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Bioactive Secondary Metabolites from Terrestrial *Streptomyces baarnensis* MH4

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Authors' contributions

This work was carried out in collaboration between all authors. Authors HN, MOR and MS performed fermentation, solvent extraction, compounds purification, spectroscopic studies and compounds identification. Author MSAA isolated and identified the strain Streptomyces baarnensis MH4, and shared in fermentation, solvent extraction and compounds purification. Author MS has supervised, fully written and finalized the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Isolation, screening and identification of potent bioactive compounds producing *Streptomyces* sp. from the terrestrial soil of Egypt. Fermentation study, product recovery, purification and its characterization was also taken into consideration.
Study Design: Cultivation in lab fermenter, solvent extraction and purification of the compounds by column chromatography, identification of the compounds by mass spectrometry and NMR spectroscopy, determination of the antimicrobial activity.

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Place and Duration of Study: Institute of Organic and Biomolecular Chemistry, University of Göttingen, Germany; Department of Microbial Chemistry, National Research Centre, Egypt and Chemistry of Natural Compounds Department, National Research Centre, Egypt, between January, 2009 and December, 2010.

Methodology: The strain was cultivated at 25-liter shaker culture and the fermented broth was filtered and the filtrate was extracted by XAD-16 resin and then by methanol-water, and the concentrated water residue was extracted by ethyl acetate followed by evaporation till dryness. The resultant crude extract was fractionated on silica gel and the components were purified by column chromatography (silica gel, Sephadex column and preparative TLC). The pure component was identified by mass spectrometry (ESI and HRESI-MS), NMR analysis (^{1}H,^{13}C NMR, and 2D NMR) and by comparison with reference data. The antimicrobial activity was determined by disc diffusion assay.

Results: The morphological, biochemical and physiological characterization suggested that isolate MH4 belongs to the genus *Streptomyces*. Comparison the obtained data with literature data and showed 99% identity coverage towards *Streptomyces baarnensis*. The scale up fermentation of the isolate MH4 yielded eight known metabolites, nonactic acid (2), homononactic acid (3), 3-(3,3-Di-indolyl)propane-1,2-diol (4), turbomycin A (5), indolyl-3-acetic acid, indolyl-3-carbaldehyde, indolyl-3-carboxylic acid and 2'–deoxyadenosine. Structures of the isolated compounds were assigned by intensive studies of nuclear magnetic resonance (NMR) and mass spectrometry and comparison with corresponding literatures. The taxonomical characterization, fermentation, and biological activity of the *Streptomyces baarnensis* MH4 were investigated.

Conclusion: The isolate *Streptomyces baarnensis* MH4 is a potent producer of several antibiotic compounds which can be exploited for their commercial production.

Keywords: Terrestrial streptomyces sp.; taxonomy; N-ethylphenethyl amine; antimicrobial activity.

1. INTRODUCTION

Microorganisms cultured from soil have provided most of the antibiotics and many other medicinal agents that have dramatically improved human health in the latter half of the 20th century [1,2]. Despite this fruitful history, traditional searches for new natural products from soil microorganisms are now confronting diminishing returns for the discovery of new compounds [3]. In light of the need for new antibiotics to combat the multidrug-resistant pathogens that have recently emerged, new approaches to antibiotic discovery are needed. One of the richest sources of new antibiotics may be the uncultured microorganisms of soil. The number of microorganisms typically cultured from soil represents 1% or fewer of the total microbial community [4,5]. DNA-DNA reassociation measurements and other culture-independent methods reveal that the total genetic diversity in a soil sample of 100 g or less is likely between 4,000 [4] and 13,000 species. Recent analyses of 16S rRNA genes amplified directly from soil indicated that novel phyla of *Bacteria* and *Archaea* are present [6-12]. If the diversity of chemistry produced by the culturable bacteria is an indicator of the chemical capacity of the uncultured bacteria, then many molecules, and perhaps useful drugs, remain to be discovered from soil microorganisms.

In the present investigation, the terrestrial *Streptomyces baarnensis* MH4 was selected according to the chemical and biological screening. Based on chemical screening using thin layer chromatography (TLC), several UV absorbing bands of diverse polarity, were investigated. These bands showed different staining colourations (pink, red, orange and blue) on anisaldehyde/sulphuric acid and heating. Working up of the strain extract using series of different chromatographic techniques, nine metabolic compounds were isolated (Fig. 1), among them N-ethylphenethyl amine (1), and the known metabolites, nonactic acid (2), homononactic acid (3), 3-(3,3-Di-indolyl) propane-1,2-diol (4), turbomycin A (5), indolyl-3-acetic acid, indolyl-3-carbaldehyde, indolyl-3-carboxylic acid and 2'–deoxyadenosine; and their structures were intensively studied by nuclear magnetic resonance (NMR) and mass spectrometry. In addition to the above, we report the taxonomical characterization, fermentation, and the antimicrobial activity of the terrestrial *Streptomyces baarnensis* MH4.

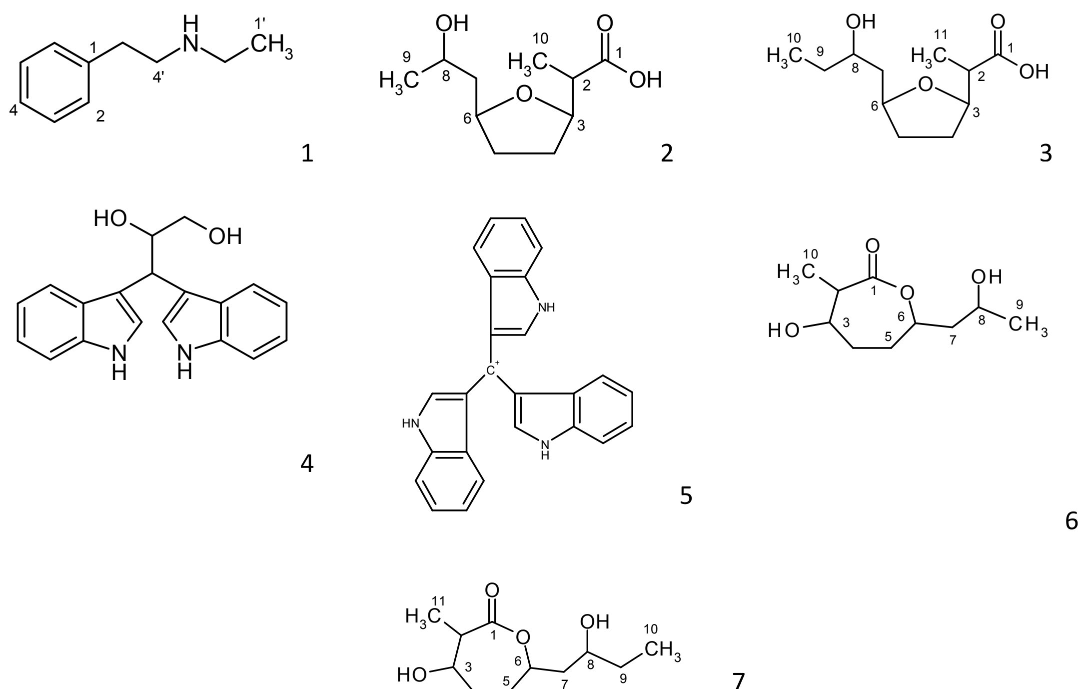


Fig. 1. Chemical structures for some of isolated (1-5) and related (6-7) compounds from *streptomyces baarnensis* MH4

2. MATERIALS AND METHODS

The NMR spectra were measured on Varian Unity 300 (300.145 MHz) and Varian Inova 600 (150.820 MHz) spectrometers. ESI MS was recorded on a Finnigan LCQ with quaternary pump Rheos 4000 (Flux Instrument). EI mass spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV). Flash chromatography was carried out on silica gel (230-400 mesh). Rate of flow (R_f) values were measured on Polygram SIL G/UV_{254} TLC cards (Macherey-Nagel & Co.). Size exclusion chromatography was done on Sephadex LH-20 (Lipophilic Sephadex, Amersham Biosciences Ltd; purchased from Sigma-Aldrich Chemie, Steinheim, Germany). All chemicals served in the biological study were of analytical grade, which were purchased from Sigma, Merck and Aldrich.

2.1 Terrestrial *Streptomyces* sp. MH4

2.1.1 Collection and isolation

The terrestrial *Streptomyces* sp. isolate MH4 was isolated from a soil sample collected from Dekrnis region (Mansoura providence) at depth of 10~20 cm and kept in sterile conditions. Starch-Casein Nitrate (SCN) agar medium (soluble starch, 10 g/L; casein, 0.3 g/L; KNO_3, 2 g/L; $MgSO_47H_2O$, 2 g/L; K_2HPO_4, 2 g/L; $CaCO_3$, 0.02 g/L; $FeSO_47H_2O$, 0.01 g/L; agar, 20 g/L; distilled water, 1L) supplemented with 100 µg/mL cycloheximide was used for isolation and enumeration of *Actinomycetes*. In conventional dilution plate technique, 1gm of soil sample was suspended in 9 mL of sterile water and successive dilution was made up to $10^{-4.}$ An aliquot (0.1 mL) of suspension from the last dilution test tube was spread over starch-agar-casein agar medium and incubated for 7-9 days at 30°C [13].

2.1.2 Fermentation and isolation

A 25-liter shaker culture of the terrestrial *streptomyces baarnensis* MH4 was incubated at 28°C using M_2 medium (malt extract, 10 g/L; yeast, 4 g/L; glucose, 4 g/L; tape water, 1L) for 7 days. After harvesting, the resulting yellow culture broth was mixed with *ca* 1kg diatomaceous earth (Celite) and filtered during a filter press. The filtrate was extracted using XAD-16 resin followed by elution with MeOH-H_2O (80:20), and the collected aqueous methanolic extract was concentrated *in vacuo*. The remaining water residue was then extracted with

ethyl acetate. The mycelium cake was first extracted with ethyl acetate (3× v/v) and then by acetone (3×v/v). The acetone extract was evaporated *in vacuo*, and the residual aqueous solution was re-extracted by ethyl acetate. According to TLC monitoring, ethyl acetate extracts of mycelium and supernatant showed high similarity and were combined and followed by concentration *in vacuo* to afford 6.1g as reddish-brown crude extract.

The crude extract (6.1 g) was dissolved in a mixture of CH_2Cl_2/MeOH (85:15) and ca. 4 g of silica gel were added and this mixture was brought to dryness under reduced pressure. Separation was performed by a silica gel column (3×75 cm, 150 g) chromatography using CH_2Cl_2-MeOH gradient (100:0, 98:2, 95:5, 93:7, 90:10, 80:20, 50:50). After TLC monitoring, four fractions were afforded, FI (0.55 g), FII (1.2 g), FIII (1.4 g), and FIV (1.8 g). Fraction II (1.2 g) was purified on Sephadex LH-20 (CH_2Cl_2-MeOH, 60:40) to afford three colorless oils of N-enthyl-phenethylamine (1,6mg), nonactic acid (2,9 mg) and homononactic acid (3, 15 mg). Purification of fraction III (1.4g) using Sephadex LH-20 (MeOH) delivered 3-(3,3-di-indole)propane-1,2-diol (4, 11 mg) and turbomycin A (5, 12 mg). Fraction FIV (1.8 g) was purified on Sephadex LH-20 (MeOH) followed by RP-18 using $MeOH/H_2O$ gradient (10 to 30% MeOH) to deliver indolyl-3-acetic acid (13 mg), indolyl-3-carbaldehyde (8 mg), indolyl-3-carboxylic acid (9 mg) and 2'–deoxyadenosine (12 mg).

2.2 Spectroscopic and Chromatographic Data of the Main Isolated Compounds (1-5)

2.2.1 N-Ethylphenethyl amine (1)

$C_{10}H_{15}N$ (149) colourless oil, UV absorbing, turned pink by anisaldehyde/sulphuric acid and heating. –R_f = 0.38 (CH_2Cl_2/5% MeOH). – ^1H NMR (CD_3OD, 600 MHz): δ 7.35 (m, 2H, H-2,6), 7.25 (m, 3H, H-3,4,5), 3.22-3.18 (m, 4H, H_2-2',4'), 2.95 (t, 2H, H_2-5') 1.28 (t, *J* = 6.9, 3H, H_3-1'). –^{13}C NMR (CD_3OD, 150 MHz): *δ* 138.0 (C_q-1), 129.0 (CH-2,6), 128.8 (CH-3,5), 127.7 (CH-4), 47.9 (CH_2-4'), 41.6 (CH_2-2'), 34.6 (CH_2-5'), 9.5 (CH_3-1').–(-)-ESIMS: *m/z* = 148 $[M-H]^-$, 297 $[2M-H]^-$.–(+)-HRESI MS: *m/z* =172. 10914 $[M+Na]^+$([calcd 172.10912 for $C_{10}H_{15}NNa$).

2.2.2 Nonactic acid (2)

$C_{10}H_{18}O_4$ (202), colourless oil, UV inactive stained to violet by anisaldehyde/sulphuric acid and heating. – R_f = 0.36 (CH_2Cl_2/5% MeOH). – ^1H NMR (CD_3OD, 300 MHz): δ 3.99 (m, 2H, H-6,8), 3.89 (m, 1H, H-3), 2.42 (dt, *J* = 13.4, 6.8 Hz, 1H, H-2), 1.99 (m, 2H, CH_2-7), 1.58 (m, 4H, CH_2-4,5), 1.15 (d, *J* = 6.2 Hz, 3H, CH_3-9), 1.09 (d, *J* = 6.9 Hz, 3H, CH_3-10). –^{13}C NMR (CD_3OD, 125 MHz): *δ* 179.2 (C_q-1), 82.0 (CH-3), 77.9 (CH-6), 66.1 (CH-8), 46.1 (CH-2), 32.2 (CH_2-5), 29.4 (CH_2-4), 24.1 (CH_3-9), 14.1 (CH_3-10). – (+)-ESIMS: *m/z* = 225 $[M+Na]^+$, 427 $[2M+Na]^+$. −(-)-ESIMS: *m/z* = 201 $[M-H]^-$, 403 $[2M-H]^-$. –(+)-HRESI MS: m/z = 225.109714 $[M+Na]^+$ ([calcd 225.11027 for $C_{10}H_{18}O_4Na$).

2.2.3 Homononactic acid (3)

$C_{11}H_{20}O_4$ (216) UV inactive colourless oil, turned violet with anisaldehyde/sulphuric acid and heating. – R_f = 0.39 (CH_2Cl_2/5% MeOH). – ^1H NMR (CD_3OD, 300 MHz): *δ* 4.02 (m, 2H, H-6,8), 3.64 (m, 1H, H-3), 2.42 (m, 1H, H-2), 1.99-120 (m, 8H, CH_2-4,5,7,9), 1.09 (d, *J* = 6.9, 3H, CH_3-11), 0.91 (t, *J* = 6.7, 3H, CH_3-10).– ^{13}C NMR (CD_3OD, 125 MHz): *δ* 179.2 (C_q-1), 81.8 (CH-3), 77.8 (CH-6), 71.1 (CH-8), 46.9 (CH-2), 43.8 (CH_2-7), 32.1 (CH_2-5), 31.4 (CH_2-9), 29.3 (CH_2-4), 13.9 (CH_3-11), 10.17 (CH_3-10).–(+)-ESI MS: m/z (%) = 239 $[M+Na]^+$, 455 $[2M+Na]^+$, 477 $[2M-H+2Na]^+$.–(-)-ESI MS: *m/z* = 215 $[M-H]^-$.–(+)-HRESI MS: *m/z* = 239.12538 $[M+Na]^+$ (calcd 239.125375 for $C_{11}H_{20}O_4Na$).

2.2.4 3-(3,3-Bisindolyl)propane-1,2-diol (4)

$C_{19}H_{18}N_2O_2$ (306) red oil, UV absorbance, turned red with anisaldehyde/sulphuric acid and heating. –R_f = 0.19 (CH_2Cl_2/7% Me OH). – ^1H NMR (CD_3OD, 300 MHz): *δ* 7.54 (dd, *J* = 7.9, 2.7 Hz, 2H, H-4), 7.28 (dd, *J* = 8.1, 1.0 Hz, 2H, H-7), 7.29 (s, 2H, H-2), 7.01 (dt, *J* = 7.0, 4.7 Hz, 2H, H-6), 6.90 (dt, *J* = 8.9, 1.0 Hz, 2H, H-5), 4.68 (d, *J* = 6.7 Hz, 1H, H-1'), 4.48 (dt, *J* = 7.0, 4.1 Hz, 1H, H-2'), 3.61 (ABX, J_{AB} = 11.1, J_{AX} = 4.1 Hz, 1H, H-3'). 3.48 (ABX, J_{AB} = 11.1, $^3J_{BX}$ = 7.1 Hz, 1H, H-3').–(+)-ESIMS: *m/z* = 329 $[M+Na]^+$, 635 $[2M+Na]^+$.–(-)-ESIMS: *m/z* = 305 $[M-H]^-$.–(+)-HRESIMS: *m/z* = 329.1263 (calcd. 329.1260 for $[M+Na]^+$).

2.2.5 Turbomycin A (5)

$C_{25}H_{17}N_3$ (359): Red oily substance, UV active turned red by spraying with anisaldehyde regent and heating. –R_f = 0.27 (CH_2Cl_2/ 7% Me OH). – ^1H NMR (CD_3OD, 300 MHz): *δ* 8.24 (s, 3H, H-2), 7.64 (d, *J* = 9.5 Hz, 3H, H-4), 7.28 (t, *J* = 7.6 Hz, 3H, H-5), 7.01 (t, *J* = 7.5 Hz, 3H, H-6), 6.90

(d, J = 8.4 Hz, 3H, H-7).–(+)-ESIMS: m/z = 360.2 $[M+H]^+$.–(-)-ESIMS: m/z = 358.1 $[M-H]^-$.−(+)-HRESIMS: m/z = 360.1501 (calcd. 360.1495 for $[M+H]^+$, $C_{25}H_{18}N_3$).

2.3 Antimicrobial Activity

Antimicrobial assays were conducted utilizing the disc-agar method (Phadungkit, Rattarom & Rattana, 2010) [14] against *Staphylococcus aureus*, *Escherichia coli* and *Saccharomyces cerevisiae*. The bacterial extract was dissolved in CH_2Cl_2/10% Me OH at a concentration of 5 mg/mL. Aliquots of 5 μL were soaked on filter paper discs (5mm∅, no. 2668, Schleicher & Schüll, Germany) and dried for 1 h at room temperature under sterilized conditions. The paper discs were placed on inoculated agar plats and incubated for 24 h at 37°C. The antibacterial activity was evaluated by measuring the zone of inhibition (in mm) against the test organisms.

3. RESULTS AND DISCUSSION

3.1 Taxonomical Characterization

Aerial mycelia of isolate MH4 were related to flexuous/spiral category as examined by light microscope (Fig. 2a). The mature spore mass was belonging to white series and had smooth surface ornamentation as detected using transmission electron microscopy (TEM) (Fig. 2b). Moreover, the cultural properties of the chosen isolate revealed its rich growth on most of the tested organic and synthetic media. The colour of the aerial and substrate mycelia was varied depending on the used medium. The strain could liquefy gelatine but couldn't coagulate skim milk; however, it showed positive results for nitrate reduction and negative results for hydrogen sulphide production. In contrast, the strain showed negative reaction for melanin formation, while it could hydrolyze chitin, cellulose, starch and pectin. The isolate could grow on media containing upto 7% NaCl (w/v). It also could tolerate pHs; 4.0-10.0, temperatures; 10-40ºC, however, no growth was observed at 50ºC. This strain can utilize glucose, L-arabinose, D-xylose, mannitol, fructose, sucrose, mannose, salicin, trehalose inisitol, rhamnose and raffinose (Table 1). L, L-Diaminopemelic acid was contained in cell wall. After incubation period, the plates were examined showing typical colonies of *Streptomyces*. The typical round, small, opaque, compact, frequently pigmented colonies were examined under a light microscope (100×). The colonies that bear typical *Streptomyces* morphology were purified and sub-cultured on Yeast extract-glucose agar plates and stored for further study [15]. Based on its application to ISP technique [16], morphological study, cultural (aerial colour, reverse side pigments, melanoid pigments and soluble pigments followed by NaCl characteristics) [17], physiological characteristics and examination of the spore surface by electron microscope, the isolate MH4 was identified as *Streptomyces baarnensis* [18,19] .

An antimicrobial assay of the *Streptomyces baarnensis* MH4 revealed the presence of high antibacterial activities against Gram positive *St. aureus* (12 mm) and the Gram negative *E. coli* (activity 13 mm) and of *S. cerevisiae* (activity 10 mm). Chemical screening of the strain extract displayed numerous bands during TLC ranged between orange and violet colours after spraying with anisaldehyde/sulphuric acid. Large scale fermentation (25L) of the strain as shaker culture on M_2 medium was carried out revealing a yellow culture broth. After harvesting, working up and isolation using different chromatographic techniques, the obtained bacterial reddish brown crude extract was found to deliver nine bioactive metabolites including one new compound (N-ethylphenethylamine,1). Structures of the obtained compounds were discussed as shown below.

3.2 Structural Elucidation

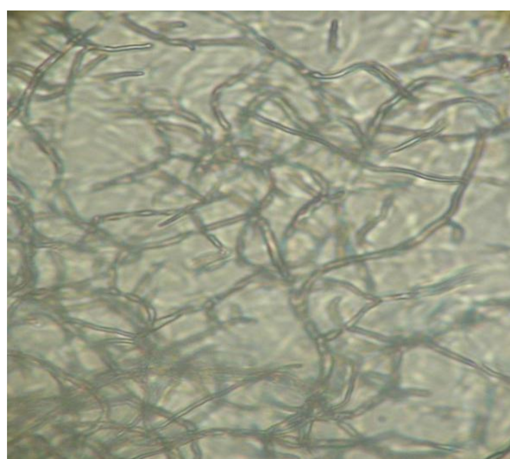
3.2.1 *N*-ethylphenethylamine

As low polar colourless oil, compound 1 was obtained from fraction II after its application to a series of chromatographic purifications, showing UV absorbance and pink coloration on spraying with anisaldehyde/sulphuric acid on TLC. According to Electrospray ionization-Mass spectrometry (ESI MS), the molecular weight of 1 was deduced as 149 Dalton with a corresponding molecular formula of $C_{10}H_{15}N$. Based on the proton nuclear magnetic resonance spectroscopy (^{1}H NMR), a pattern of 5H representing a phenyl residue was deduced in the region of δ 7.35-7.25 (m), among with two hetero atom -bounded methylene protons were visible as multiplet pattern in the region of 3.22-3.18, and one sp^2-bounded triplet methylene (δ 2.95) in addition to triplet 3H methyl group (δ 1.28). On the bases of 13°C NMR, four aromatic signals, representing one quaternary carbon (δ 138.0), three methine signals at δ 129.0 (CH-2,6), 128.8 (CH-3,5) and 127.7 (CH-

4), confirming the phenyl ring. In contrast, four sp^3 carbon signals being of three methylene carbons (δ 47.9, 41.6 and 34.6) and one methyl carbon (δ 9.5) were established, deducing the existence of ten carbon signals as matched with the revealed molecular formula. Finally, structure of 1 was established as *N*-ethylphenethyl amine on the bases of H,C (HMQC, HMBC) and H,H (HHCOSY) Connectivities (Fig. 3). *N*-ethyl phenethyl amine is reported her to first time as natural product. However, it was detected by GC-MS along with methamphetamine and related regioisomeric phenethylamines [20-22].

Table 1. Morphological and nutritional characteristics of *Streptomyces baarnensis* MH4

Characteristics	Results
Spore chain	Straight
Spore surface	Smooth
Aerial spore-mass colour	Yellow
Soluble pigment	Light brown
Growth on agar medium: Yeast extract-Malt extract, tyrosine, oatmeal, asparagine and inorganic salt-starch	Positive
Growth at 25ºC to 40ºC	Positive
Growth at 50ºC	Negative
Growth at pH 4 to 10	Positive
Growth in 0 to 10% NaCl	Up to 7
Decomposition of chitin and cellulose	Negative
Decomposition of gelatine and starch	Positive
Coagulation of skim milk	Negative
Production of hydrogen sulphide	Negative
Melanin production	Negative
Reduction of nitrate	Positive
Utilization of D-xylose, fructose, glucose, L-arabinose, mannitol, salicin, trehalose, sucrose, raffinose, rhamnose and inisitol	All positive
Cell wall peptidoglycan	L,L-diaminopimelic acid



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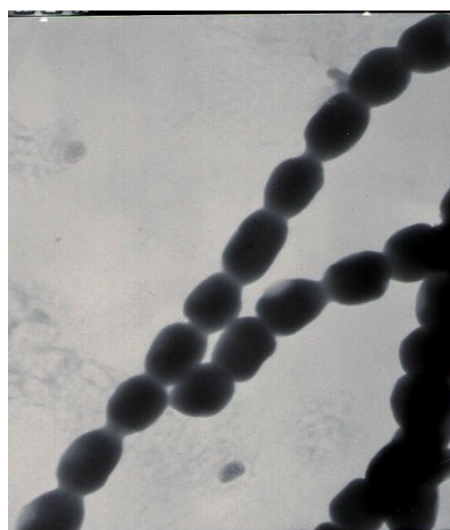

b

Fig. 2 (a). Photograph showing spore chain morphology of *Streptomyces baarnensis* MH4 (X1000), (b). Transmittance electron micrograph (TEM) of spores of *Streptomyces baarnensis* MH4

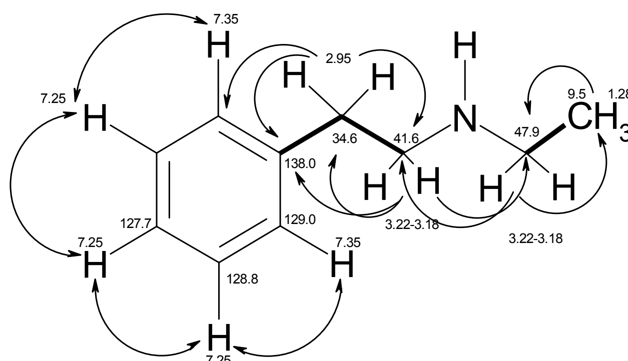

Fig. 3. H,H COSY (▬) and HMBC (→) connectivities of N-ethyl phenethyl amine (1)

3.2.2 Nonactic acid and homononactic acid

As colourless oils, compounds 2 and 3 were obtained from fraction II, showing UV non-absorbance, which was detected as reddish-brown and changed latter to violet on spraying with anisaldehyde/sulphuric acid. The molecular weights (202, 216 Daltons) and the corresponding formula ($C_{10}H_{18}O_4$, $C_{11}H_{20}O_4$) of 2 and 3 were obtained by ESI MS and high resolution electrospray ionization mass spectrometry (HRESIMS), respectively. The 1H NMR spectrum of 2 exhibited three multiplets of oxy-methines at δ 3.99 (2H) and 3.89 (1H). In the region of δ 2.42~1.58 a series of multiplets with integration of 7H was present corresponding to three methylene groups and one methine proton (2.42). The latter methine appeared as quartet, pointing to its direct connection with a methyl group, which gave a doublet at δ 1.15, this was together with a further doublet methyl was observed at δ 1.09. 13ºC NMR spectrum of 2 revealed 10 carbon signals, among them a quaternary carbon at δ 179.2 of a carboxylic acid carbonyl, and three oxygenated methines (δ 82.0, 77.9, 66.1). One methine (δ 46.1), two methylene (δ 32.2, 29.4) and two methyl (δ 24.1, 14.1) carbon signals were finally deduced as well. Alternatively, the ^{1}H NMR spectrum of 3 was identical to those of 2, except that one of the methyl doublet present in the side chain of 2 was replaced by a terminal ethyl group in 3, which was responsible for the methyl triplet at δ 0.91. Based on the revealed spectroscopic means and comparison with literature, structures of 2 and 3 were established as nonactic acid [23] and homononactic acid [24], respectively. Nonactic acid (2) was reported as moderate inhibitor of 3α-hydroxysteroid dehydrogenase [25]. The reported structurally related feigrisolides A-B (6, 7) by Tang et al. [25] which were considered as building blocks of nonactic acid (2) and homononactic acid (3), respectively, were excluded by synthesis [26]. Therefore, the reported 6 and 7 were actually 2 and 3, respectively.

3.2.3 3-(3,3-Bisindolyl)propane-1,2-diol and turbomycin A

As middle polar red oil, compound 4 was obtained, showing UV absorbance during TLC. The molecular weight of 4 was deduced as 306 daltons on the bases of ESI MS modes, and the corresponding molecular formula $C_{19}H_{18}N_2O_2$ was established by HRESI MS. According to the ^{1}H NMR spectrum, two overlapping 2H doublets (δ 7.54 and 7.28) and 2H triplets (δ 7.01, 6.90), together with two singlets (δ 7.29 and 7.13) were visible, giving evidence of two 3-substituted indole moieties. In the aliphatic region, diastereotopic methylene protons (δ 3.61, 3.48) were visible, indicating their neighbor to a stereogenic centre being attached to a *sp*^{2} carbon or heteroatom. Finally, two oxymethine protons were observed as doublet (δ 4.68) and multiplet (δ 4.48), respectively. Based on these features and comparing with literature values and authentic data, compound 4 was fixed as 3-(3,3-bisindolyl) propane-1,2-diol [27,28], a cytotoxic agent to fertilized leghorn eggs [29].

Compound 5, as closely structural analogue of 4, was obtained as reddish oil, which gave a red color with anisaldehyde/sulfuric acid. The proton NMR spectrum indicated two doublets (7.64, 6.90) and two triplets (7.28, 7.01) of four consecutive protons, each of 3H intensity in addition to a singlet (δ 8.24) of 3 protons in position 2 of an indole system. The (+)-ESI MS delivered surprisingly a *quasi-molecular* ion at *m/z* 360 $[M]^+$ instead of the expected 361

$[M+H]^+$), corresponding to an ion formula $C_{25}H_{18}N_3$ by high resolution, pointing to a symmetrical molecule. Based on these data, compound 5 was concluded as turbomycin A (5). Tris- arylmethanes are easily oxidized; this explains their red tailing on TLC. Turbomycin A (5) is well known for its broad-spectrum antibiotic activity against Gram-negative and positive bacteria [30]. Turbomycin A (5) is formed by interaction of indole with indolyl-3-carbaldehyde [31].

3.2.4 Indolyl-3-acetic acid, 3-indolylcarbaldehyde, indole carboxylic acid and 2'-deoxyadenosine

Structures of remaining known compounds; indolyl-3-acetic acid [32], indolyl-3-carbaldehyde [32], indolyl-3-carboxylic acid [32] and 2'-deoxyadenosine [32,33] were deduced on the bases of their chromatographic properties, and spectroscopic means (NMR and MS) (see the experimental part) and comparison with corresponding literatures.

4. CONCLUSION

Actinomycetes especially *Streptomyces* species are widely recognized as industrially important microorganisms as they are a rich source of several useful bioactive natural products with potential applications and are prolific producers of secondary metabolites, many of which have commercial importance as antibiotics, anti-parasitics and antifungal agents, herbicides, pesticides, anticancer or immunosuppressive agents as well as industrially important enzymes. In the present study, the new N- ethylphenethyl amine (1) together with nonactic acid (2), homononactic acid (3), 3- (3,3-Di-indolyl) propane-1,2-diol (4), turbomycin A (5), indolyl-3-acetic acid, indolyl-3-carbaldehyde, indolyl-3-carboxylic acid and 2'–deoxyadenosine, were isolated from the terrestrial *Streptomyces baarnensis* MH4. Structures of the isolated compounds were determined using intensive studies of their NMR and mass spectrometry and comparison with corresponding literatures. Taxonomical characterization of the terrestrial *Streptomyces baarnensis* MH4 along with large scale fermentation and examination of the antimicrobial activity of its extract were reported. The crude extract of terrestrial *Streptomyces baarnensis* MH4 was deduced to exhibit high antibacterial activity against Gram positive (*St. Aureus)* and Gram negative bacteria (*E. coli* and *S. cerevisiae*).

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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