterated chloroform |
| CFU | = | Colony forming unit |
| $CHCl_3$ | = | Chloroform |
| Cm | = | Centimeter |
| EDTA | = | Disodiummethylenediaminetetraacetate |
| EMBL | = | European Molecular Biology Laboratory |
| EtOAc | = | Ethyl acetate |
| GenBank | = | National Institute of Health genetic sequence database |
| h | = | Hour |
| HCl | = | Hydrochloric acid |
| $^1H$-NMR | = | Proton nuclear magnetic resonance |
| HPLC | = | High performance liquid chromatography |
| HPTLC | = | High performance thin layer chromatography |
| $H_2O$ | = | Water |
| ISP | = | International Streptomyces Project |
| $KNO_3$ | = | Potassium nitrate |