

APPENDIX A
Media formulation

Basal medium

| | |
|--|-------|
| KH ₂ PO ₄ | 2.38g |
| K ₂ HPO ₄ ·3H ₂ O | 5.65g |
| MgSO ₄ ·7H ₂ O | 1.0g |
| Salt solution | 6.2ml |
| Distilled water | 1.0L |

Glycerol-Asparagine agar medium (ISP5)

| | |
|--------------------------------------|--------|
| L-asparagine monohydrate | 1.0g |
| Potassium phosphate, Dibasic | 1.0g |
| Iron (II) Sulphate Heptahydrate | 0.001g |
| Manganese (II) Chloride Tetrahydrate | 0.001g |
| Zinc Sulphate Heptahydrate | 0.001g |
| Agar | 15.0g |
| Glycerol | 10.0g |
| pH 7.0-7.4 | |

Glycerol solution (30% v/v)

| | |
|-----------------|-------|
| Glycerol | 300ml |
| Yeast extract | 1.0g |
| Glucose | 3.75g |
| Casein | 1.25g |
| Distilled water | 0.7L |

Inorganic salt- starch agar medium (ISP 4)

| | |
|--------------------------------------|--------|
| Soluble starch | 10.0g |
| Potassium phosphate Dibasic | 1.0g |
| Magnesium Sulphate Heptahydrate | 1.0g |
| Sodium chloride | 1.0g |
| Ammonium sulphate | 2.0g |
| Calcium carbonate | 2.0g |
| Iron (II) Sulphate Heptahydrate | 0.001g |
| Manganese (II) Chloride Tetrahydrate | 0.001g |
| Zinc Sulphate Heptahydrate | 0.001g |
| Agar | 15.0g |
| Distilled water | 1.0L |
| pH 7.0 ~ 7.4 | |

Nitrate Reduction test:

Solution I:

| | |
|-------------------|-------|
| CaCl ₂ | 0.5g |
| Distilled water | 100ml |

Solution II:

| | |
|---|-------|
| K ₂ HPO ₄ (anhydrous) | 0.5g |
| MgSO ₄ ·7H ₂ O | 0.2g |
| KNO ₃ | 1.0g |
| Glucose | 10.0g |
| Distilled water | 900ml |

Non-sporulating media

| | |
|----------------|-------|
| Casamino acid | 20.0g |
| Soluble starch | 20.0g |
| Yeast extract | 4.0g |
| Agar | 18.0g |

Oatmeal agar (ISP 3)

| | |
|-----------------|-------|
| Oatmeal | 60.0g |
| Agar | 12.5g |
| Distilled water | 1.0L |
| pH 6.0 ± 0.2 | |

Peptone Iron agar medium

| | |
|-------------------------|--------|
| Peptone | 15.0 g |
| Proteose Peptone | 5.0 g |
| Ferric Ammonium Citrate | 0.5 g |
| Sodium Glycerophosphate | 1.0 g |
| Sodium Thiosulfate | 0.08 g |
| Agar | 15.0 g |

Peptone-Yeast Extract-Iron agar medium (ISP 6)

| | |
|------------------------------|-------|
| Peptone | 15.0g |
| Proteose peptone | 5.0g |
| Ammonium iron (III) citrate | 0.5g |
| Potassium phosphate, Dibasic | 1.0g |
| Sodium Thiosulphate | 0.08g |
| Yeast extract | 1.0g |
| Agar | 15.0g |
| pH 7.0 – 7.2 | |

Peptone-Yeast Medium (ISP 1)

| | |
|-----------------|------|
| Polypepton | 5.0g |
| Yeast extract | 3.0g |
| Distilled water | 1.0L |
| pH 7.0 - 7.2 | |

Sabouraud Dextrose Agar (SDA)

| | |
|----------------------------|-------|
| Enzymatic digest of casein | 10.0g |
| Dextrose | 20.0g |
| Agar | 15.0g |
| Distilled water | 1.0L |
| pH 5.4 – 5.8 | |

Salt solution

| | |
|--------------------------------------|-------|
| CuSO ₄ .5H ₂ O | 102mg |
| FeSO ₄ .7H ₂ O | 176mg |
| MnCl ₂ .4H ₂ O | 126mg |
| ZnSO ₄ .7H ₂ O | 24mg |
| Distilled water | 100ml |

Starch-Glucose-Yeast Extract Yeast (SGY)

| | |
|-------------------------|-------|
| Soluble starch | 10.0g |
| D(+)-glucose | 10.0g |
| Glycerol | 10.0g |
| Corn steep solids | 2.5g |
| Bacteriological peptone | 5.0g |
| Yeast extract | 2.0g |
| CaCO ₃ | 3.0g |
| Instant ocean | 16.7g |
| Distilled water | 1.0L |
| pH 7.3 | |

Tyrosine agar medium (ISP 7)

| | |
|--------------------------------------|--------|
| L-Tyrosine | 0.5g |
| L-Asparagine Monohydrate | 1.0g |
| Potassium phosphate | 0.5g |
| Magnesium sulfate Heptahydrate | 0.5g |
| Sodium chloride | 0.5g |
| Iron (II) sulfate Heptahydrate | 0.011g |
| Manganese (II) chloride Tetrahydrate | 0.001g |
| Zinc sulfate Heptahydrate | 0.001g |
| Agar | 15.0g |
| Glycerol | 15.0g |
| pH 7.0 – 7.4 | |

Yeast extract-malt extract agar medium (ISP 2)

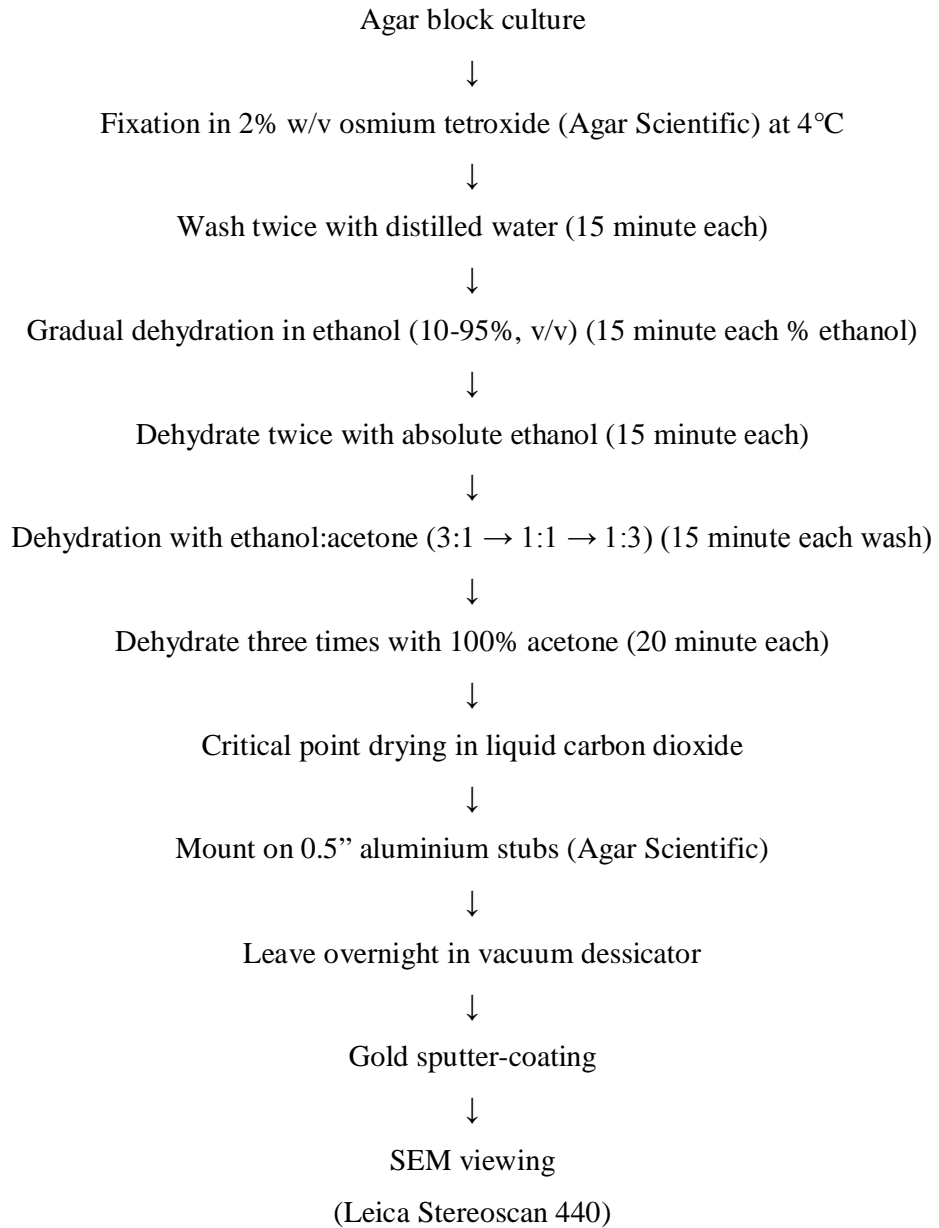
| | |
|-----------------|-------|
| Yeast extract | 4.0g |
| Malt extract | 10.0g |
| Glucose | 4.0g |
| Agar | 15.0g |
| Distilled water | 1.0L |
| pH 7.2 - 7.4 | |

Yeast extract-peptone-Glucose agar (YPG)

| | |
|-----------------|-------|
| Yeast extract | 10.0g |
| Peptone | 20.0g |
| Glucose | 20.0 |
| Agar | 18.0g |
| Distilled water | 1.0L |
| pH 6.8 | |

APPENDIX B

Scanning electron microscopy (SEM) procedure



APPENDIX C

Forward and reverse sequences of strains X34, X42 and X77 obtained from 1st Base using ABI system.

>1st_BASE_214384_strain X34_27f_

GNNNNNANTGGCTTACATATGTTCGAGTCGAACGATGAACCACTTCGGTGGG
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>1st_BASE_214385_strain X34_1525r

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CAGCCATGCACCACCTGTACACCGACCACAAGGGGGACCCTGTCTCCAGGG
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CGATTGTGGCAATTATTTCCCCAACCTGGCCTGCCCTCCGTAAGGA

>1st_BASE_244193_strain X42_27f

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>1st_BASE_244194_strain X42_1525r

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ATGCTGATCTGCGATTACTAGCGACTCCGACTTCATGGGGTCGAGTTGCAGA
CCCCAATCCGAACTGAGACCGGCTTTTTGAGATTGCTCCACCTCACGGTAT
CGCAGCTCATTGTACCGGCCATTGTAGCACGTGTGCAGCCCAAGACATAAG
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GACTACCAGGGTATCTAATCCTGTTTCGCTCCCCACGCTTTCGCTCCTCAGCG
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>1st_BASE_244195_strain X77_27f

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>1st_BASE_244196_strain X77_1525r

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ACAACGCTTTGCGTCCCTACGTATTATCCGCGGGCTGCTGGCACGTAGNAGC
CGGCGCTTCTTTCTGCAAGTACNNCACTGCGCTNTNCTTGCTGAAAGAGGTT
TACANNCGAGGGCCGT

APPENDIX D

Combined forward and reverse sequences of strains X34, X42 and X77

>consensus_strain X34 (1109 nucleotides)

GTCGAACGATGAACCACTTCGGTGGGGATTAGTGGCGAACGGGTGAGTAAC
ACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTA
ATACCGGATACTGATCCTCGCAGGCATCTGCGAGGTTTCGAAAGCTCCGGCG
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TgGGCGaAAgCCTGATGCAGCgACGCcGcgtgaGGGATGACGgCCTTCGGGTTGT
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CCTGGAGACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTC
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GCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCC
TTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCGAT
ACCGCGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGT
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>consensus_strain X42 (1342 nucleotides)

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>consensus_ strain X77 (1278 nucleotides)

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APPENDIX E

BLAST results (top 20) of strains X34, X42 and X77

BLAST Strain X34

(Formatting Results - 60HVH2YZ01S)

| <u>Accession</u> | <u>Description</u> | <u>Max score</u> | <u>Total score</u> | <u>Query coverage</u> | <u>E value</u> | <u>Max ident</u> |
|----------------------------|--|----------------------|--------------------|-----------------------|----------------|------------------|
| GU263851.1 | Streptomyces sp. 35034 16S ribosomal RNA gene, partial sequence | 2593 | 2593 | 100% | 0 | 99% |
| GQ392058.1 | Streptomyces rochei strain A-1 16S ribosomal RNA gene, partial sequence | 2593 | 2593 | 100% | 0 | 99% |
| AB184529.2 | Streptomyces fungicidicus gene for 16S rRNA, partial sequence, strain: NBRC 13848 | 2593 | 2593 | 100% | 0 | 99% |
| GQ289142.1 | Bacterium Kwm1 16S ribosomal RNA gene, partial sequence | 2593 | 2593 | 100% | 0 | 99% |
| FJ792577.1 | Streptomyces rochei strain fcfc3165 16S ribosomal RNA gene, partial sequence | 2593 | 2593 | 100% | 0 | 99% |
| FJ792558.1 | Streptomyces rochei strain fcfc3131 16S ribosomal RNA gene, partial sequence | 2593 | 2593 | 100% | 0 | 99% |
| FJ792552.1 | Streptomyces rochei strain fcfc3115 16S ribosomal RNA gene, partial sequence | 2593 | 2593 | 100% | 0 | 99% |
| FJ481066.1 | Streptomyces avidinii strain xsd08150 16S ribosomal RNA gene, partial sequence | 2593 | 2593 | 100% | 0 | 99% |
| FJ481055.1 | Streptomyces rochei strain xsd08098 16S ribosomal RNA gene, partial sequence | 2593 | 2593 | 100% | 0 | 99% |
| FJ481635.1 | Streptomyces enissocaesilis strain HBUM174552 16S ribosomal RNA gene, partial sequence | 2593 | 2593 | 100% | 0 | 99% |
| FJ224362.1 | Streptomyces sp. P-13 16S ribosomal RNA gene, partial sequence | 2593 | 2593 | 100% | 0 | 99% |
| EU841560.1 | Streptomyces rochei strain HBUM174096 16S ribosomal RNA gene, partial sequence | 2593 | 2593 | 100% | 0 | 99% |
| EU621883.1 | Streptomyces sp. YDG17 16S ribosomal RNA gene, partial sequence | 2593 | 2593 | 100% | 0 | 99% |
| EU593731.1 | Streptomyces rochei strain 173672 16S ribosomal RNA gene, partial sequence | 2593 | 2593 | 100% | 0 | 99% |
| EU593730.1 | Streptomyces rochei strain 173260 16S ribosomal RNA gene, partial sequence | 2593 | 2593 | 100% | 0 | 99% |
| EU593640.1 | Streptomyces avidinii strain 173969 16S ribosomal RNA gene, partial sequence | 2593 | 2593 | 100% | 0 | 99% |
| EU570711.1 | Streptomyces rochei strain 173609 16S ribosomal RNA gene, partial sequence | 2593 | 2593 | 100% | 0 | 99% |
| EU570623.1 | Streptomyces rochei strain 173342 16S ribosomal RNA gene, partial sequence | 2593 | 2593 | 100% | 0 | 99% |
| EU570372.1 | Streptomyces rochei strain 173315 16S ribosomal RNA gene, partial sequence | 2593 | 2593 | 100% | 0 | 99% |
| EU257263.1 | Streptomyces sp. A53Ydz-XM 16S ribosomal RNA gene, partial sequence | 2593 | 2593 | 100% | 0 | 99% |

BLAST Strain X42**(Formatting Results - DNYC2HWM01S)**

| <u>Accession</u> | <u>Description</u> | <u>Max score</u> | <u>Total score</u> | <u>Query coverage</u> | <u>E value</u> | <u>Max ident</u> |
|----------------------------|---|----------------------|--------------------|-----------------------|----------------|------------------|
| GU002076.1 | Streptomyces sp. FXJ6.047 16S ribosomal RNA gene, partial sequence | 2405 | 2405 | 100% | 0 | 99% |
| AB362247.1 | Streptomyces sampsonii gene for 16S rRNA, partial sequence | 2405 | 2405 | 100% | 0 | 99% |
| EU384262.1 | Streptomyces sp. A556 Ydz-TA 16S ribosomal RNA gene, partial sequence | 2405 | 2405 | 100% | 0 | 99% |
| EU368806.1 | Streptomyces sp. A52 Ydz-XM 16S ribosomal RNA gene, partial sequence | 2405 | 2405 | 100% | 0 | 99% |
| EU257255.1 | Streptomyces sp. A93Ydz-QZ 16S ribosomal RNA gene, partial sequence | 2405 | 2405 | 100% | 0 | 99% |
| EF063487.1 | Streptomyces sp. JXHM-A-12 16S ribosomal RNA gene, partial sequence | 2405 | 2405 | 100% | 0 | 99% |
| EF063445.1 | Streptomyces sp. C19-17 16S ribosomal RNA gene, partial sequence | 2405 | 2405 | 100% | 0 | 99% |
| EU257240.1 | Streptomyces sp. A46Ydz-XG 16S ribosomal RNA gene, partial sequence | 2399 | 2399 | 100% | 0 | 99% |
| AM412550.1 | Streptomyces sp. 45zhy partial 16S rRNA gene, strain 45zhy | 2399 | 2399 | 100% | 0 | 99% |
| AB045867.1 | Streptomyces griseus subsp. griseus gene for 16S rRNA | 2399 | 2399 | 100% | 0 | 99% |
| EF588223.2 | Streptomyces griseus strain NRRL B-2165 16S ribosomal RNA gene, partial sequence | 2394 | 2394 | 100% | 0 | 99% |
| EU181249.1 | Streptomyces sp. 94P32-5 16S ribosomal RNA gene, partial sequence | 2394 | 2394 | 100% | 0 | 99% |
| AM412551.1 | Streptomyces sp. 41zhy partial 16S rRNA gene, strain 41zhy | 2394 | 2394 | 100% | 0 | 99% |
| HQ132794.1 | Streptomyces albus strain N64 16S ribosomal RNA gene, partial sequence | 2388 | 2388 | 100% | 0 | 99% |
| HM797397.1 | Streptomyces sp. L131D 16S ribosomal RNA gene, partial sequence | 2388 | 2388 | 100% | 0 | 99% |
| HM481475.1 | Streptomyces albus strain XJ-17 16S ribosomal RNA gene, partial sequence | 2388 | 2388 | 100% | 0 | 99% |
| GU722183.1 | Streptomyces sp. JV186 16S ribosomal RNA gene, partial sequence | 2388 | 2388 | 100% | 0 | 99% |
| HM222696.1 | Streptomyces sp. 401K3-1 16S ribosomal RNA gene, partial sequence | 2388 | 2388 | 100% | 0 | 99% |
| GU213490.1 | Streptomyces sp. HB094 16S ribosomal RNA gene, partial sequence | 2388 | 2388 | 100% | 0 | 99% |
| HM007152.1 | Actinomycetales bacterium B3(2010) strain B3 16S ribosomal RNA gene, partial sequence | 2388 | 2388 | 100% | 0 | 99% |

BLAST Strain X77**(Formatting Results - DNWTHD5701N)**

| <u>Accession</u> | <u>Description</u> | <u>Total score</u> | <u>Query coverage</u> | <u>E value</u> | <u>Max ident</u> |
|-------------------|--|--------------------|-----------------------|----------------|------------------|
| <u>HM018120.1</u> | Streptomyces sp. VAN21 16S ribosomal RNA gene, partial sequence | 2220 | 100% | 0 | 99% |
| <u>HM018109.1</u> | Streptomyces sp. WALP25 16S ribosomal RNA gene, partial sequence | 2220 | 100% | 0 | 99% |
| <u>HM018108.1</u> | Streptomyces sp. WALP24 16S ribosomal RNA gene, partial sequence | 2220 | 100% | 0 | 99% |
| <u>HM018107.1</u> | Streptomyces sp. WALP23 16S ribosomal RNA gene, partial sequence | 2220 | 100% | 0 | 99% |
| <u>HM018106.1</u> | Streptomyces sp. WALP22 16S ribosomal RNA gene, partial sequence | 2220 | 100% | 0 | 99% |
| <u>HM018082.1</u> | Streptomyces sp. MEC01 16S ribosomal RNA gene, partial sequence | 2220 | 100% | 0 | 99% |
| <u>HM018079.1</u> | Streptomyces sp. LEN01 16S ribosomal RNA gene, partial sequence | 2220 | 100% | 0 | 99% |
| <u>HM018076.1</u> | Streptomyces sp. HS7003 16S ribosomal RNA gene, partial sequence | 2220 | 100% | 0 | 99% |
| <u>AB184777.2</u> | Streptomyces roseochromogenus gene for 16S rRNA, partial sequence, strain: NBRC 3442 | 2220 | 100% | 0 | 99% |
| <u>EU647478.1</u> | Streptomyces anulatus strain Malaysia 16S ribosomal RNA gene, partial sequence | 2220 | 100% | 0 | 99% |
| <u>EU741221.1</u> | Streptomyces globisporus subsp. globisporus strain 13669A 16S ribosomal RNA gene, partial sequence | 2220 | 100% | 0 | 99% |
| <u>EU741191.1</u> | Streptomyces sp. 13661T 16S ribosomal RNA gene, partial sequence | 2220 | 100% | 0 | 99% |
| <u>EU741124.1</u> | Streptomyces globisporus subsp. globisporus strain 13638A 16S ribosomal RNA gene, partial sequence | 2220 | 100% | 0 | 99% |
| <u>FJ792550.1</u> | Streptomyces bacillaris strain cfcc3101 16S ribosomal RNA gene, partial sequence | 2220 | 100% | 0 | 99% |
| <u>FJ572022.1</u> | Actinomycetales bacterium JH105 16S ribosomal RNA gene, partial sequence | 2220 | 100% | 0 | 99% |
| <u>FJ238114.1</u> | Streptomyces sp. DS3024 16S ribosomal RNA gene, partial sequence | 2220 | 100% | 0 | 99% |
| <u>EU368787.1</u> | Streptomyces sp. A306 Ydz-ZZ 16S ribosomal RNA gene, partial sequence | 2220 | 100% | 0 | 99% |
| <u>EU273549.1</u> | Streptomyces globisporus subsp. globisporus isolate XSD-114 16S ribosomal RNA gene, partial sequence | 2220 | 100% | 0 | 99% |
| <u>EU257275.1</u> | Streptomyces sp. A10Ydz-XM 16S ribosomal RNA gene, partial sequence | 2220 | 100% | 0 | 99% |
| <u>EF192235.1</u> | Streptomyces griseus 16S ribosomal RNA gene, partial sequence | 2220 | 100% | 0 | 99% |

APPENDIX F

Ribosomal Database Project (RDP) results (top 20 lists) of strains X34, X42 and X77

SeqMatch :: Detail Hierarchy –Strain X34

Query Sequence: unknown, 1309 unique oligos

short ID, orientation, similarity score, S_ab score, unique common oligomers and sequence full name.

Lineage:

| | | | | | |
|---|--------------------------|----------------------------|----------------|--------------------------------------|-----------------------------------|
| Lineage: | | | | | |
| + | no rank | Root | (0/20/609523) | (selected/match/total RDP sequences) | |
| + | domain | Bacteria | (0/20/600316) | | |
| + | phylum | "Actinobacteria" | (0/20/115554) | | |
| + | class | Actinobacteria | (0/20/115554) | | |
| + | subclass | Actinobacteridae | (0/20/110191) | | |
| + | order | Actinomycetales | (0/20/109342) | | |
| + | suborder | Streptomycineae | (0/20/6703) | | |
| + | family | Streptomycetaceae | (0/20/6703) | | |
| + | genus | Streptomyces | (0/20/6449) | | |
| | <input type="checkbox"/> | S000015282 | not_calculated | 0.995 | 1382 Streptomyces maritimus; |
| BD26; AF233338 | | | | | |
| | <input type="checkbox"/> | S000607425 | not_calculated | 0.995 | 1368 Streptomyces sp.; |
| NT90(K15); AJ002089 | | | | | |
| | <input type="checkbox"/> | S000651961 | not_calculated | 0.995 | 1354 Streptomyces rochei (T); |
| NBRC 12908; AB184237 | | | | | |
| | <input type="checkbox"/> | S000652015 | not_calculated | 0.995 | 1371 Streptomyces plicatus; NBRC |
| 13071; AB184291 | | | | | |
| | <input type="checkbox"/> | S000652035 | not_calculated | 0.995 | 1364 Streptomyces |
| vinaceusdrappus; NBRC 13099; AB184311 | | | | | |
| | <input type="checkbox"/> | S000652337 | not_calculated | 0.995 | 1341 Streptomyces |
| rubrocyano-diastaticus subsp. piger; NBRC 14692; AB184613 | | | | | |
| | <input type="checkbox"/> | S000652454 | not_calculated | 0.995 | 1371 Streptomyces olivaceus; |
| NBRC 3119; AB184730 | | | | | |
| | <input type="checkbox"/> | S000711645 | not_calculated | 0.995 | 1378 Streptomyces enissocaesilis; |
| NRRL B-16365; DQ026641 | | | | | |
| | <input type="checkbox"/> | S000712498 | not_calculated | 0.995 | 1384 Streptomyces sp. 3194; |
| DQ663150 | | | | | |
| | <input type="checkbox"/> | S000892542 | not_calculated | 0.995 | 1378 Streptomyces rochei; NRRL |
| B-1559; EF626598 | | | | | |
| | <input type="checkbox"/> | S000967652 | not_calculated | 0.995 | 1338 Streptomyces sp. A53Ydz- |
| XM; EU257263 | | | | | |
| | <input type="checkbox"/> | S001096907 | not_calculated | 0.995 | 1342 Streptomyces rochei; 173315; |
| EU570372 | | | | | |
| | <input type="checkbox"/> | S001097158 | not_calculated | 0.995 | 1335 Streptomyces rochei; 173342; |
| EU570623 | | | | | |
| | <input type="checkbox"/> | S001097246 | not_calculated | 0.995 | 1341 Streptomyces rochei; 173609; |
| EU570711 | | | | | |

| | | | | | | |
|----------------------|--------------------------|----------------------------|----------------|-------|------|------------------------------|
| 173969; EU593640 | <input type="checkbox"/> | S001097744 | not_calculated | 0.995 | 1337 | Streptomyces avidinii; |
| EU593730 | <input type="checkbox"/> | S001097834 | not_calculated | 0.995 | 1340 | Streptomyces rochei; 173260; |
| EU593731 | <input type="checkbox"/> | S001097835 | not_calculated | 0.995 | 1344 | Streptomyces rochei; 173672; |
| EU621883 | <input type="checkbox"/> | S001098348 | not_calculated | 0.995 | 1359 | Streptomyces sp. YDG17; |
| HBUM174096; EU841560 | <input type="checkbox"/> | S001154764 | not_calculated | 0.995 | 1344 | Streptomyces rochei; |
| FJ224362 | <input type="checkbox"/> | S001189021 | not_calculated | 0.995 | 1328 | Streptomyces sp. P-13; |

Query Sequence: unknown, 1233 unique oligos

short ID, orientation, similarity score, S_ab score, unique common oligomers and sequence full name.

Lineage:

| | | | | | | |
|-------------------------------------|-------------------------------------|---|----------------|-------|------|-----------------------------|
| <input checked="" type="checkbox"/> | no rank | Root (0/20/615916) (selected/match/total RDP sequences) | | | | |
| <input checked="" type="checkbox"/> | | domain Bacteria (0/20/606188) | | | | |
| <input checked="" type="checkbox"/> | | phylum "Actinobacteria" (0/20/115729) | | | | |
| <input checked="" type="checkbox"/> | | class Actinobacteria (0/20/115729) | | | | |
| <input checked="" type="checkbox"/> | | subclass Actinobacteridae (0/20/110300) | | | | |
| <input checked="" type="checkbox"/> | | order Actinomycetales (0/20/109449) | | | | |
| <input checked="" type="checkbox"/> | | suborder Streptomycineae (0/20/6711) | | | | |
| <input checked="" type="checkbox"/> | | family Streptomycetaceae (0/20/6711) | | | | |
| <input checked="" type="checkbox"/> | | genus Streptomyces (0/20/6457) | | | | |
| <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | <u>S000004207</u> | not_calculated | 0.989 | 1382 | Streptomyces griseus; IFO |
| 13550; AB045867 | | | | | | |
| <input type="checkbox"/> | <input type="checkbox"/> | <u>S000006304</u> | not_calculated | 0.982 | 1377 | Streptomyces sp.; |
| ATCC10246; Y15503 | | | | | | |
| <input type="checkbox"/> | <input type="checkbox"/> | <u>S000393985</u> | not_calculated | 0.982 | 1413 | Streptomyces sp. VTT E-99- |
| 1329 (A52); AF429393 | | | | | | |
| <input type="checkbox"/> | <input type="checkbox"/> | <u>S000439349</u> | not_calculated | 0.982 | 1425 | Streptomyces sampsonii (T); |
| ATCC25495; D63871 | | | | | | |
| <input type="checkbox"/> | <input type="checkbox"/> | <u>S000651942</u> | not_calculated | 0.982 | 1353 | Streptomyces sp.; NBRC |
| 12883; AB184218 | | | | | | |
| <input type="checkbox"/> | <input type="checkbox"/> | <u>S000652367</u> | not_calculated | 0.982 | 1353 | Streptomyces albidoflavus; |
| NBRC 15392; AB184643 | | | | | | |
| <input type="checkbox"/> | <input type="checkbox"/> | <u>S000711646</u> | not_calculated | 0.982 | 1426 | Streptomyces champavatii |
| (T); NRRL B-5682; DQ026642 | | | | | | |
| <input type="checkbox"/> | <input type="checkbox"/> | <u>S000770062</u> | not_calculated | 0.989 | 1388 | Streptomyces sp. 45zhy; |
| AM412550 | | | | | | |
| <input type="checkbox"/> | <input type="checkbox"/> | <u>S000770063</u> | not_calculated | 0.983 | 1388 | Streptomyces sp. 41zhy; |
| AM412551 | | | | | | |
| <input type="checkbox"/> | <input type="checkbox"/> | <u>S000770111</u> | not_calculated | 0.994 | 1392 | Streptomyces sp. C19-17; |
| EF063445 | | | | | | |
| <input type="checkbox"/> | <input type="checkbox"/> | <u>S000770153</u> | not_calculated | 0.994 | 1392 | Streptomyces sp. JXHM-A- |
| 12; EF063487 | | | | | | |
| <input type="checkbox"/> | <input type="checkbox"/> | <u>S000966532</u> | not_calculated | 0.986 | 1392 | Streptomyces sp. 94P32-5; |
| EU181249 | | | | | | |
| <input type="checkbox"/> | <input type="checkbox"/> | <u>S000967629</u> | not_calculated | 0.989 | 1344 | Streptomyces sp. A46Ydz- |
| XG; EU257240 | | | | | | |
| <input type="checkbox"/> | <input type="checkbox"/> | <u>S000967644</u> | not_calculated | 0.994 | 1348 | Streptomyces sp. A93Ydz- |
| QZ; EU257255 | | | | | | |
| <input type="checkbox"/> | <input type="checkbox"/> | <u>S000981689</u> | not_calculated | 0.994 | 1347 | Streptomyces sp. A52 Ydz- |
| XM; EU368806 | | | | | | |
| <input type="checkbox"/> | <input type="checkbox"/> | <u>S001015127</u> | not_calculated | 0.994 | 1347 | Streptomyces sp. A556 Ydz- |
| TA; EU384262 | | | | | | |
| <input type="checkbox"/> | <input type="checkbox"/> | <u>S001093294</u> | not_calculated | 0.994 | 1306 | Streptomyces albidoflavus; |
| JAM-AC0101; AB362247 | | | | | | |

| | | | | | | |
|------------------|--------------------------|-------------------|----------------|-------|------|----------------------------|
| GU002076 | <input type="checkbox"/> | <u>S001613869</u> | not_calculated | 0.994 | 1301 | Streptomyces sp. FXJ6.047; |
| B-2165; EF588223 | <input type="checkbox"/> | <u>S001793115</u> | not_calculated | 0.984 | 1305 | Streptomyces griseus; NRRL |
| GU263861 | <input type="checkbox"/> | <u>S001873751</u> | not_calculated | 0.983 | 1362 | Streptomyces sp. 163003; |

.....

SeqMatch :: Detail Hierarchy -X77

Query Sequence: unknown, 1147 unique oligos

short ID, orientation, [similarity score](#), [S_ab score](#), unique common oligomers and sequence full name.

Lineage:

| | | | | |
|--|--|----------------------------|----------------|---|
| <input checked="" type="checkbox"/> | no rank Root (0/20/615916) (selected/match/total RDP sequences) | | | |
| <input checked="" type="checkbox"/> | domain Bacteria (0/20/606188) | | | |
| <input checked="" type="checkbox"/> | phylum "Actinobacteria" (0/20/115729) | | | |
| <input checked="" type="checkbox"/> | class Actinobacteria (0/20/115729) | | | |
| <input checked="" type="checkbox"/> | subclass Actinobacteridae (0/20/110300) | | | |
| <input checked="" type="checkbox"/> | order Actinomycetales (0/20/109449) | | | |
| <input checked="" type="checkbox"/> | suborder Streptomycineae (0/20/6711) | | | |
| <input checked="" type="checkbox"/> | family Streptomycetaceae (0/20/6711) | | | |
| <input checked="" type="checkbox"/> | genus Streptomyces (0/20/6457) | | | |
| | <input type="checkbox"/> | S000406301 | not_calculated | 0.997 1391 Streptomyces sp. YIM 80038; |
| AY299622 | | | | |
| | <input type="checkbox"/> | S000587694 | not_calculated | 0.997 1383 Actinomycetales bacterium |
| HPA66; DQ144230 | | | | |
| | <input type="checkbox"/> | S000651790 | not_calculated | 0.997 1376 Streptomyces globisporus |
| subsp. globisporus; NBRC 12208; AB184066 | | | | |
| | <input type="checkbox"/> | S000651988 | not_calculated | 0.997 1383 Streptomyces cavourensis |
| subsp. cavourensis; NBRC 13026; AB184264 | | | | |
| | <input type="checkbox"/> | S000652149 | not_calculated | 0.997 1373 Streptomyces albolongus (T); |
| NBRC 13465; AB184425 | | | | |
| | <input type="checkbox"/> | S000652163 | not_calculated | 0.997 1373 Streptomyces bacillaris (T); |
| NBRC 13487; AB184439 | | | | |
| | <input type="checkbox"/> | S000652202 | not_calculated | 0.997 1338 Streptomyces tsusimaensis; |
| NBRC 13782; AB184478 | | | | |
| | <input type="checkbox"/> | S000711020 | not_calculated | 0.997 1361 Streptomyces griseobrunneus |
| (T); NBRC 12775; AB249912 | | | | |
| | <input type="checkbox"/> | S000770104 | not_calculated | 0.997 1388 Streptomyces sp. M10; |
| EF034030 | | | | |
| | <input type="checkbox"/> | S000805317 | not_calculated | 0.997 1332 Streptomyces griseus; |
| EF192235 | | | | |
| | <input type="checkbox"/> | S000967664 | not_calculated | 0.997 1350 Streptomyces sp. A10Ydz- |
| XM; EU257275 | | | | |
| | <input type="checkbox"/> | S000968181 | not_calculated | 0.997 1371 Streptomyces globisporus |
| subsp. globisporus; XSD-114; EU273549 | | | | |
| | <input type="checkbox"/> | S000981670 | not_calculated | 0.997 1347 Streptomyces sp. A306 Ydz- |
| ZZ; EU368787 | | | | |
| | <input type="checkbox"/> | S001198885 | not_calculated | 0.997 1408 Streptomyces sp. DS3024; |
| FJ238114 | | | | |
| | <input type="checkbox"/> | S001292987 | not_calculated | 0.997 1320 Actinomycetales bacterium |
| JH105; FJ572022 | | | | |
| | <input type="checkbox"/> | S001351242 | not_calculated | 0.997 1359 Streptomyces bacillaris; |
| cfcc3101; FJ792550 | | | | |
| | <input type="checkbox"/> | S001416303 | not_calculated | 0.997 1373 Streptomyces anulatus; |

| | | | | | | |
|--------------------------------------|--------------------------|-------------------|----------------|-------|------|--------------------------|
| Malaysia; EU647478 | <input type="checkbox"/> | <u>S001416786</u> | not_calculated | 0.997 | 1395 | Streptomyces globisporus |
| subsp. globisporus; 13638A; EU741124 | | | | | | |
| | <input type="checkbox"/> | <u>S001416853</u> | not_calculated | 0.997 | 1395 | Streptomyces sp. 13661T; |
| EU741191 | | | | | | |
| | <input type="checkbox"/> | <u>S001416883</u> | not_calculated | 0.997 | 1392 | Streptomyces globisporus |
| subsp. globisporus; 13669A; EU741221 | | | | | | |

APPENDIX G

Details information of closely related *Streptomyces* spp. to *S. rochei* (strain X34), *S. albidoflavus* (strain X42) and *S. cavourensis* (strain X77) – from NCBI GenBank

***Streptomyces rochei* gene for 16S rRNA, partial sequence, strain: NBRC 12908**

GenBank: AB184237.1
LOCUS AB184237, 1472 bp DNA linear BCT 01-APR-2006
DEFINITION *Streptomyces rochei* gene for 16S rRNA, partial sequence, strain: NBRC 12908.
ACCESSION AB184237
VERSION AB184237.1 GI:90960053
ORGANISM [Streptomyces rochei](#)
REFERENCE 1 AUTHORS Tamura,T., Oguchi,A., Kikuchi,T., Kikuchi,H., Nishii,T.,
Tsuji,K., Yamaguchi,Y., Tase,A., Takahashi,M., Sakane,T.,
Suzuki,K. and Hatano,K.
TITLE Phylogenic analysis of the genus *Streptomyces*
JOURNAL Unpublished
REFERENCE 2 AUTHORS Tamura,T., Oguchi,A., Kikuchi,T., Kikuchi,H., Nishii,T., Tsuji,K.,
(bases 1 to 1472) Yamaguchi,Y., Tase,A., Takahashi,M., Sakane,T., Suzuki,K. and
Hatano,K.
TITLE Direct Submission
JOURNAL Submitted (14-JUL-2004) Tomohiko Tamura, National Institute of
Technology and Evaluation, Department of Biotechnology; 2-5-8,
Kazusakamatari, Kisarazu, Chiba 292-0818, Japan

ORIGIN

```
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1021 cgtgagatgt tgggtaagt cccgcaacga gcgcaaccct tgtcccgtgt tgccagcagg
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1381 accggaagcc ggtggcccaa ccccttggg gagggagctg tcgaaggtgg gactggcagat
1441 tgggacgaag tcgtaacaag gtgaccgtac cg
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***Streptomyces albidoflavus* (DSM 40455T) 16S rRNA gene**

GenBank: Z76676.1

LOCUS Z76676, 1476 bp DNA linear BCT 25-APR-1997
 DEFINITION S.albidoflavus (DSM 40455T) 16S rRNA gene.
 ACCESSION Z76676
 VERSION Z76676.1 GI:2052194
 ORGANISM [Streptomyces albidoflavus](#)
 REFERENCE 1 AUTHORS Hain,T., Ward-Rainey,N., Kroppenstedt,R.M., Stackebrandt,E. and Rainey,F.A.
 TITLE Discrimination of Streptomyces albidoflavus strains based on the size and number of 16S-23S ribosomal DNA intergenic spacers
 JOURNAL Int. J. Syst. Bacteriol. 47 (1), 202-206 (1997)
 REFERENCE 2 AUTHORS Rainey,F.A.
 (bases 1 to 1476) TITLE Direct Submission
 JOURNAL Submitted (16-JUL-1996) F.A. Rainey, DSM - Dt.Sammlung von Mikroorganismen, und Zellkulturen, Mascheroder Weg 1b, D-38124 Braunschweig, FRG

ORIGIN

1 aacgtggcg gegtcttaa cacatgcaag tcgaacgatg aaccgcttc gggcggggat
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 181 gcggtgcagg atgagcccgc ggccatcag cttgtggtg aggtagtggc tcaccaaggc
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 1321 ggtgaatag ttccgggccc ttgtacacac cggcgtcac gtcacgaaag tcggtaacac
 1381 ccgaagccgg tgcccgaacc ccttgggga gggagctgtc gaaggtggga ctggcgattg
 1441 ggacgaagtc gtaacaaggt agccgtaccg gaaggt

***Streptomyces cavourensis* subsp. *cavourensis* gene for 16S rRNA, partial sequence, strain: NBRC 13026**

GenBank: AB184264.1 1479 bp DNA linear BCT 01-APR-2006

LOCUS AB184264
 DEFINITION *Streptomyces cavourensis* subsp. *cavourensis* gene for 16S rRNA, partial sequence, strain: NBRC 13026.
 ACCESSION AB184264
 VERSION AB184264.1 GI:90960080
 ORGANISM [Streptomyces cavourensis subsp. cavourensis](#)
 REFERENCE 1 AUTHORS Tamura,T., Oguchi,A., Kikuchi,T., Kikuchi,H., Nishii,T., Tsuji,K., Yamaguchi,Y., Tase,A., Takahashi,M., Sakane,T., Suzuki,K. and Hatano,K.
 TITLE Phylogenic analysis of the genus Streptomyces
 JOURNAL Unpublished
 REFERENCE 2 AUTHORS Tamura,T., Oguchi,A., Kikuchi,T., Kikuchi,H., Nishii,T., Tsuji,K., Yamaguchi,Y., Tase,A., Takahashi,M., Sakane,T., Suzuki,K. and Hatano,K.
 (bases 1 to 1479)
 TITLE Direct Submission
 JOURNAL Submitted (14-JUL-2004) Tomohiko Tamura, National Institute of Technology and Evaluation, Department of Biotechnology; 2-5-8, Kazusakamatari, Kisarazu, Chiba 292-0818, Japan

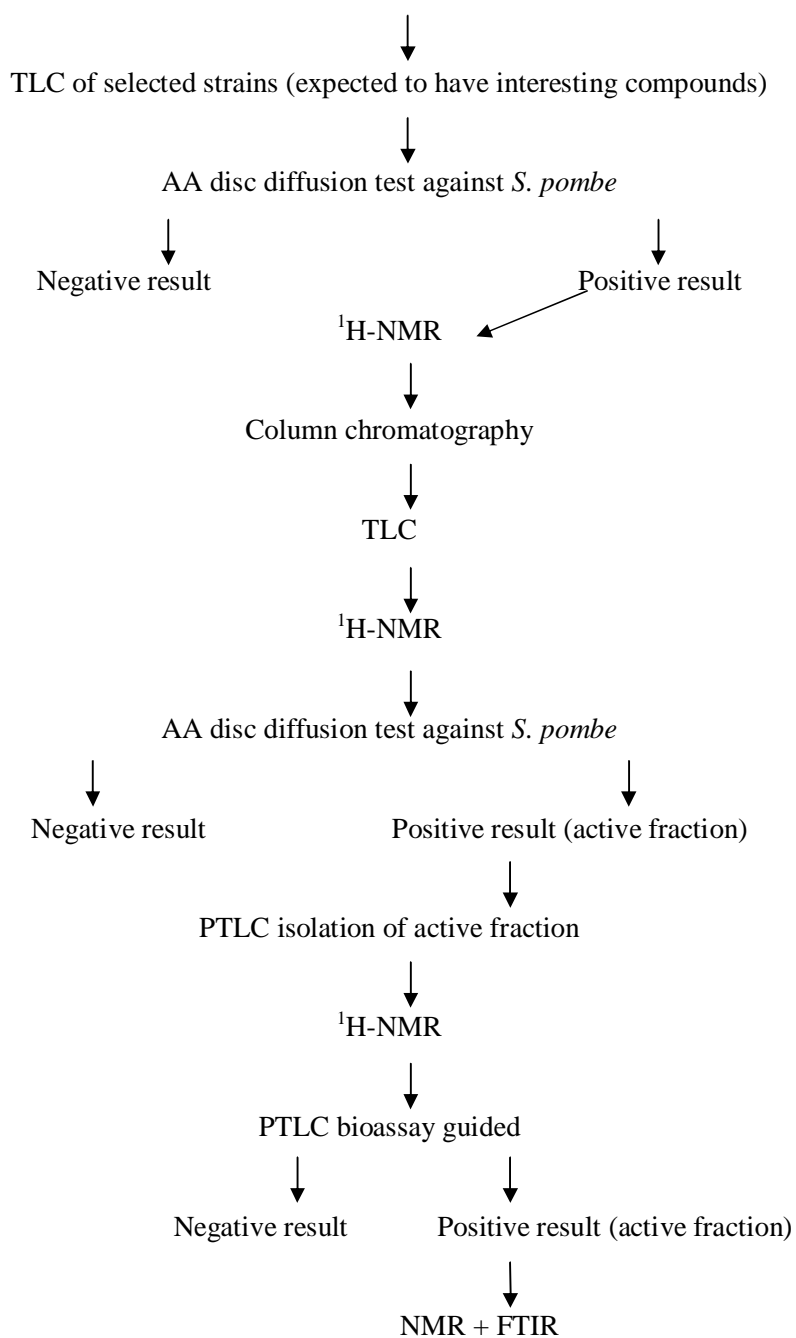
ORIGIN

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 121 ctggaacgg ggtctaatac cggataatac tctgcctgc atgggtgggg gttgaaagct
 181 ccggcgggtga aggatgagcc cgcggcctat cagctgttg gtggggtaat ggcctaccaa
 241 ggcgacgacg ggtagccggc ctgagagggc gaccggccac actgggactg agacacggcc
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 661 aacaccggtg gcgaaggcgg atctctgggc cattactgac gctgaggagc gaaagcgtgg
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 1141 gacgacgta agtcatcatg ccccttatgt cttgggtgc acacgtgcta caatggccgg
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 1321 ctgcggtgaa tacgttccc ggcctgtac acaccgccc tcacgtcacg aaagtcgta
 1381 acaccggaag ccggtggccc aacccttgt gggagggagc tgtcgaaggt gggactggcg
 1441 attgggacga agtcgtaaca aggtagccgt accggaagg

APPENDIX H

Flow chart of chemical profiling and compound isolation procedure

HPLC of potential strains (based on primary screening for antifungal activity)



APPENDIX I

^1H -NMR chromatograms of fractions F1 – F6 of *S. rochei* after column chromatography

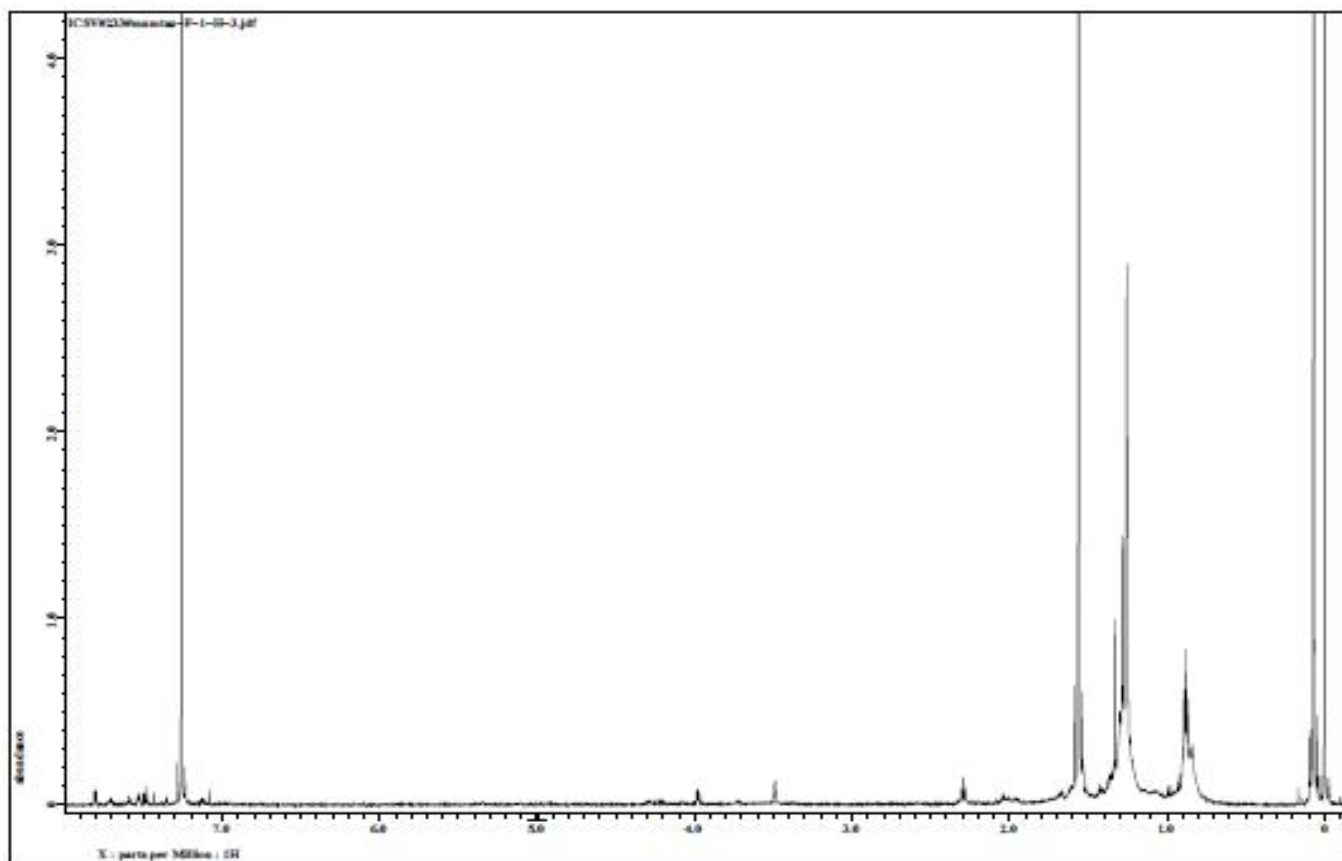


Figure I-1: ^1H -NMR chromatograms of fraction F1 of *S. rochei* (strain X34)

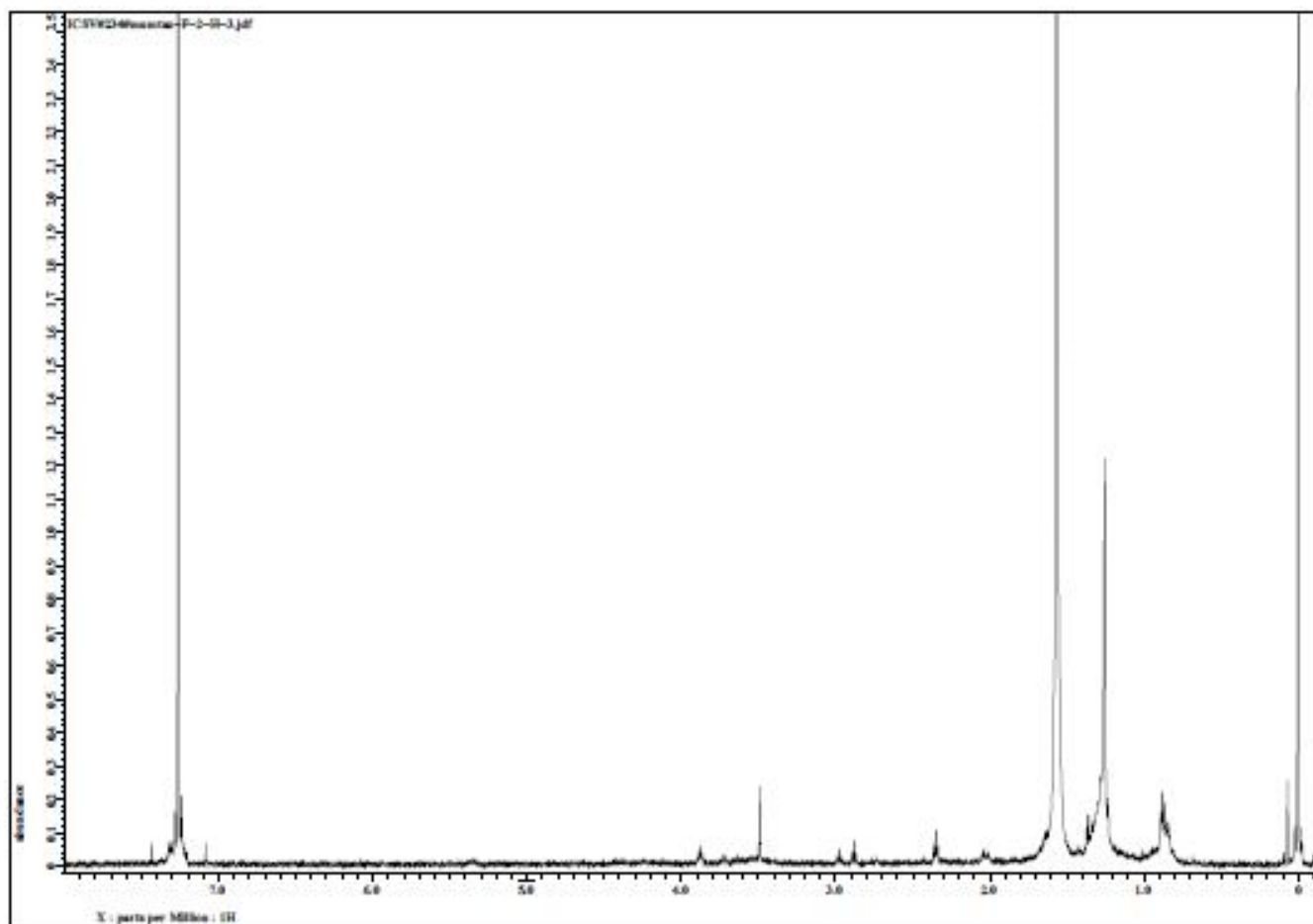


Figure I-2: ^1H -NMR chromatograms of fraction F2 of *S. rochei* (strain X34)

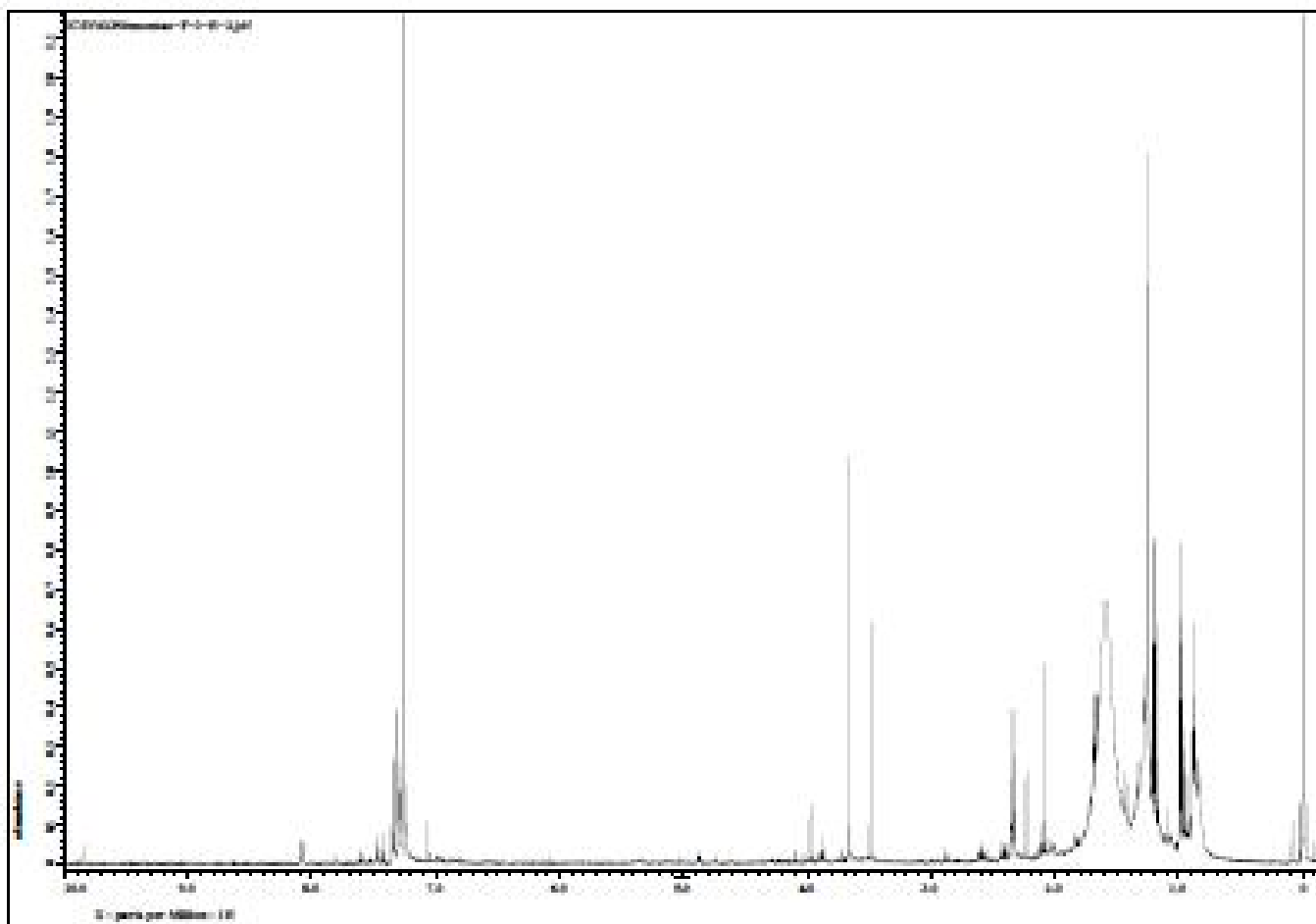


Figure I-3: ^1H -NMR chromatograms of fraction F3 of *S. rochei* (strain X34)

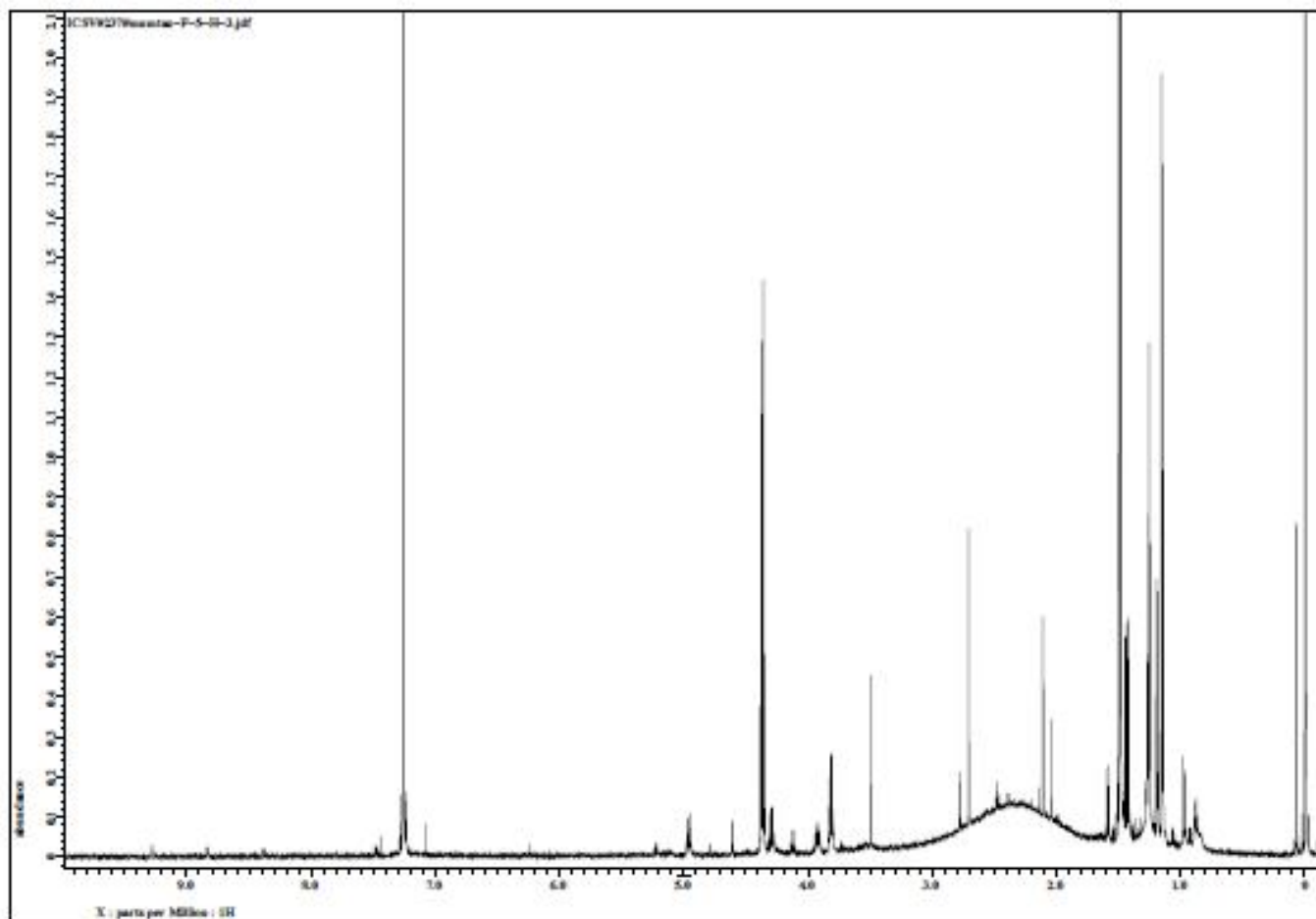


Figure I-4: ^1H -NMR chromatograms of fraction F4 of *S. rochei* (strain X34)

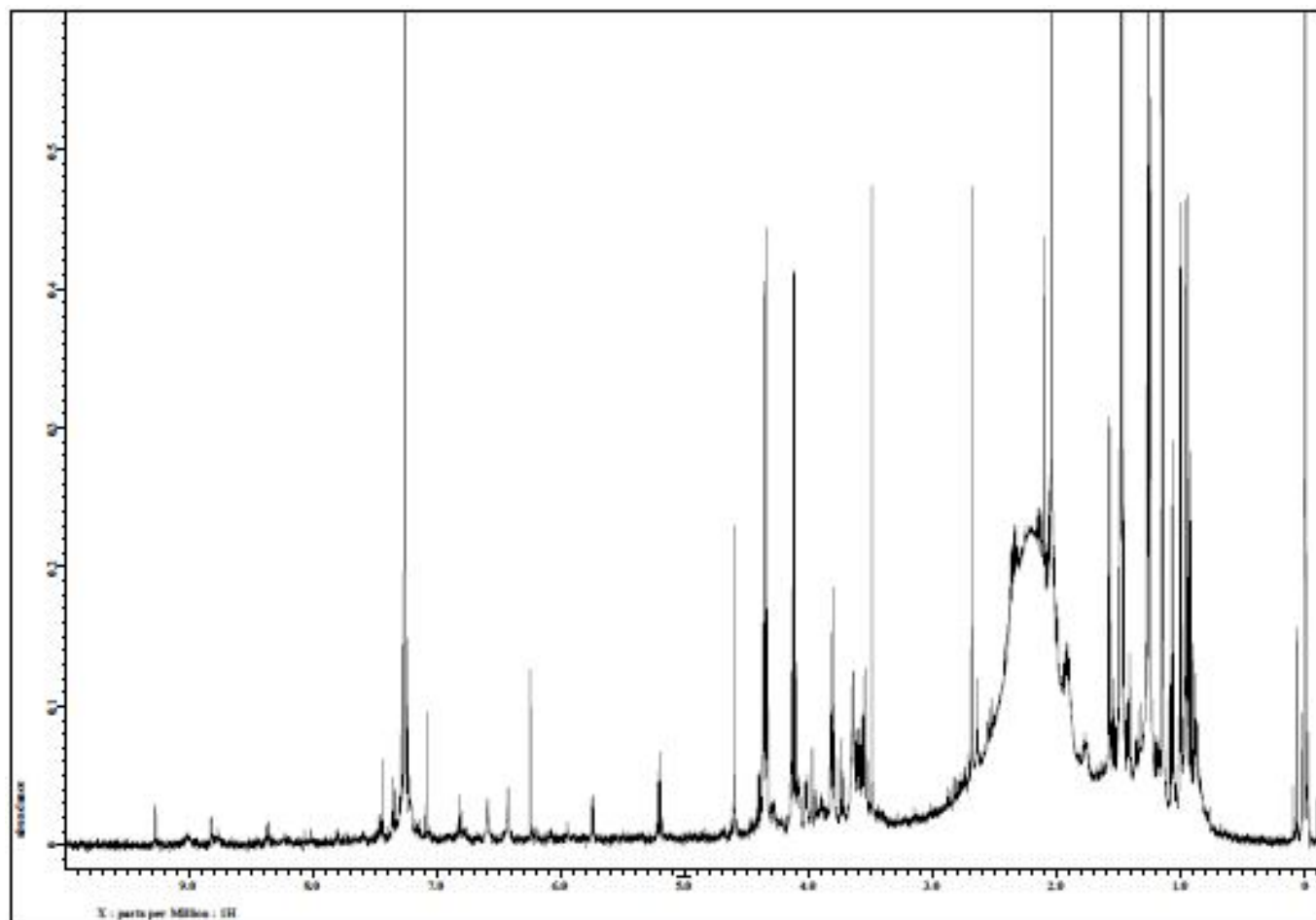


Figure I-5: ^1H -NMR chromatograms of fraction F5 of *S. rochei* (strain X34)

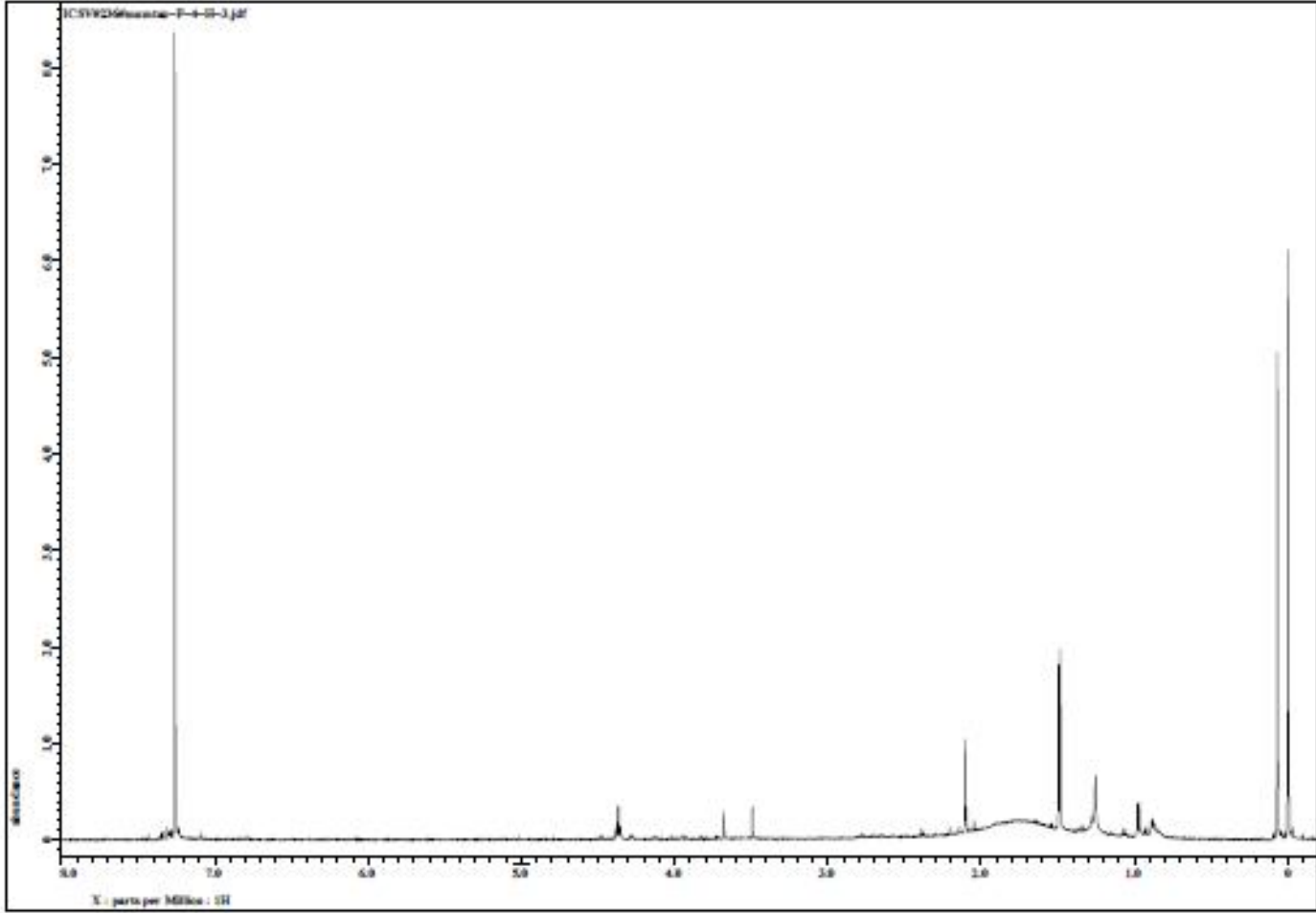
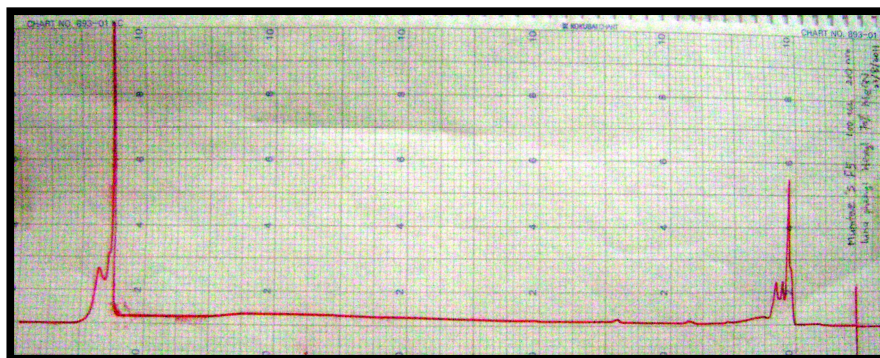


Figure I-6: ^1H -NMR chromatograms of fraction F6 of *S. rochei* (strain X34)

APPENDIX J

HPLC chromatograms of active fraction F5 of *Streptomyces rochei* (strain X34) using four parameters

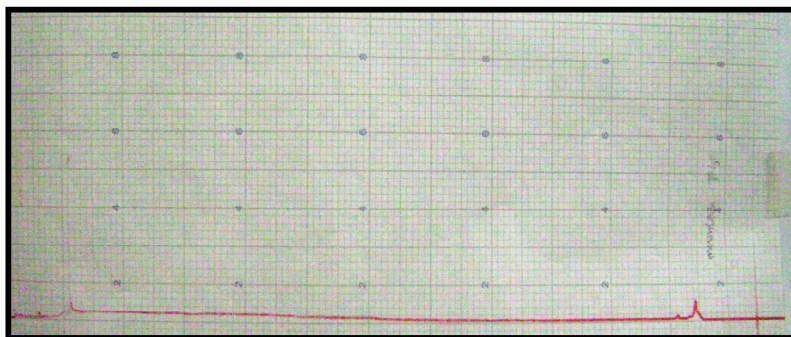
Column : Luna phenyl-Hexyl
Wavelength : 210nm
Solvent : 75% of 70% MeCN
Flow rate : 2.0ml/min
Injection volume : 100 μ l
Sample [] : 5mg/ml MeCN
Sensitivity of plotter : 0.050 AU



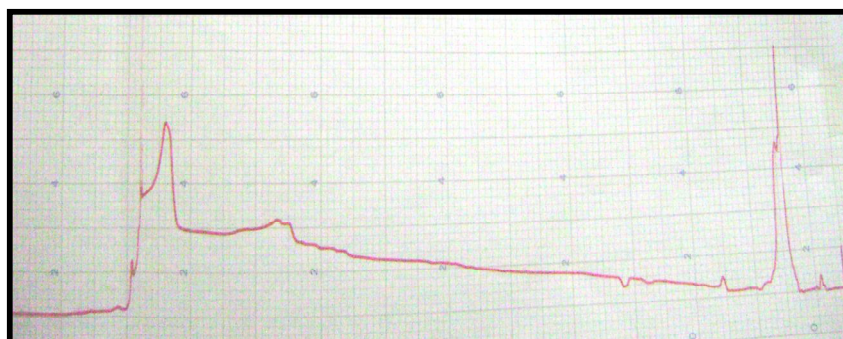
Column : Luna phenyl-Hexyl
Wavelength : 210nm
Solvent : 90% of 70% MeCN
Flow rate : 2.0ml/min
Injection volume : 100 μ l
Sample [] : 5mg/ml MeCN
Sensitivity of plotter : 0.050 AU



Column : C18 (2)
Wavelength : 254 nm
Solvent : 75% of 70% MeCN
Flow rate : 2.0ml/min
Injection volume : 100µl
Sample [] : 5mg/ml MeCN
Sensitivity of plotter : 0.050 AU



Column : C18 (2)
Wavelength : 210 nm
Solvent : 50% of 50% MeCN
Flow rate : 2.0ml/min
Injection volume : 100µl
Sample [] : 5mg/ml MeCN
Sensitivity of plotter : 0.050 AU



APPENDIX K

HPLC chromatograms of six potential strains of tested *Streptomyces* spp.

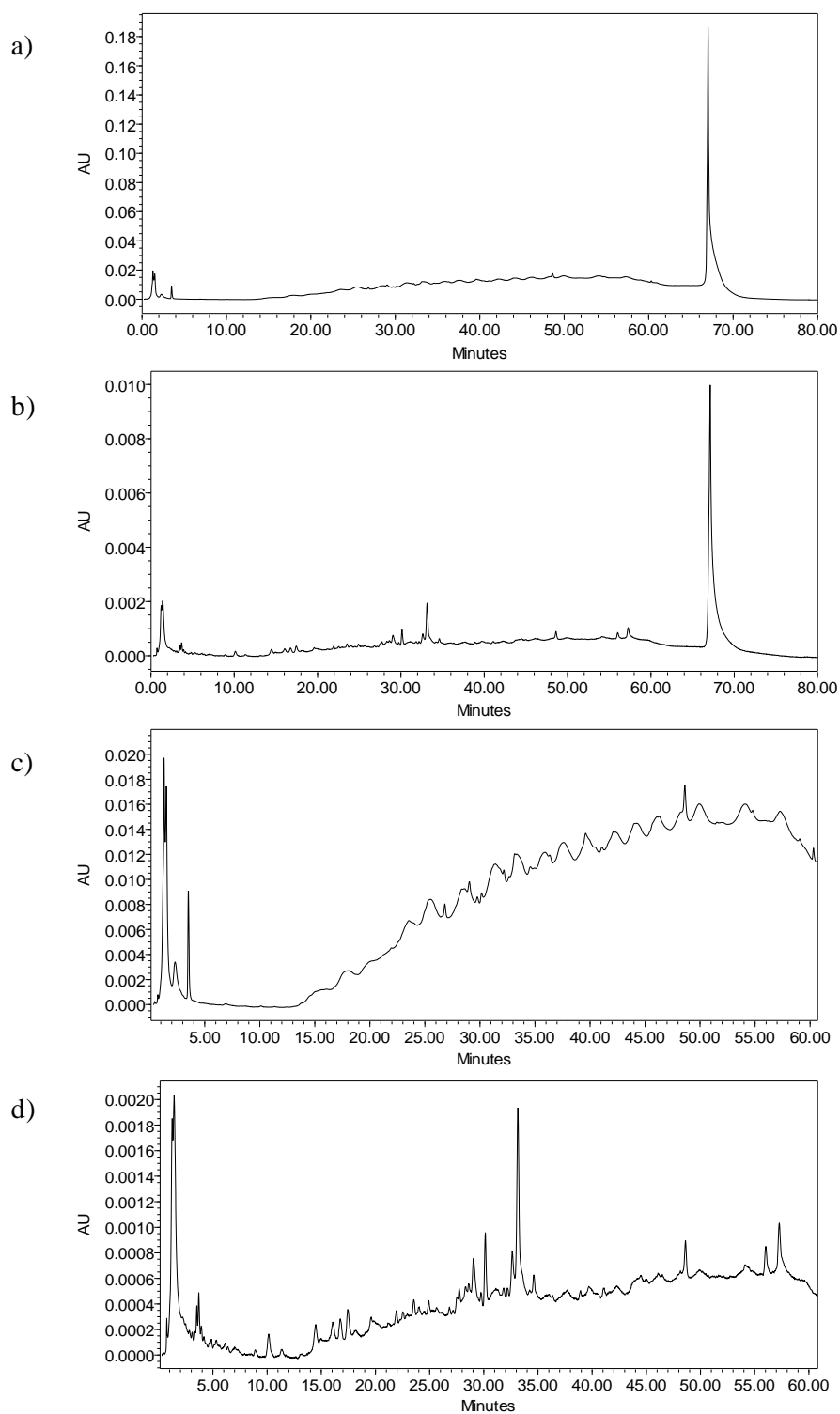


Figure K-1: HPLC chromatograms of strain X13 with different UV detector. a & c: 220nm, b & d: 254nm

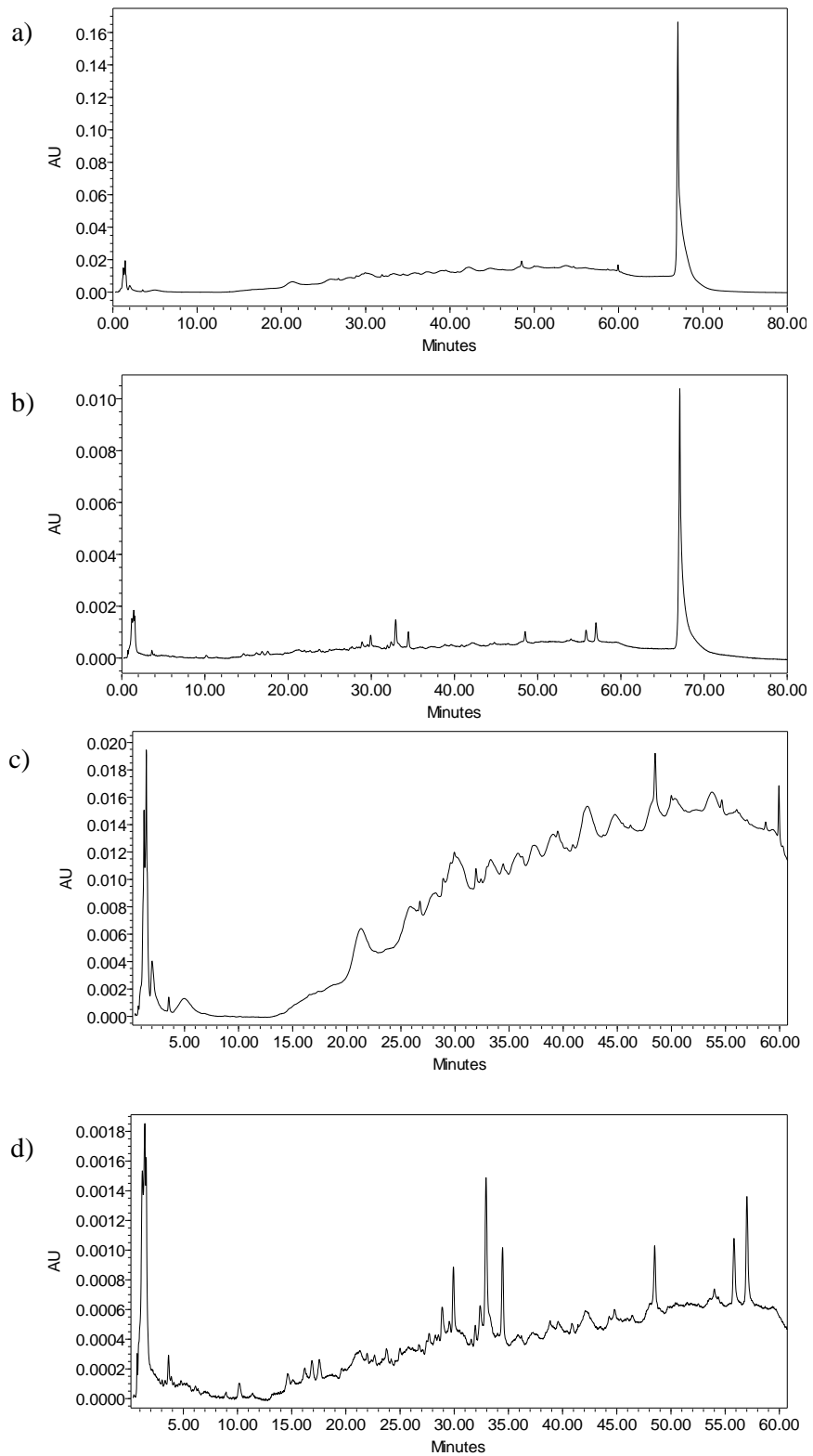


Figure K-2: HPLC chromatograms of strain X32 with different UV detector. a & c: 220nm, b & d: 254nm

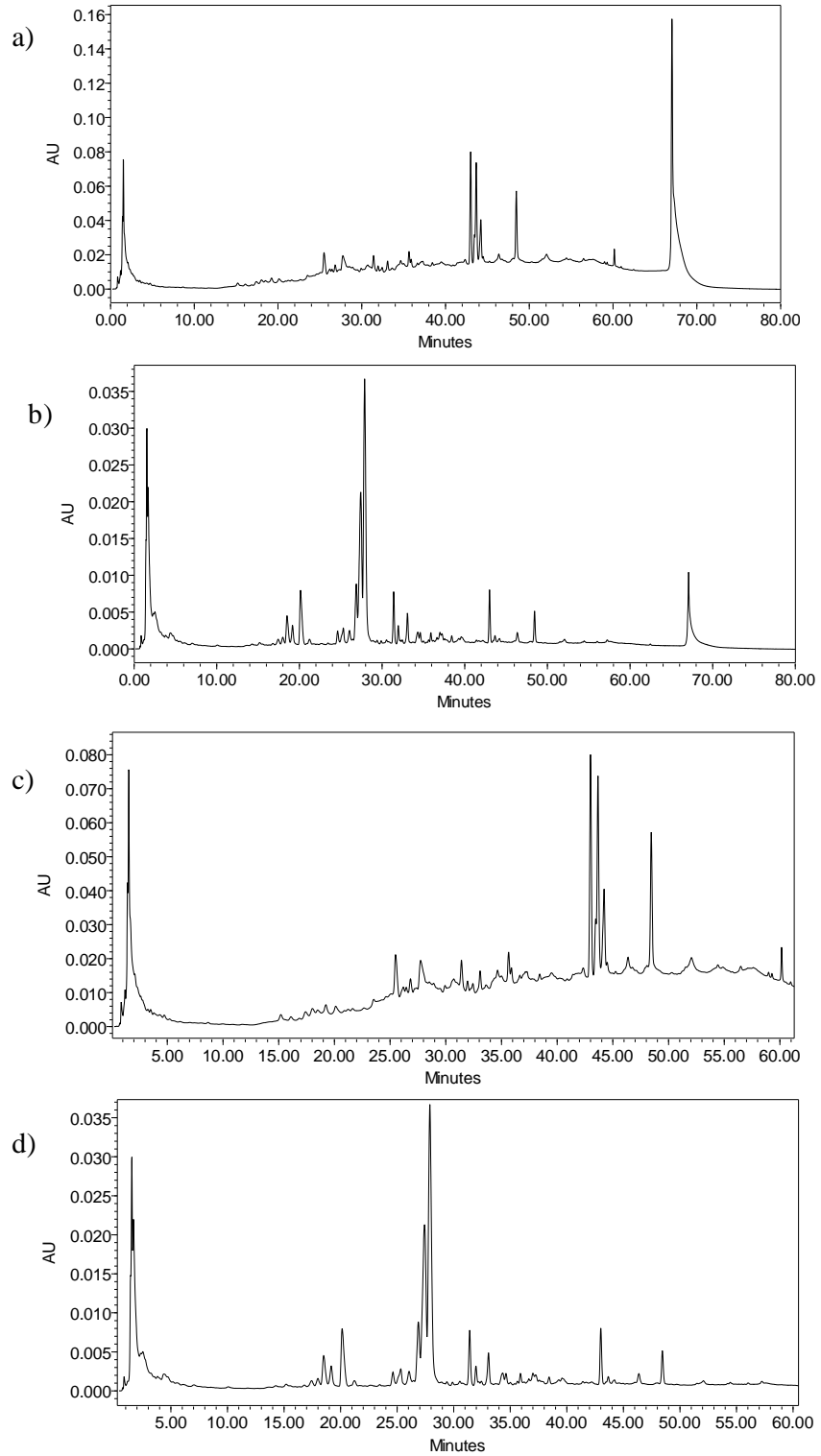


Figure K-3: HPLC chromatograms of strain X34 with different UV detector. a & c: 220nm, b & d: 254nm

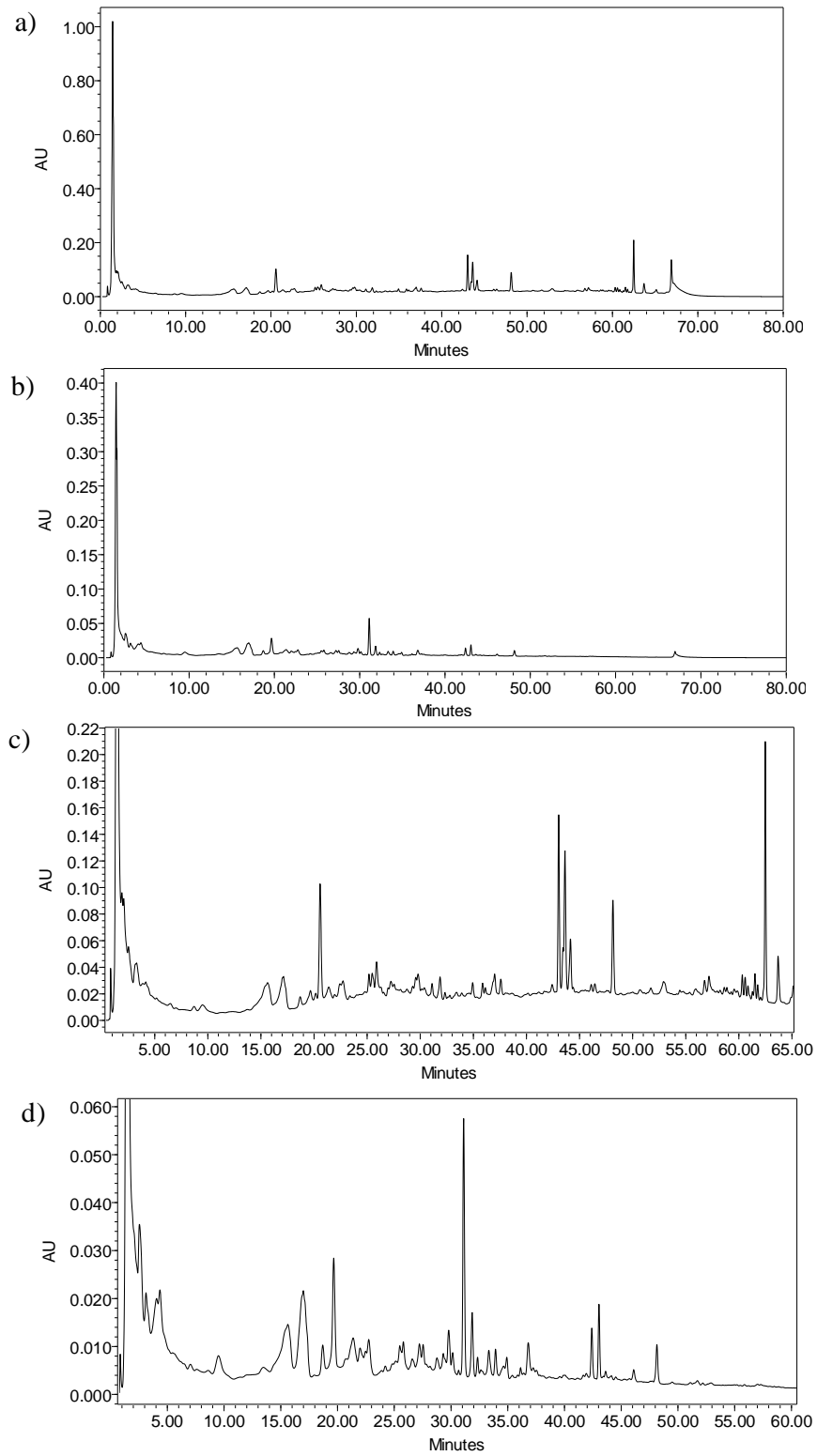


Figure K-4: HPLC chromatograms of strain X35 with different UV detector. a & c: 220nm, b & d: 254nm

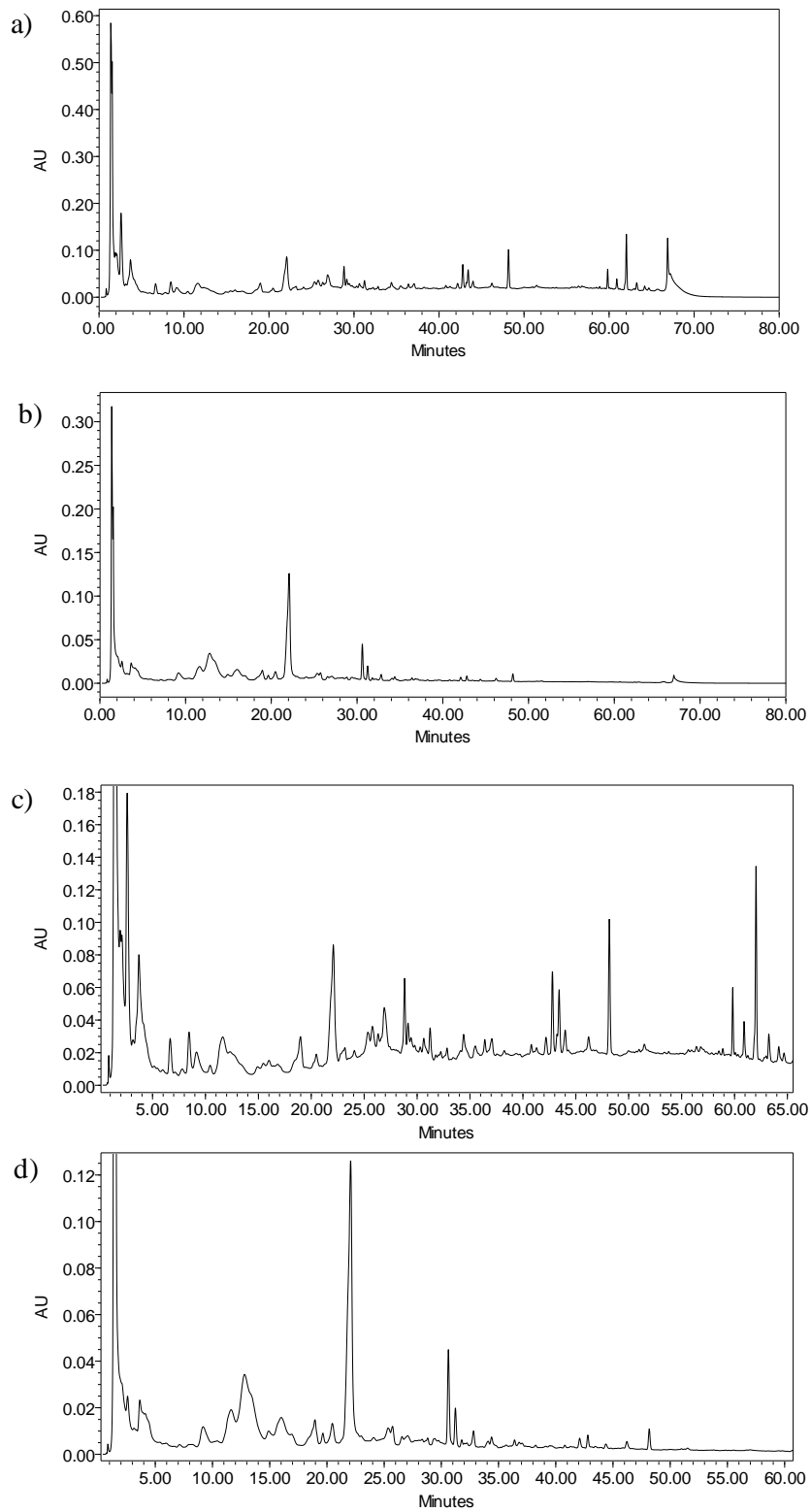


Figure K-5: HPLC chromatograms of strain X42 with different UV detector. a & c: 220nm, b & d: 254nm

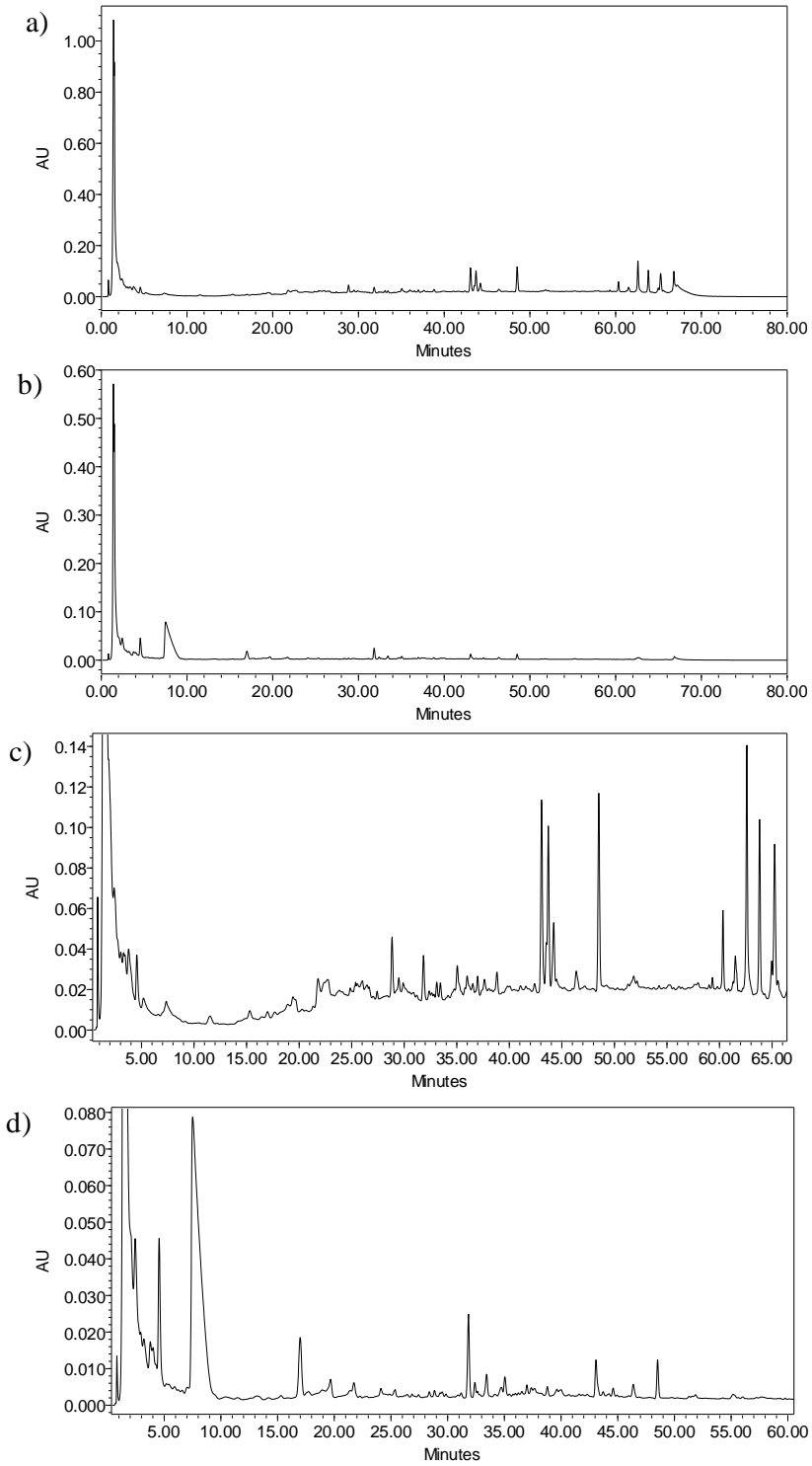


Figure K-6: HPLC chromatograms of strain X77 with different UV detector. a & c: 220nm, b & d: 254nm

APPENDIX L

$^1\text{H-NMR}$ chromatograms of sub-fractions F5.1 – F5.6 of *S. rochei* (strain X34)

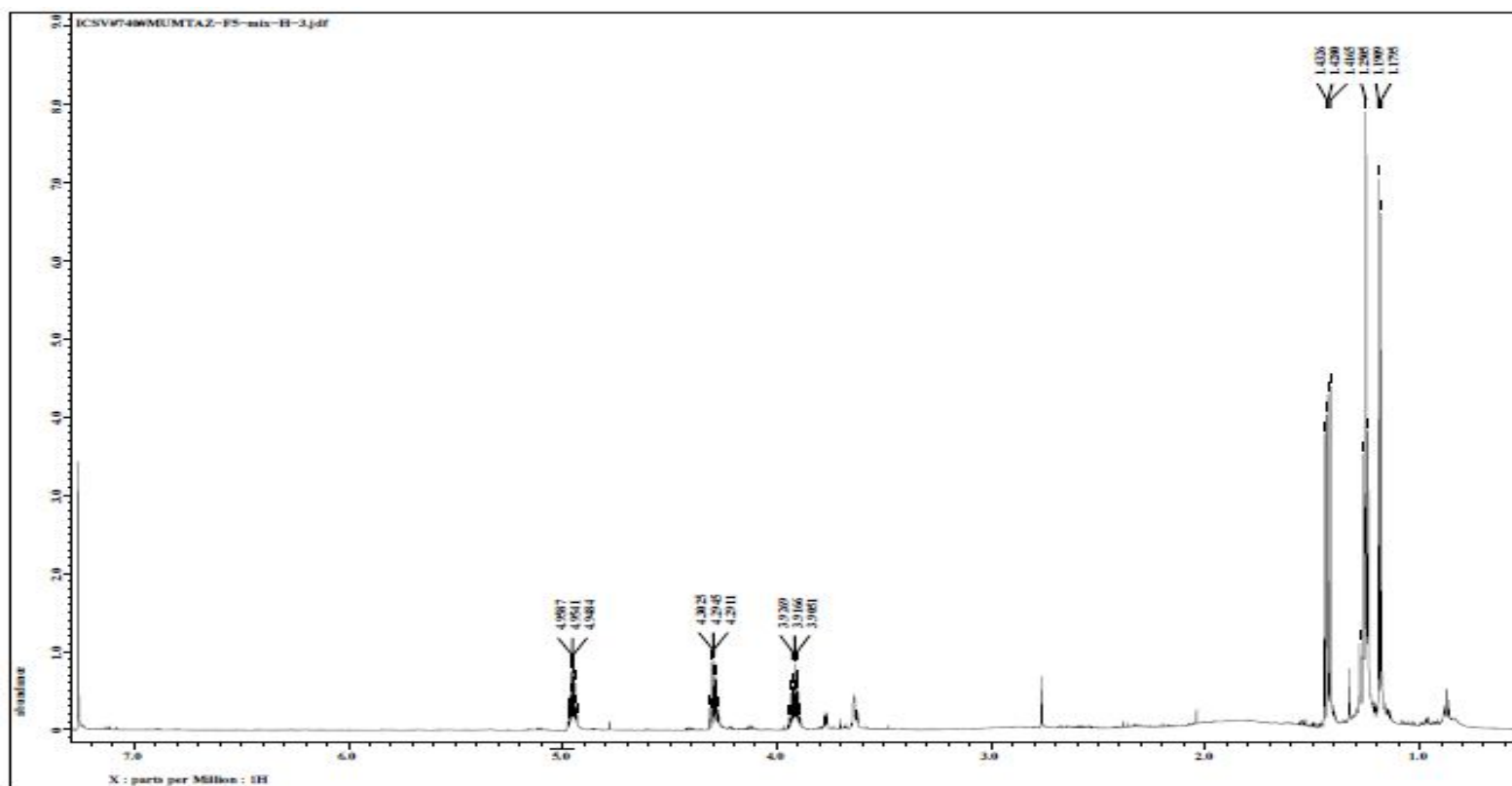


Figure L-1: $^1\text{H-NMR}$ chromatogram of sub-fraction F5.1 of *Streptomyces rochei* (strain X34)

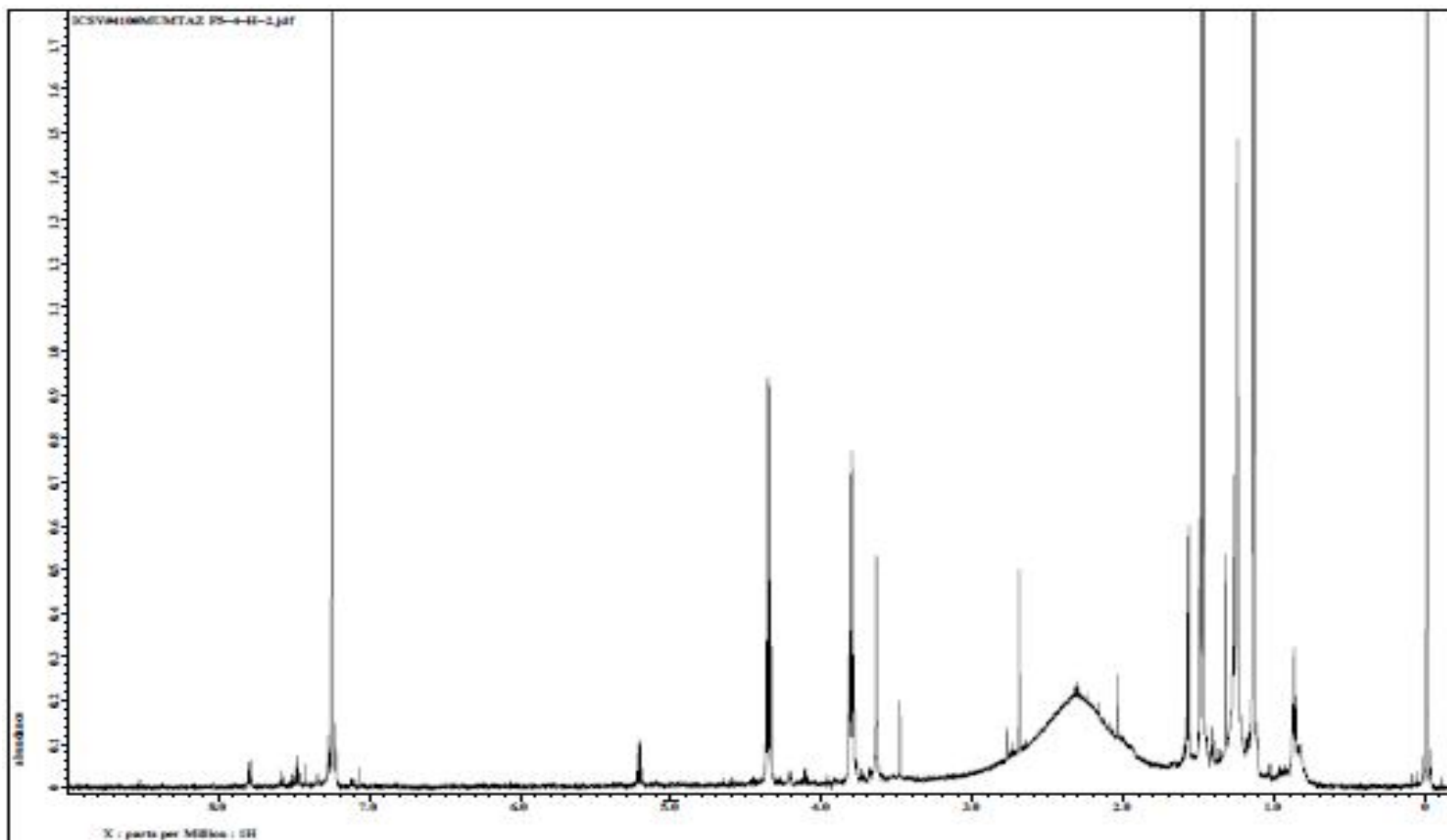


Figure L-2: ^1H -NMR chromatograms of sub-fraction F5.2 of *Streptomyces rochei* (strain X34)

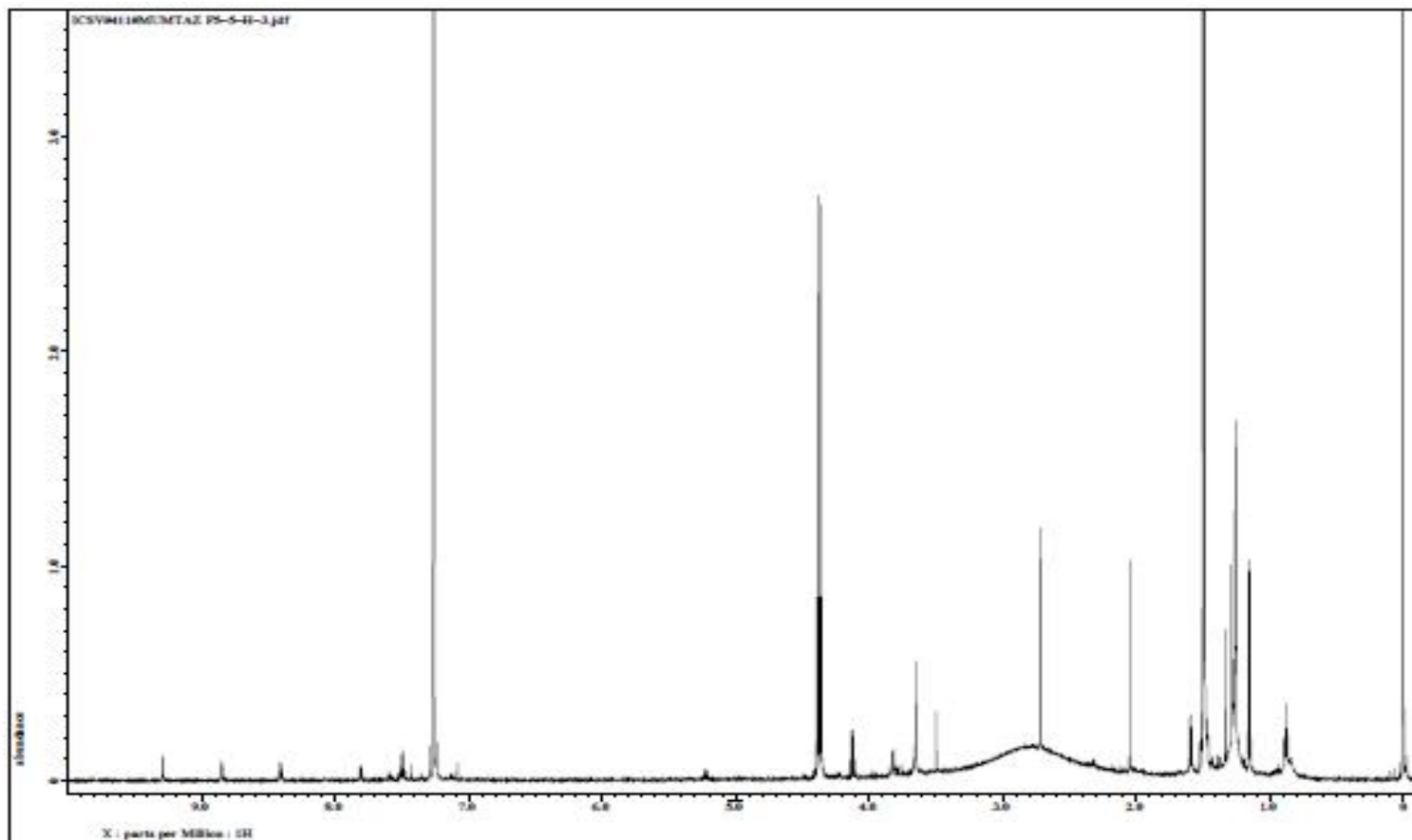


Figure L-3: ^1H -NMR chromatograms of sub-fraction F5.3 of *Streptomyces rochei* (strain X34)

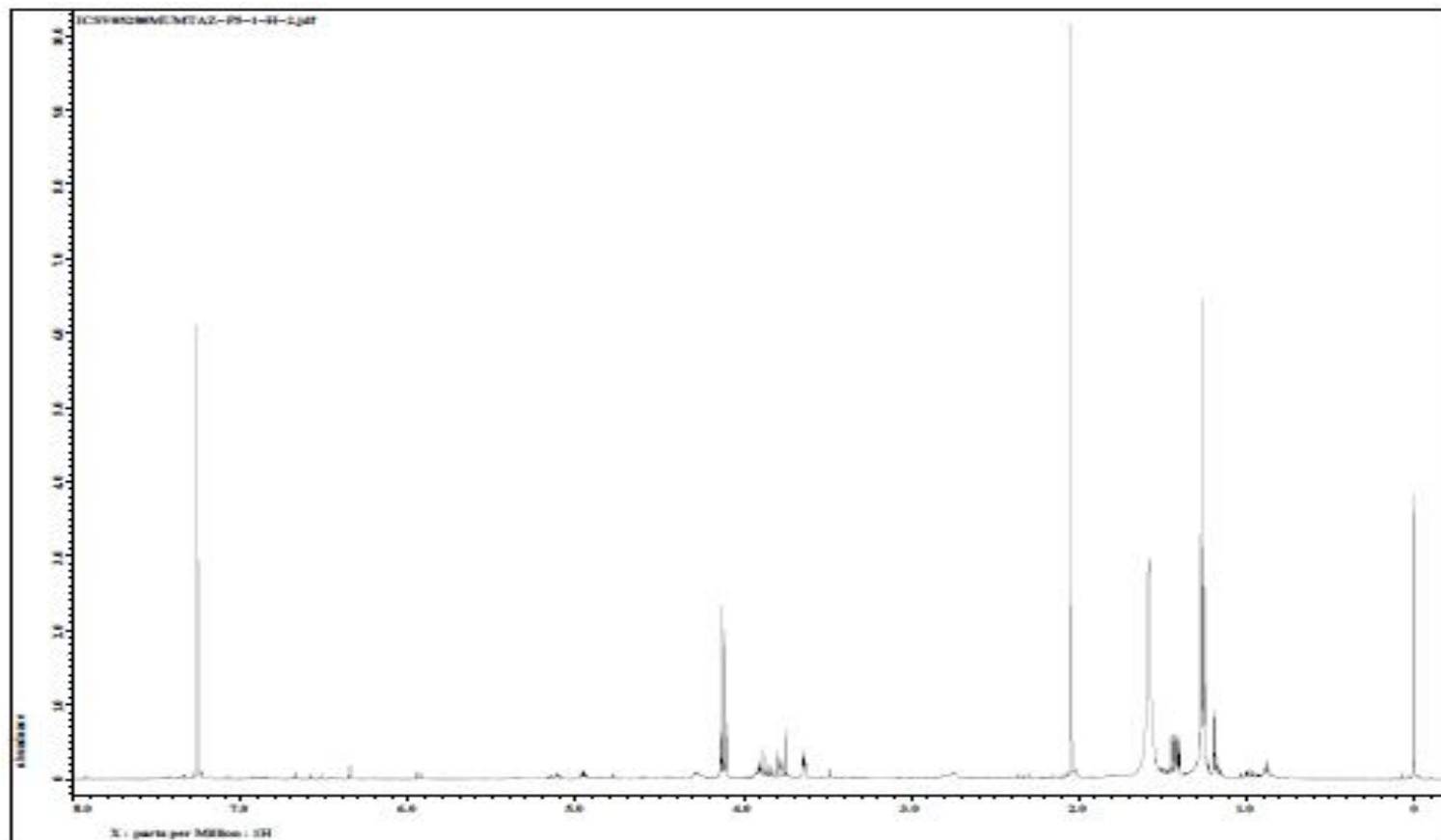


Figure L-4: ^1H -NMR chromatograms of sub-fraction F5.4 of *Streptomyces rochei* (strain X34)

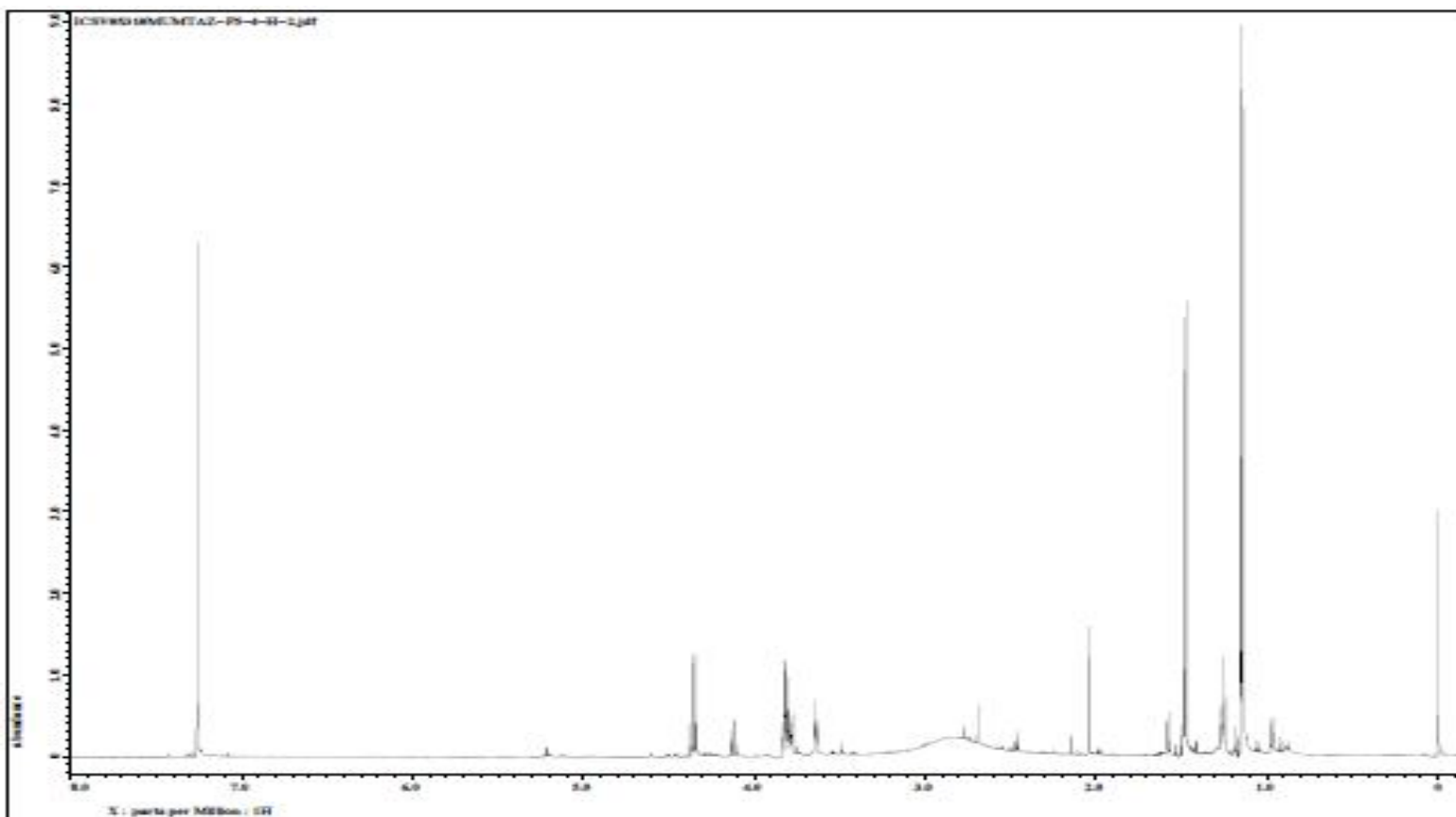


Figure L-5: ^1H -NMR chromatograms of sub-fraction F5.5 of *Streptomyces rochei* (strain X34)

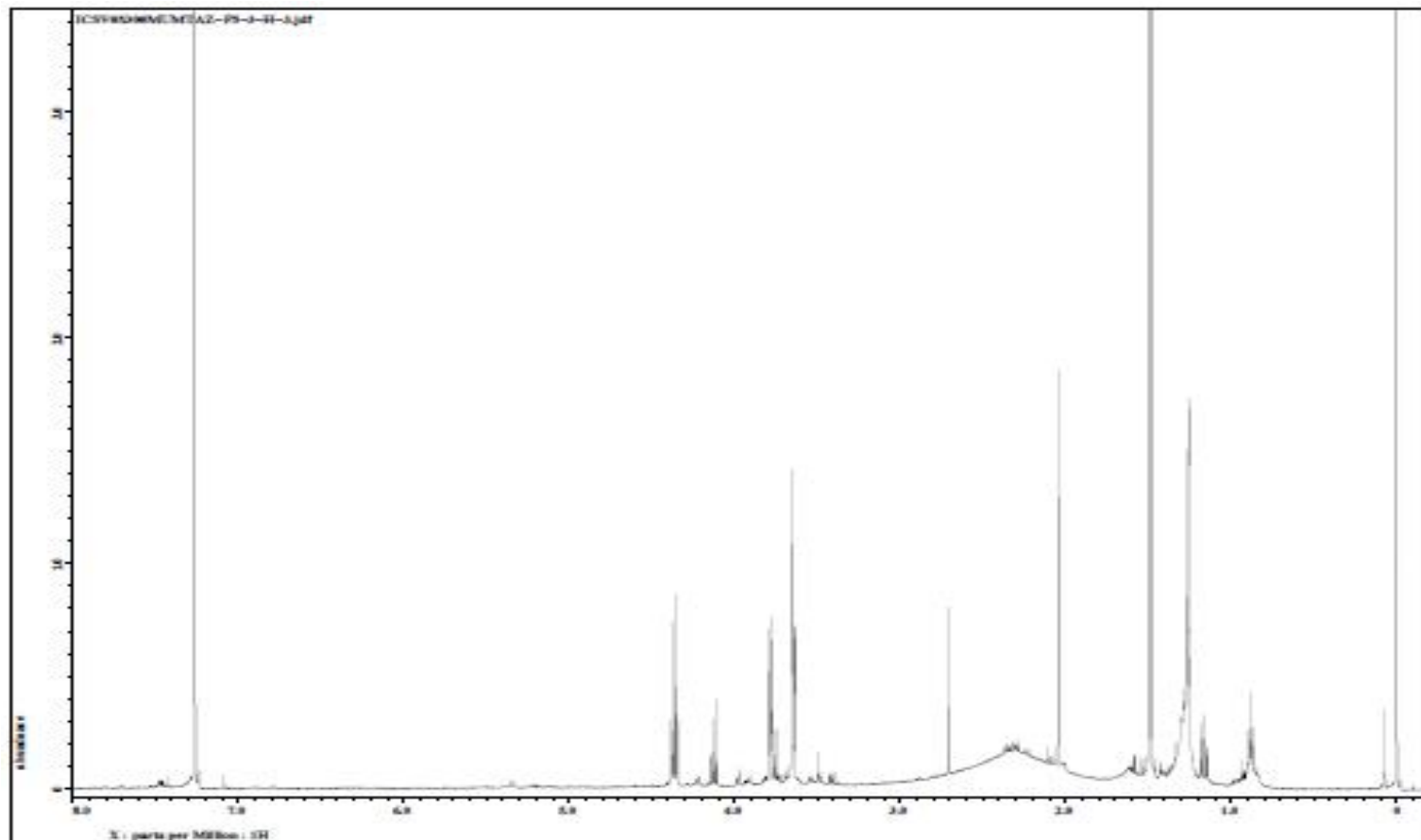


Figure L-6: ^1H -NMR chromatograms of sub-fraction F5.6 *Streptomyces rochei* (strain X34)

APPENDIX M
 ^1H -NMR and ^{13}C -NMR of active sub-fraction F5.4 of *S. rochei* (strain X34)

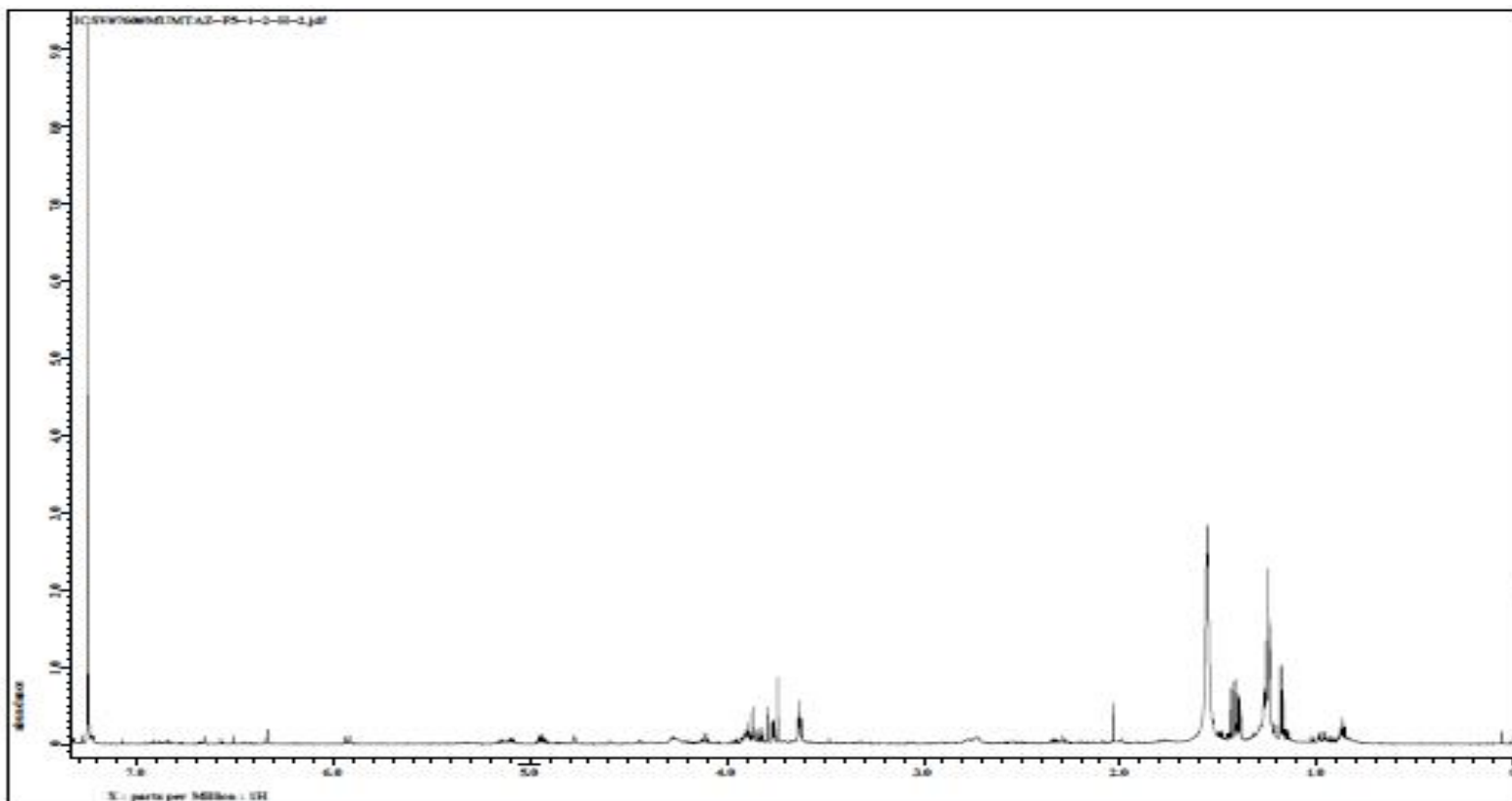


Figure M-1: ^1H -NMR chromatogram of active sub-fraction F5.4 of *S. rochei*.(strain X34)

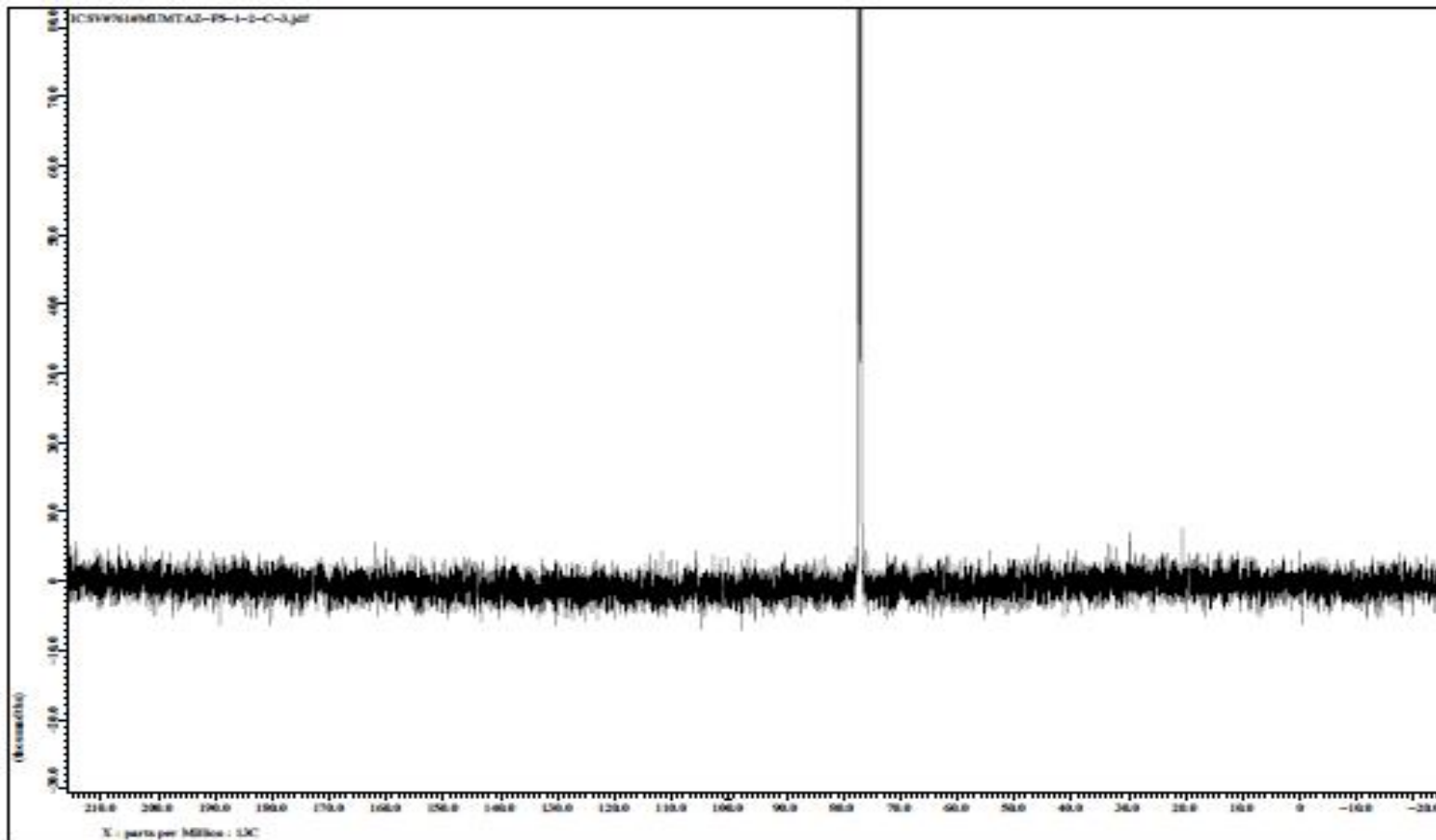


Figure M-2: ^{13}C -NMR chromatogram of active sub-fraction F5.4 of *S. rochei*.(strain X34)

APPENDIX N

Table N-1: Comparison of carbon sources utilization and NaCl tolerance between tested strains (X34, X42 and X77) and their most probably designated species.

| +; | <i>Streptomyces</i> spp. strain | Fru | Glu | Man | Xyl | Inos | Lact | Ara | Rha | Malt | Raff | Sorb | Suc | NaCl tolerance | References |
|----|---------------------------------|-----|-----|-----|-----|------|------|-----|-----|------|------|------|-----|----------------|---|
| | X34 | + | + | + | ± | ± | + | + | + | + | - | - | ± | 8% | - |
| | <i>S. rochei</i> | + | + | + | + | - | + | + | + | + | - | - | + | 5% | Reddy <i>et al.</i> (2011) Berger <i>et al.</i> (1953) |
| | <i>S. plicatus</i> | + | * | + | + | + | - | - | + | * | - | + | - | * | Aghighi <i>et al.</i> (2004) |
| | <i>S. enissocaesilis</i> | - | + | - | + | + | * | + | + | * | + | * | + | 5% | Gause <i>et al.</i> (1983) |
| | X42 | + | + | + | ± | ± | - | + | ± | ± | - | + | ± | 10% | - |
| | <i>S. albidoflavus</i> | + | + | + | - | - | - | + | - | - | - | * | - | 5% | Atta <i>et al.</i> (2011) Waksman & Henrici (1948) |
| | <i>S. somaliensis</i> | + | + | - | - | * | - | - | - | - | + | * | - | * | Lee <i>et al.</i> (2005) |
| | <i>S. hydrogenans</i> | - | + | - | - | - | * | + | - | * | - | * | - | 5% | Lindner <i>et al.</i> (1958) |

good growth, ±; poor growth, -; no growth, *; not determined

Fru; D-fructose, Glu; D-glucose, Man; D-mannitol, Xyl; D-xylose, Inos; Inositol, Lact; Lactose, Ara; L-arabinose, Rha; L-rhamnose, Malt; Maltose, Raff; Raffinose, Sorb; Sorbitol, Suc; Sucrose.

Continuation of Table N-1.

| <i>Streptomyces</i> spp. strain | Fru | Glu | Man | Xyl | Inos | Ara | Rha | Raff | Suc | NaCl Tolerance | References |
|---------------------------------|-----|-----|-----|-----|------|-----|-----|------|-----|----------------|--------------------------------|
| X77 | + | + | + | + | ± | ± | ± | - | - | 6% | - |
| <i>S. cavourensis</i> | + | + | + | + | - | - | - | - | - | 7.5% | Skarbek & Brady (1978a) |
| <i>S. celluloflavus</i> | + | + | + | + | + | + | + | + | + | 2.5% | Nishimura <i>et al.</i> (1953) |
| <i>S. albolongus</i> | - | + | - | - | - | + | - | - | - | * | Tsukiura <i>et al.</i> (1964) |

+, good growth, ±; poor growth, -; no growth, *; not determined

Fruc; D-fructose, Glu; D-glucose, Man; D-mannitol, Xyl; D-xylose, Inos; Inositol, Lact; Lactose, Ara; L-arabinose, Rha; L-rhamnose, Malt; Maltose, Raff; Raffinose, Sorb; Sorbitol, Suc; Sucrose.

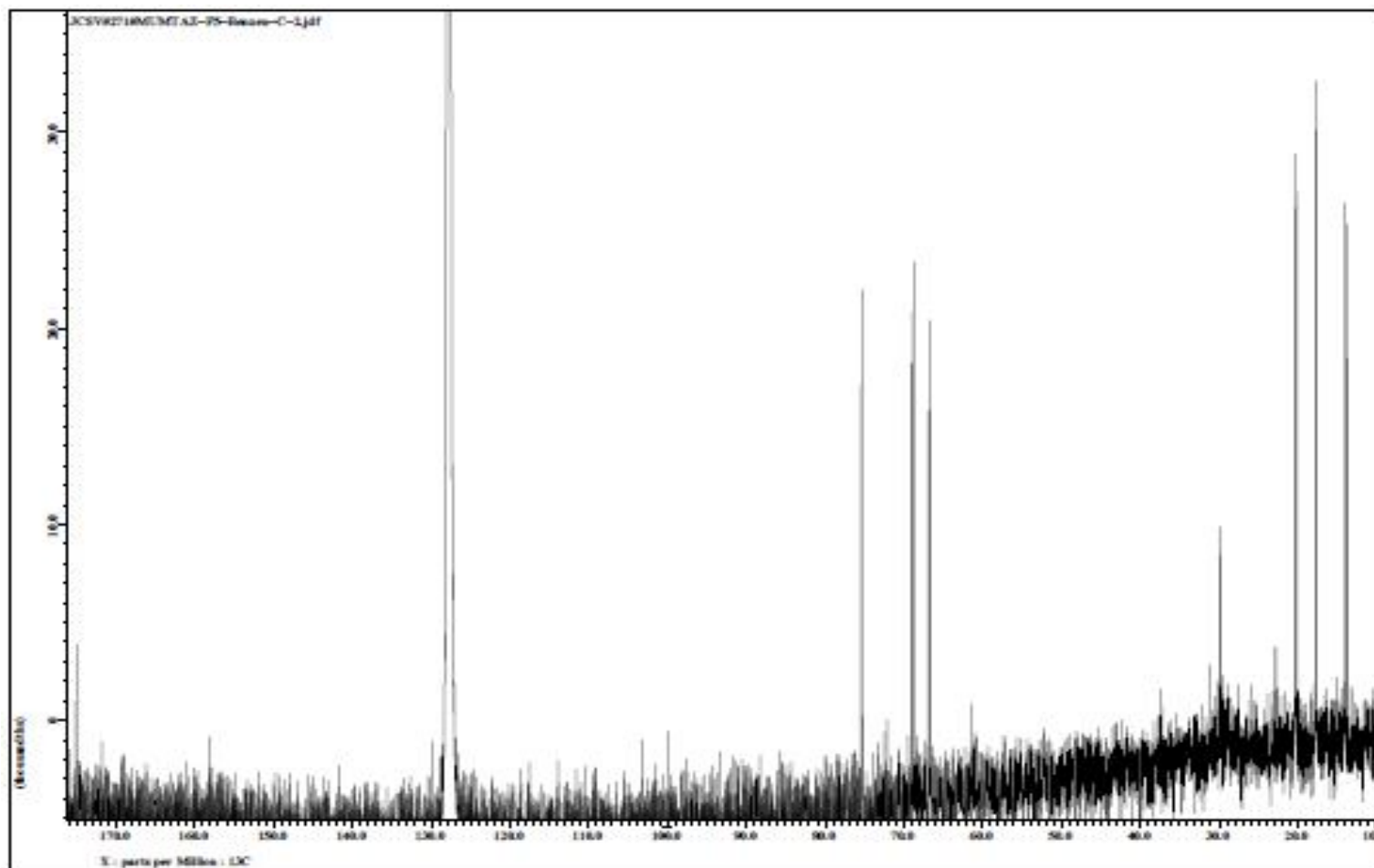


Figure O-2: ^{13}C -NMR chromatogram of active sub-fraction F5.1

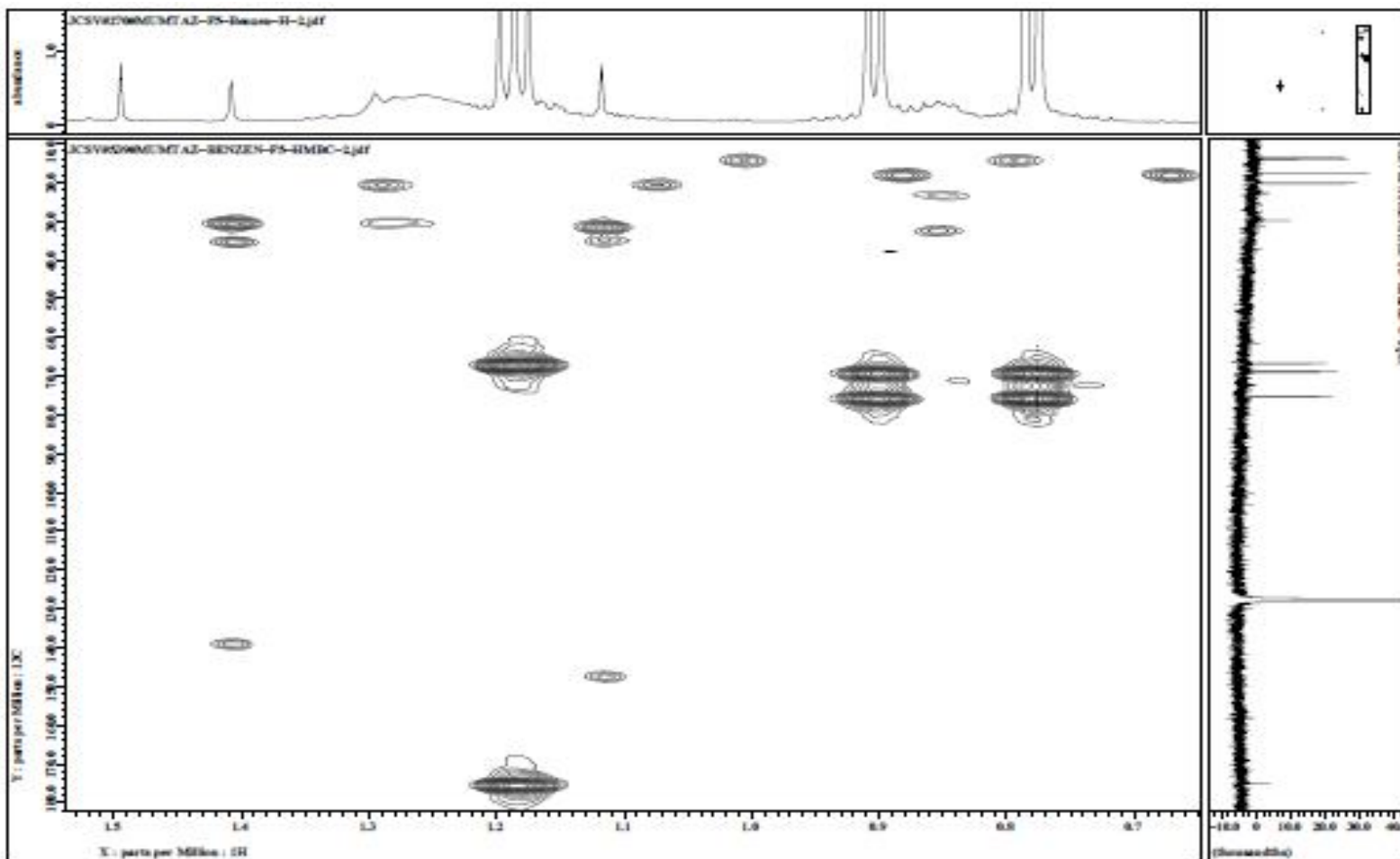


Figure O-3: HMBC chromatogram of active sub-fraction F5.1

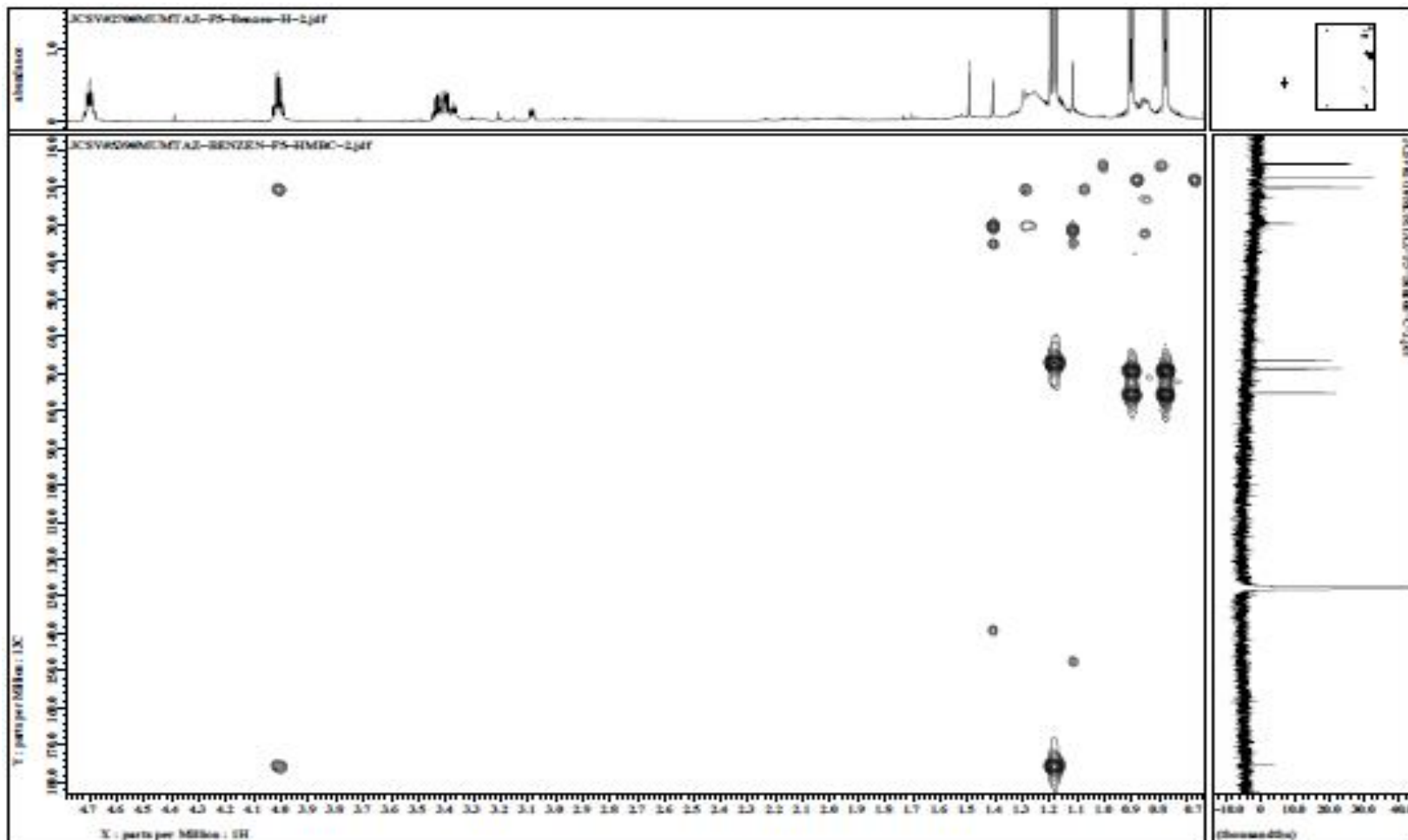


Figure O-4: HMBC chromatogram of active sub-fraction F5.1

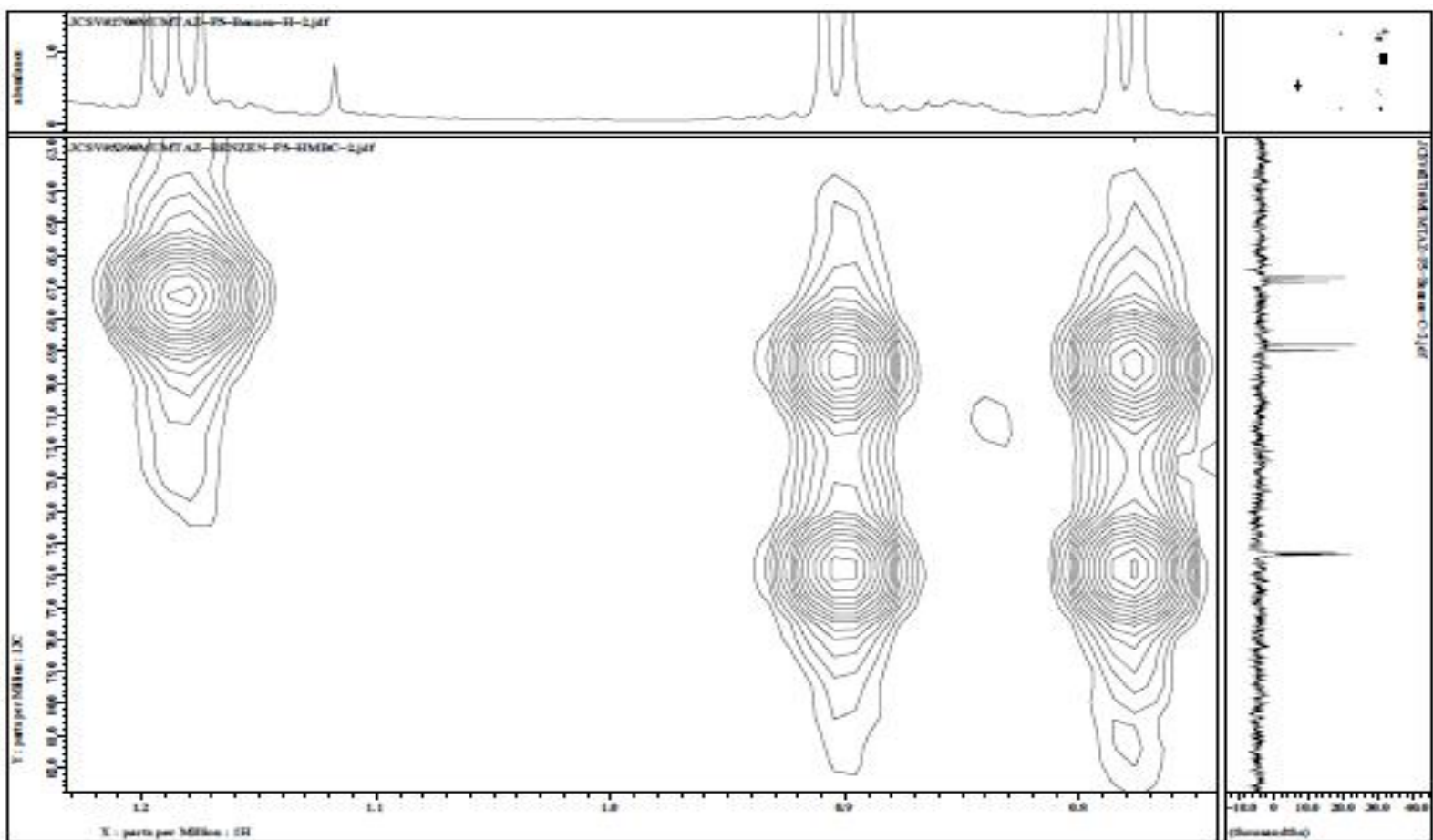


Figure O-5: HMBC chromatogram of active sub-fraction F5.1

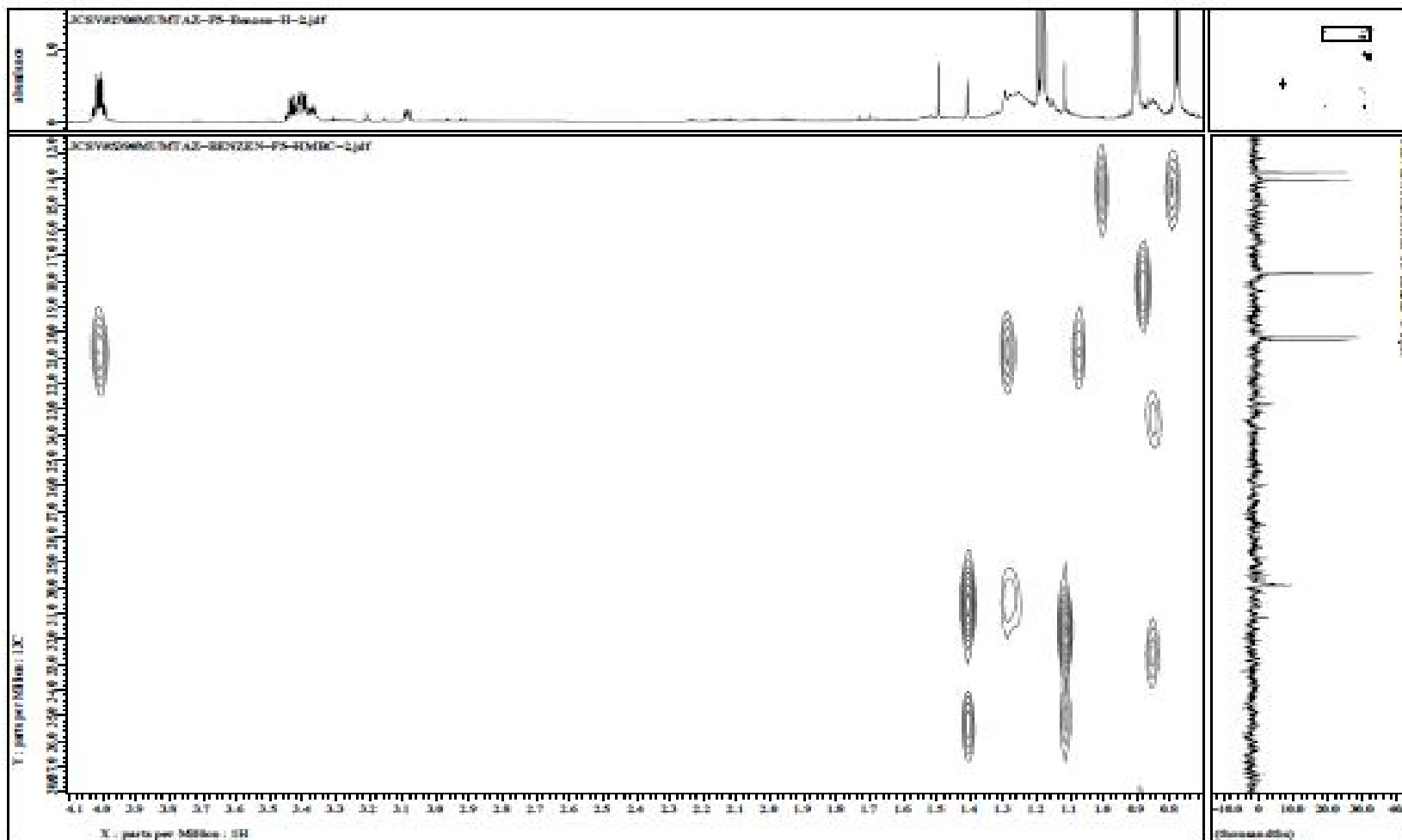


Figure O-6: HMBC chromatogram of active sub-fraction F5.1

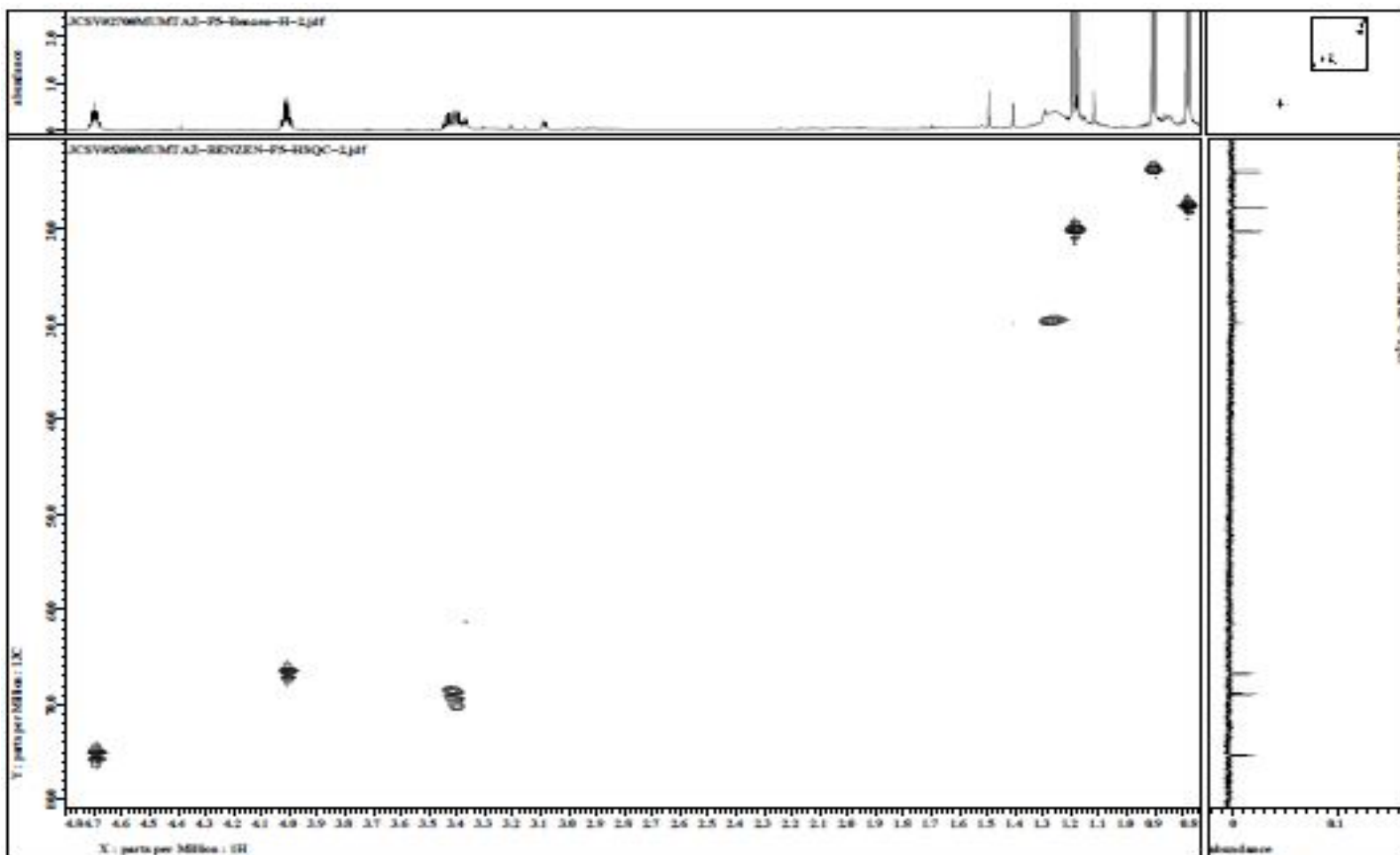


Figure O-7: HSQC chromatogram of active sub-fraction F5.1

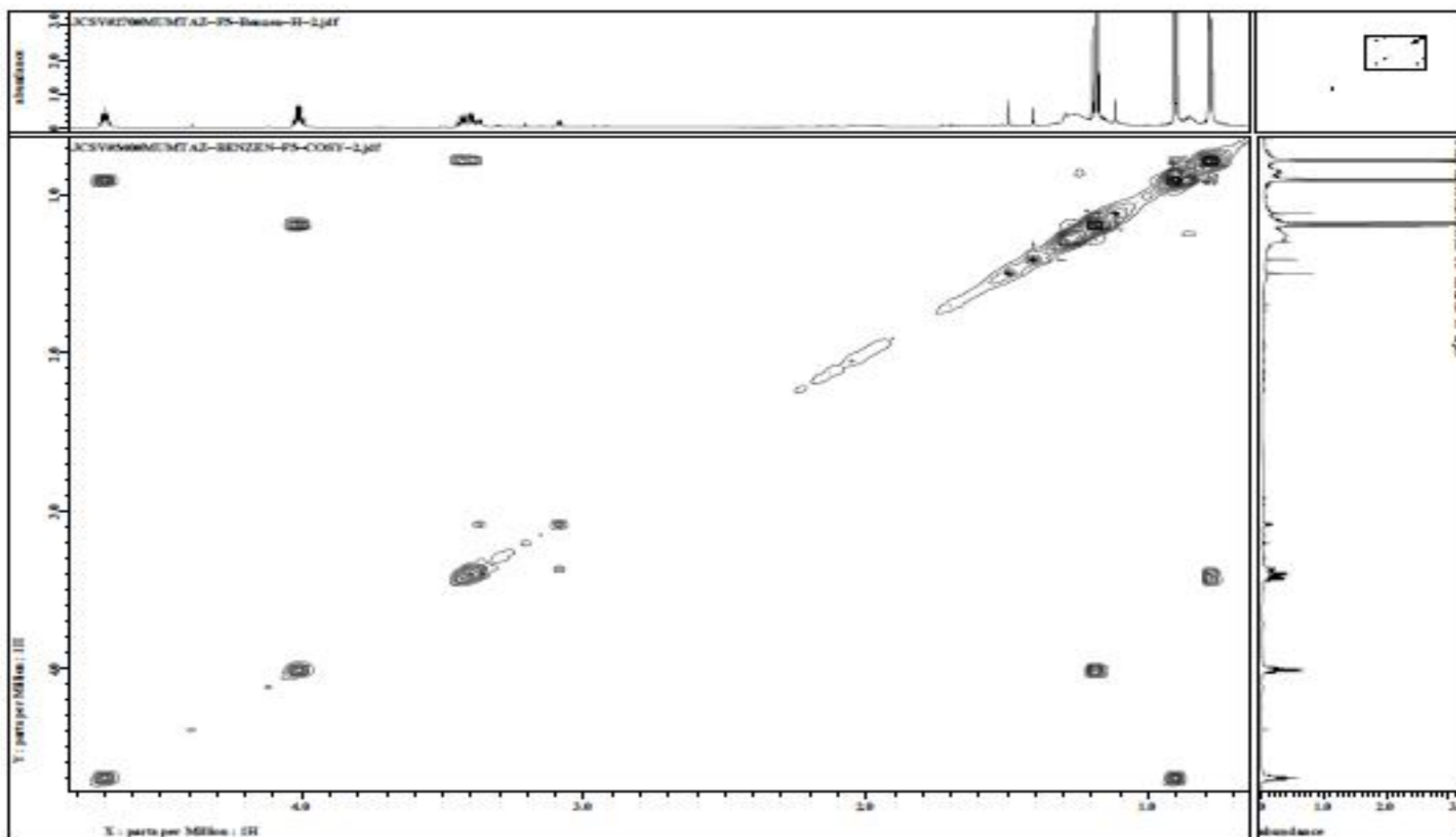


Figure O-8: COSY chromatogram of active sub-fraction F5.1

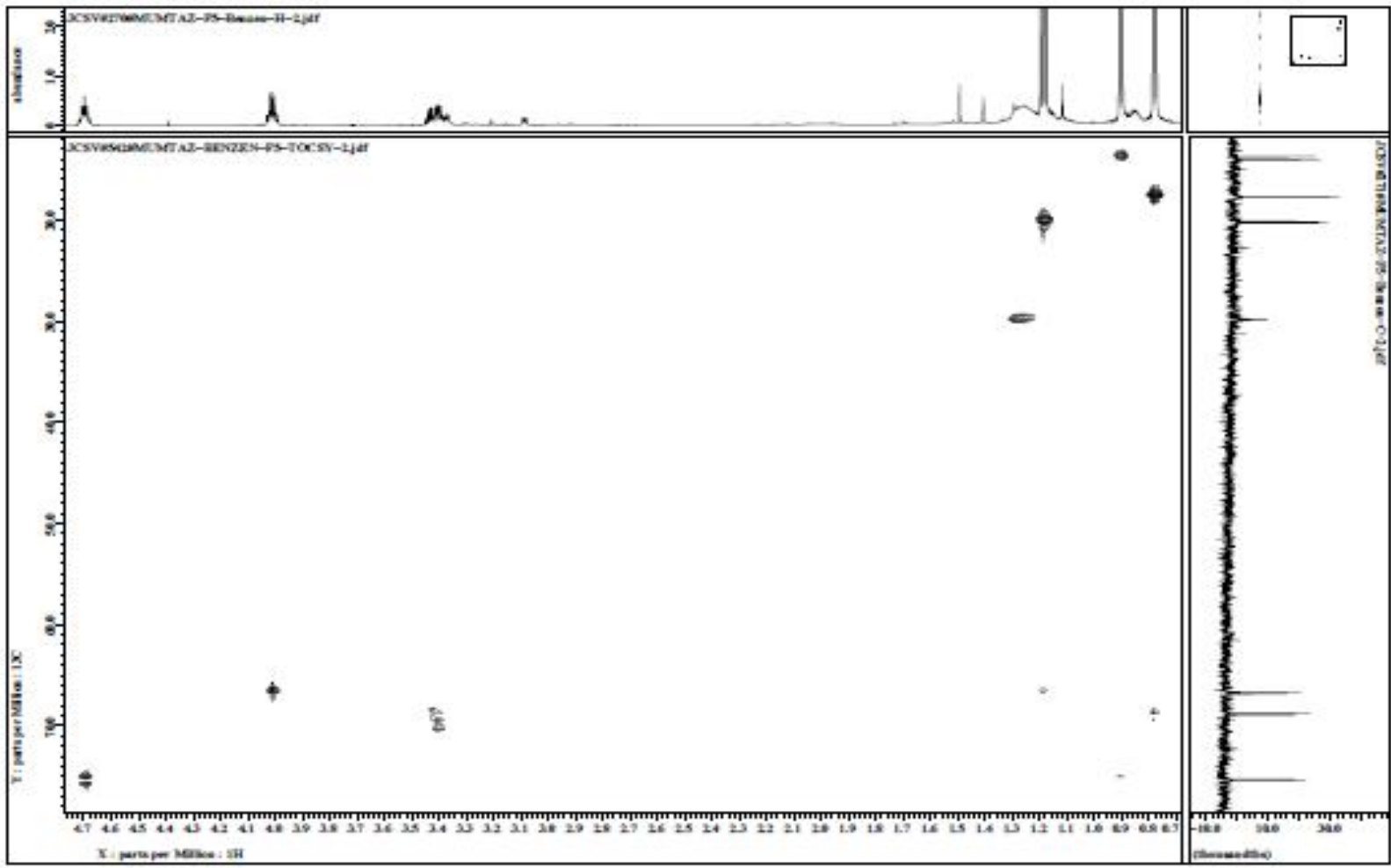


Figure O-9: TOCSY chromatogram of active sub-fraction F5.1

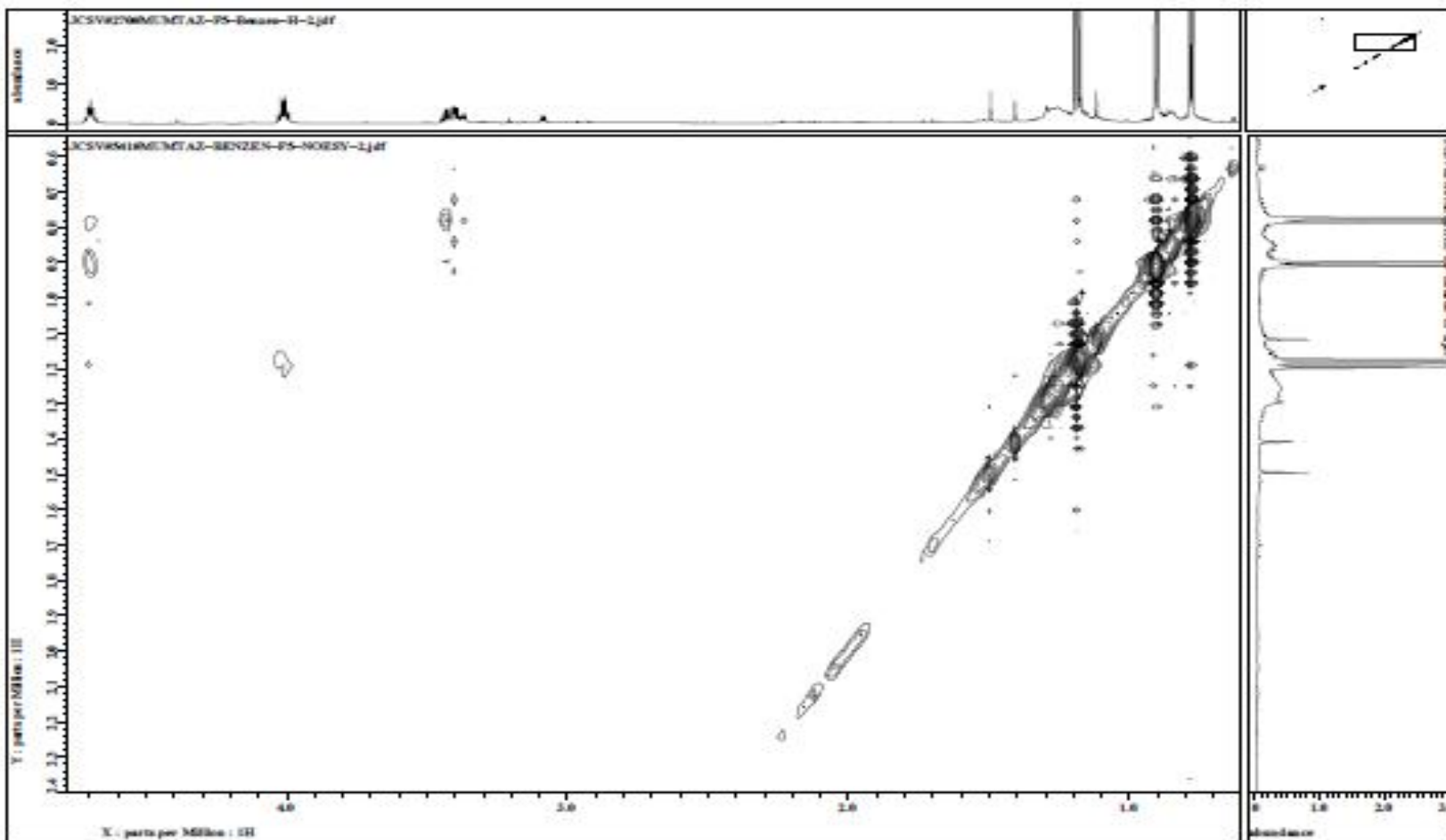
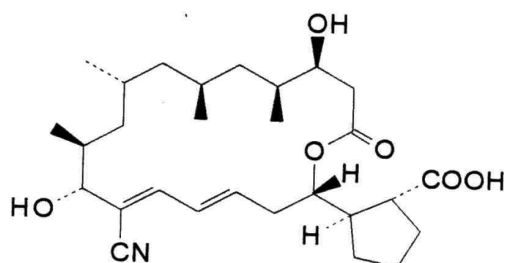


Figure O-10: NOESY chromatogram of active sub-fraction F5.1

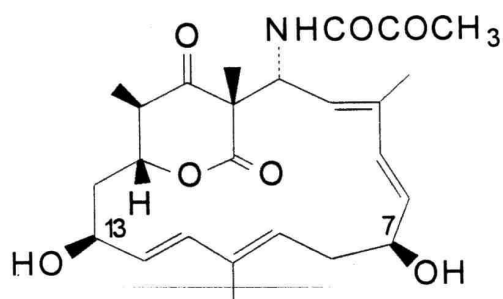
APPENDIX P

Secondary metabolites from *Streptomyces rochei* (Berger *et al.*, 1953; Kotake *et al.*, 1992)

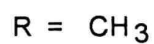
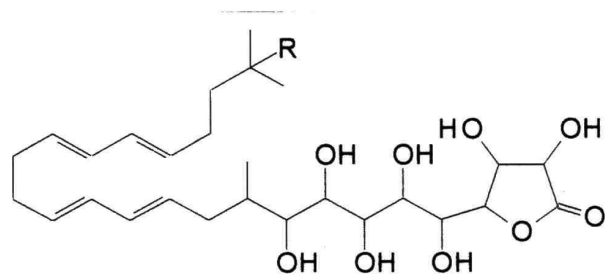
Borrelidin, macrolide antibiotic with antiviral activity



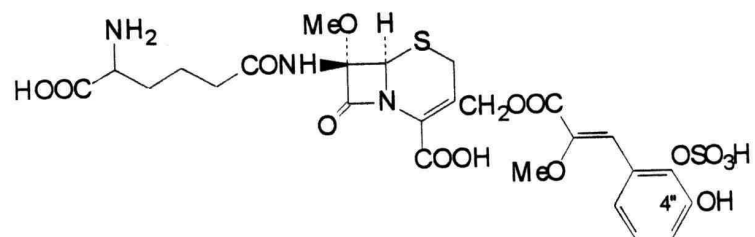
Bundlin, a macrocyclic compound active against gram-positive bacteria



Butyrolactol A, active against fungi



Cephalosporin antibiotic



Lankacyclinol, polyene-type antibiotic with antimicrobial activity

