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Full Length Research Paper

Cyclic lipopeptides and other bioactive secondary metabolites from a new terrestrial *Streptomyces* sp. TN272

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The ethyl acetate extract of the new terrestrial *Streptomyces* sp. isolate TN272 delivered eighteen bioactive compounds, including four homologues cyclic lipopeptides. Structures of the first fourteen compounds (1 to 14) were established on the basis of different spectroscopic techniques, including GC-MS, ESI-MS, HRESI/EIMS, 1D NMR experiments, and by comparison with related compounds. They are: 2,4-bis (1,1-dimethylethyl) phenol (1) 1-hexadecene (2), 5-octadecen (3), *cis*-cyclo (L-prolyl-L-valyl) (4), hexadecanoic acid (5), cyclo (leucyl-prolyl) (6), *cis*-cyclo(phenyl- prolyl) (7), 3(Z)-tetradecene (8), *trans*-1,10-dimethyl-trans-9-decal (9) and 1-nonadecene (10) together with a mixture of four hydroxy fatty acids; 7-hydroxy-tetradecanoic acid (11), 7-hydroxy-pentadecanoic acid (12), 9-hydroxy-hexadecanoic acid (13) and 9-hydroxy-heptadecanoic acid (14). The UV non absorbing three known cyclic lipopeptides homologues (15_1 to 15_5, 16_1 to 16_4, 17_1 to 17_2) and unknown cyclic lipopeptides (18) (m/z 662) were detected during hyphenated HPLC-UV/Vis-ESI MS screening. According to our biological studies, these unknown cyclic lipopeptides possess antifungal activities against the two tested fungi, *Fusarium* sp. and *Candida tropicalis* R2 CIP203.

Key words: New *Streptomyces* sp.TN272, identification, bioactive compounds, purification, structure elucidation, unknown cyclic lipopeptides, antifungal activity.

INTRODUCTION

Actinomycetes are Gram-positive bacteria that are widely spread in nature and play a significant role in the production of bioactive metabolites. At least 90% actinomycetes isolated from soil have been reported to be *Streptomyces* spp. (Anderson and Wellington, 2001).

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These bacteria are characterized by a complex morphologic differentiation cycle accompanied by the production of numerous extracellular enzymes as well as, many kinds of bioactive secondary metabolites having great structural and functional diversity including, antifungal, antiviral, and anticancer, immunosuppressant agents, insecticides, herbicides, antibiotics, etc. This fact has made *Streptomyces* the most used bacteria in fermentation manufacturing of active pharmaceutical compounds.

During our search program for bioactive compounds

from actinomycetes, a new terrestrial bacterium strain, called TN 272, was isolated and selected due its inhibitory activities against Gram positive and Gram negative bacteria and fungi. In this article, we describe the identification of the new *Streptomyces* sp. isolate TN272 using its cultural characteristics, the nucleotide sequence of the corresponding 16S rRNA gene and the phylogenetic analysis, followed by optimization, large-scale fermentation, isolation of the bioactive compounds and determination of their structures. The antimicrobial activity of the unknown cyclic lipopeptides (18) was addressed as well.

MATERIALS AND METHODS

Micro-organisms and plasmids

Escherichia coli DH5α was used as host strain. Gram positive bacteria, *Micrococcus luteus* LB 14110 and *Staphylococcus aureus* ATCC 6538, and Gram negative bacterium *Agrobacterium tumefaciens* were used as indicator microorganisms for the antibacterial activity assays. Antifungal activity was determined against *Fusarium* sp. and *C. tropicalis* R2 CIP203. pIJ2925 derivative of pUC18 (Janssen and Bibb, 1993) and pCR-Blunt vector (Invitrogen) were used as the cloning vectors.

Culture conditions and biological assays of antimicrobial activities

Transformation of *E. coli* DH5α with pIJ2925 derivatives was carried out according to (Hanahan, 1983). For the isolation of actinomycete strains, soil and water samples collected from different Tunisian regions were spread on solid boiled barley bran medium (Mellouli et al., 1996). After incubation at 30 or 40°C for several days, colonies showing sporulation and filamentous morphology were picked and propagated on the same solid medium. The TN272 strain was grown in tryptic soy broth (TSB) at 30 g/l for the preparation of genomic DNA (Hopwood et al., 1985). Cultural characteristics of TN272 strain were compared on the basis of observations made after 7, 14 and 21 days incubation on nutrient agar, Sabouraud agar and yeast malt agar media. To investigate the influence of the medium on antimicrobial production, spores at 10^7 g/ml were used to inoculate 500 ml Erlenmeyer baffled flasks containing 100 ml of TSB medium (30 g/l). After incubation at 30°C for 24 h, this pre-culture was used to inoculate 1/10 (v/v) 1000 ml Erlenmeyer flasks with four indents, containing 200 ml of modified Bennett medium (peptone 2 g/l, yeast extract 1 g/l, beef extract 1 g/l) supplemented at 1% (w/v) with one of the five tested carbon sources (starch, fructose, glycerol, glucose and sucrose). After incubation at 30°C for 72 h in an orbital incubator with shaking at 200 rpm, biological activities were assayed for each culture supernatant. Influence of magnesium, potassium and trace mineral oligoelements on active molecules production was also investigated by addition of these chemical additives to the culture medium, additive by additive, the combination of two additives and the addition of all three additives. The final magnesium and potassium concentration was 3.5 and 1 mM respectively. For trace mineral oligoelements (40 mg $ZnCl_2$, 200 mg $FeSO_4 \cdot 7H_2O$, 6.5 mg H_3BO_3 and 13.5 mg $MoNa_2O_4 \cdot 2H_2O$ per 100 ml distilled water), 7.5 ml were added to 1 l of growth medium. Eight different concentrations (% w/v) of glycerol were tested: 0.2; 0.5; 0.75; 1; 1.25; 1.5; 1.75 and 2. The effect of culture conditions: temperatures (25, 30, 35 and 0°C), initial pH (6.0, 6.5, 7.0, 7.2, 7.5, and 8.0), incubation times (24, 36, 48, 60, 72, 84, 96, 110, 120 and 144 h) and rotary shaker (100, 150, 200, 250 and 300 rpm) on growth and antimicrobial activities production was studied. Biomass of the *Streptomyces* sp. TN272 strain was determined by measuring the dry weight after drying at 105°C. Antimicrobial activities studies were performe d according to Fourati et al. (2005) and Smaoui et al. (2010).

DNA manipulation

PCR amplification of the 16S rRNA gene of TN272 strain was performed in an automated thermocycler (Perkin Elmer) using two primers 5'-AGAGTTTGATCCTGGCTCAG-3' and 5'-AAGGAGGTGATCCAGCCGCA-3' according to the amplification profile described by (Elleuch et al., 2010). Nucleotide sequence of the 16S rRNA gene was determined on both strands by an automated 3100 Genetic Analyser (Applied Biosystems) using specific primers. Homology search was performed using Blast Search algorithm. The nucleotide sequence of the whole 16S rRNA gene (1457 bp) of TN272 strain has been assigned GenBank (EMBL) under accession number FN687756. Multiple sequence alignment was carried out using clustal W (Thompson et al., 1994) at the European Bioinformatics Institute website (http://www.ebi.ac.uk/clustalw/). Phylogenetic analyses were performed using programs from the PHYLIP package (Felsenstein, 1989) and phylogenetic tree was constructed by the neighbour joining (NJ) algorithm (Saitou and Nei, 1987) using Kimura 2-parameter distance. The robustness of the inferred tree was evaluated by bootstrap (100 replication).

Fermentation, working up and isolation of the bioactive compounds

Spores at 10^7/ml of *Streptomyces* strain TN272 were used to inoculate 1000 ml Erlenmeyer flasks, each containing 200 ml of TSB medium at 30 g/l. After incubation at 30°C for 24 h in an orbital incubator shaking at 200 rpm, the pre-culture was used to inoculate 25 l of modified Bennett medium, supplemented with: glycerol at 0.75% (w/v), magnesium and potassium at 3.5 and 1 mM respectively and trace mineral oligoelements (40 mg $ZnCl_2$, 200 mg $FeSO_4 \cdot 7H_2O$, 6.5 mg H_3BO_3 and 13.5 mg $MoNa_2O_4 \cdot 2H_2O$ per 100 ml distilled water), 7.5 ml of this solution were added to 1 l of growth medium. For this purpose, we have prepared five cultures in 7 l (working capacity of 5 l) fermentor (INFORS AG CH-4103 Bottmingen/Switzerland). After three days fermentation (30°C, 200 rpm), the culture broths were subjected to filtration over celite using a filter press. The filtrate was exhaustively extracted by ethyl acetate (3 ×) and the obtained organic extract was concentrated *in vacuo* to dryness, affording 1.26 g crude extract. TLC of the strain extract is referred to a polar and oily nature. A sample of the strain extract was first applied to GC-MS analysis, delivering 12 intensive peaks at the following retention times: 7.98 (broad, 25%) for 1, 2, 3-propanetriol (MI), 9.20 (15%,) for 1,2,3-propanetriol monoacetate (MII), 15.00 (33%) for 2, 4-bis(1,1-dimethylethyl)phenol (1), 15.96 (24%) for 1-hexadecene (2), 18.23 (27 %) for 5-octadecen (3), 18.64 (45%) for *Cis*-cyclo (L-prolyl-L-valyl) (4), 18.69 (37%) for hexadecanoic acid (5), 19.69 (50%) for unknown structure, 19.87 (100%) for cyclo (leucyl-prolyl) (6), 19.95 (41%) for unknown structure, 20.66 (36%) for unknown structure, 24.06 (30%) for cyclo (phenyl-prolyl) (7). An alternative application of the strain extract to HPLC-ESI/MS^2-MS^3 delivered four homologues with molecular weights at *m/z*: 1035, 1049, 1078 and 662 corresponding to three isomeric known cyclo-lipopetides and unknown lipopeptide (*m/z* 662). The crude extract (1.26 g) was alternatively applied to fractionation by silica gel column chromatography (60 × 3 cm, cyclohexane-DCM-MeOH). After TLC monitoring and visualization by UV-light and anisaldehyde/sulphuric acid spraying reagent, five

fractions were collected: F1 (250 mg), F2 (110 mg), F3 (120 mg), F4 (300 mg) and F5 (390 mg). The first unpolar fraction showed several unpolar UV-non absorbing bands, turned mostly between blue and violet on spraying with anisaldehyde/sulphuric acid spraying reagent. The fraction 1 was applied to GC-MS analysis and displayed six major peaks of the retention time; 13.46 (43%) for 3(Z)-tetradecene (8), 14.05 (36%) for trans-1,10-dimethyl-trans-9-decal (4), 14.99 (100%) for unknown structure, 15.97 (69%) for 1-hexadecene (2), 18.22 (48%) for 1-nonadecene (10) and 20.27 (18%) for 1-nonadecene (10). Consequently, fraction 1 was applied to HPLC-ESI/MS^2-MS^3 analysis delivering a homologue of unknown isomeric cyclo-lipopetides of 662 Dalton. Fraction 2 was shown to exhibit a middle polar UV non absorbing substance, which on spraying with anisaldehyde stained as violet which turned after few minutes to blue. After purification on Sephadex LH-20 (DCM/40% MeOH), it afforded a colourless oil for a saturated fatty acid (8 mg). Fraction 3 showed a further middle polar band of UV non absorbing substance which on spraying with anisaldehyde/sulphuric acid was shown as violet band, turned latter as blue. Purification of the fraction using silica gel column chromatography (3 × 60 cm, DCM-MeOH) followed by Sephadex LH-20 (DCM/40% MeOH) afforded a colourless oils of glycerol; 1,2,3-Propanetriol (MI, 15 mg) and a saturated fatty acid (21 mg). Fraction 4 showed during TLC three bands, the first two of them were UV non absorbing, while the third one was shown as UV absorbing and fluorescence. The fraction was applied to fractionation by silica gel, affording two fractions, F4a (50 mg) and F4b (180 mg). By application the sub-fraction F4b to PTLC (20 × 20 cm, DCM/5%MeOH), a blue fluorescence band was isolated which was then purified by Sephadex LH-20 (DCM/40% MeOH) to give a colourless solid (7 mg) of unknown structure. A subjection of the sub fraction F4a to a further purification during silica gel column (DCM-MeOH), an UV absorbing colourless solid of cyclo(leucyl-prolyl)(6, 8 mg), and UV non absorbing colourless Solid as a mixture of four closely related hydroxy fatty acids (11 to 14, 16mg) were isolated.

MI: 1,2,3-propanetriol (glycerol), we think that this compound used as carbon source, is extracted from the medium composition. MII: 1,2,3-propanetriol monoacetate, this compound is probably formed through ester interchange reaction between glycerol and ethyl acetate.

Characterization of compounds

The NMR spectra were measured on a Bruker AMX 300 (300.135 MHz), a Varian Unity 300 (300.145 MHz), and a Varian Inova 600 (150.820 MHz) spectrometer. 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). DCI-MS: Finnigan MAT 95 A, 200 eV, reactant gas NH_3. Optical rotation was measured on a Perkin-Elmer polarimeter, model 343. High performance liquid chromatography (HPLC): Analytical: Jasco multiwavelength detector MD-910, two pumps type Jasco Intelligent Prep. Pump PU-987 with mixing chamber, injection valve (type Rheodyne) with sample loop 20 µl, Borwin HPLC-software. GC-MS was used a Trace GC-MS Thermo Finnigan, ionization mode EI eV 70, instrument equipped with a capillary column CP-Sil 8 CB for amines (length: 30 m; inside diameter: 0.25 mm; outside diameter: 0.35 mm; film thickness: 0.25 μm). The analysis was carried out at a programmed temperature: initial temperature 40°C (Kept for 1 min), then increasing at a rate of 10°C min^{-1} to a final temperature of 280°C (kept for 10 min). In the EI mode, the injector and detector temperature was set to 250°C; it was used as carrier gas at flow rate 1 ml min^{-1}, total run time 27 min and Injection volume 0.2 μl. Flash chromatography was carried out on silica gel (230 to 400 meshes). R_f-values were measured on Polygram SIL G/UV_{254} (Macherey-Nagel and Co.). Size exclusion chromatography was done on Sephadex LH-20 (Pharmacia).

RESULTS AND DISCUSSION

Isolation, identification and phylogenetic analysis of TN272 strain

Strain TN272 grew well on nutrient agar medium and yeast malt agar media and the colours of the vegetative and aerial mycelia were whitish and greyish, respectively. The spore chains were greyish in nutrient agar medium and yellow-brown in yeast malt media. Comparison of these characteristics with those of actinomycete species described in Bergey's manual of systematic bacteriology (Lechevalier et al., 1989) suggested that TN272 strain belongs to the genus *Streptomyces*. Total nucleotide sequence of 1457 bp (accession no. FN687756), of the whole 16S rRNA gene of the TN272 strain was determined in both strands. The alignment of this sequence through matching with reported 16S rRNA gene sequences in gene bank shows high similarity (97 to 99%) to the *Streptomyces* 16S rRNA genes. The organism most similar to the new isolate TN272 strain was *Streptomyces krainskii* AB184278.1 (Figure 1). Based on these data, we propose the assignment of our new isolate bacterium as *Streptomyces* sp. TN272 strain.

Optimization of nutritional and cultural conditions

Five carbohydrate sources (starch, fructose, glycerol, glucose and sucrose) were tested as sole carbon source at 1% (w/v) in the modified Bennett liquid medium. The studied strain exhibited the ability to grow on all tested carbon sources. The maximum of antimicrobial activity production was afforded when glycerol served as carbon source. To further optimize the culture conditions, three compounds (magnesium at 3.5 mM, potassium at 1 mM and trace mineral oligoelements) were tested using glycerol at 1% (w/v) as carbon source. Obtained results showed that the addition of these three chemical compounds affects positively the secretion of the active compounds.

The optimum temperature for growth and active compounds production of the *Streptomyces* sp. TN272 is 30°C. The highest antimicrobial activity against the tested microorganisms was obtained at initial pH = 7 of the culture medium. Concerning incubation time, antimicrobial activity appeared to be pronounced after 30 h of growth with a maximum at 72 h of incubation. This activity remained stable between 72 and 96 h and then decreased slowly until 120 h and disappeared after 140 h of incubation. Agitation rates of 100, 150 and 300 rpm gave a low production of active compounds, while the best result was noticed at 200 and 250 rpm. To further enhance the active compounds production, we have investigated the effect of the glycerol concentration. Eight concentrations (% w/v) were tested (0.2, 0.5, 0.75, 1, 1.25, 1.5, 1.75 and 2.0). In summary, the antimicrobial activity was correlated with biomass production. This

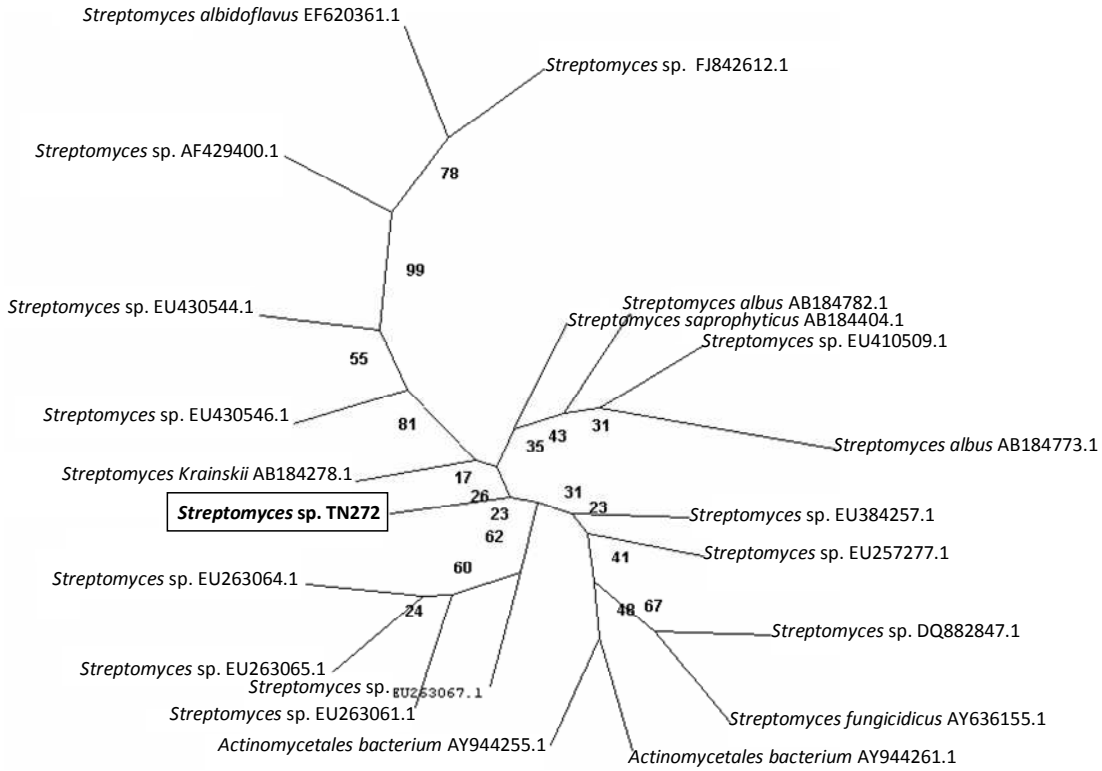


Figure 1. Phylogenetic tree of the *Streptomyces* sp. isolate TN272.

biomass increased with increasing glycerol concentrations up to 0.75% (w/v) and remained nearly constant up to 1% (w/v) with a maximum at 0.75% (w/v).

Any further increase of this substrate level resulted in a decrease in biomass and consequently in active compounds production.

Structure elucidation of the active compounds

Cyclo (leucyl-prolyl) (6)

As middle polar colourless solid, compound (6) was isolated from fraction 4 after a series of chromatographic purifications. The ^{1}H and ^{13}C NMR spectra of (6) recognized its dipeptide nature due to the existence of only two α-amino-bounded methines at δ 4.15 (δ_C 53.4), and 4.04 (δ_C 59.0) and two peptide carbonyls at δ 170.3 and 166.1. The NMR pattern of compound (6) was visible only in the aliphatic region, excluding any of the aromatic amino acids in the structure. The molecular weight of (6) was recognized as 210 Dalton during the DCI MS. According to the discussed chemical and spectroscopic data and search in AntiBase (Laatsch, 2009), compound (6) was established as cyclo(Leucyl-prolyl). This molecule has been already described from bacteria and it inhibits aflatoxin production (Yan et al., 2004). Besides this diketopiperazine (DKP) derivative, *Streptomyces* sp. TN272 produces two others DKPs, *cis*-cyclo (L-prolyl-L-valyl) (4) and cis-cyclo (phenyl-prolyl) (7). These two compounds posses antibacterial and antifungal activities and have been already described from *Streptomyces* sp. TN262 (Elleuch et al., 2010) and *Streptomyces* sp. TN97 (Ben Ameur Mehdi et al., 2006) respectively.

Hydroxy fatty acids (11 to 14)

During the isolation of compound (6), an UV non absorbing colourless solid was obtained, which on spraying with anisaldehyde/sulphuric acid was stained as bright blue band. The $^{1}H/^{13}C$ NMR of this band recognized it as a mixture either of butyrolactones or hydroxy fatty acids. The ^{1}H NMR displayed a broad singlet (δ 5.35) corresponding to four hydroxy protons, and 4 multiplets each of 1H between δ 4.10 to 3.35 for oxygenated methines. In the region of δ 2.28 to 0.78 numerous of multiplet signals corresponding to sp^2 bound methylene protons and a long chain fatty acids ended by a triplet methyl. In the ^{13}C NMR, abroad signal for a carbonyl of saturated fatty acids at δ 178.9 and 178.8.

Four signals were located between δ 74.9 to 70.3 corresponding to mainly to hydroxy methines. In the region between δ 39.8 to 11.85 multiple signals for sp^2

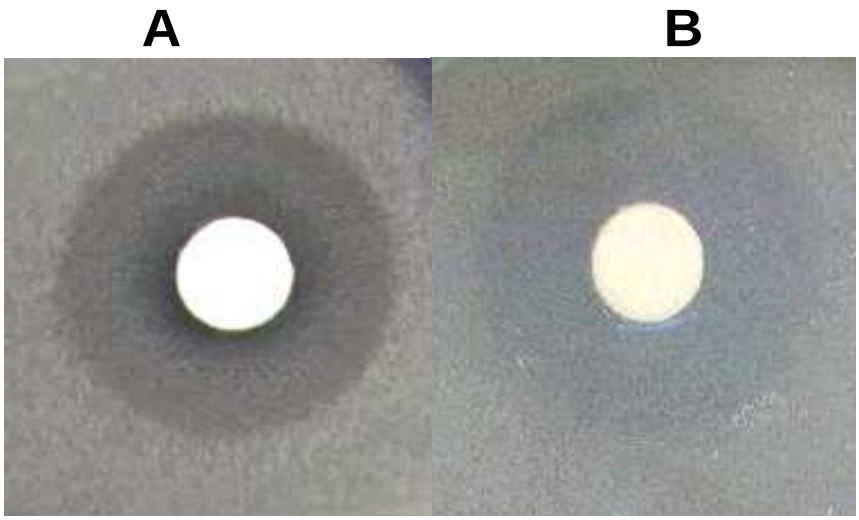
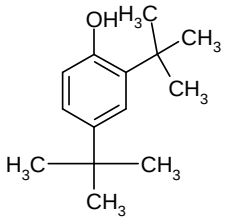


Figure 2. Antifungal activities of unknown cyclic lipopeptides (18) against Fusarium sp. (A) and *Candida tropicalis* R2 CIP203 (B) at concentration of 35 µg/disk.

bounded methylenes and long chain of sp^3 carbons for methylenes and terminal methyls were observed. The (+) ESI MS of the component recognized the existence of four molecular ion peaks at *m/z* 267 $(M1+Na)^+$, 281 $(M2+Na)^+$, 295 $(M3+Na)^+$ and 309 $(M4+Na)^+$. Their corresponding negative ions were established *via* (-) ESI MS at *m/z* 243 $(M1-H)^-$, 257 $(M2-H)^-$, 271 $(M3-H)^-$ and 285 $(M4-H)^-$, respectively. (+) HRESIMS for the afforded four molecules recognized the corresponding molecular formulas; $C_{14}H_{28}O_3Na$, $C_{15}H_{30}O_3Na$, $C_{16}H_{32}O_3Na$ and $C_{15}H_{34}O_3Na$ respectively. In accordance, using a search in AntiBase (Laatsch, 2009), two known hydorxy-fatty acids were revealed; 7-hydroxy-tetradecanoic acid (11) and 9-hydroxy-hexadecanoic acid (13) containing even carbons number. The remining two fatty acids were of an odd carbon numbers (C15 and C17), the first of them might be a close structure of (11) that is, 7-hydroxy-pentadecanoic acid (12), while the last one could be closely related to (13) that is, 9-hydroxy-heptadecanoic acid (14). The last two hydroxy fatty acids were not reported before from microorganims. These hydroxy fatty acids are mainly represented as precursors for the synthesis of butyrolactones (Beppu, 1992).

Cyclic lipopeptides (15_1-15_5; 16_1-16_4; 17_1-17_2)

An application of the crude extract of strain TN272 to TLC evaluation displayed several UV non absorbing bands, which on spraying with anisaldehyde/sulphuric acid were detected as blue of apolar and middle polar compounds. These bands turned to pink-violet on spraying with Ehrlich's reagent referring to their nitrogenous containing nature. Alternatively, the extract was applied to TLC evaluation using chlorine-*o*-dianisidin reaction (Shaaban, 2004), revealing the existence of multi-blue bands (middle-apolar) for peptides. On the basis of spraying the extract with ninhydrine during TLC, these components were mostly turned yellow, referring to them as cyclic lipopeptides. Consequently, the crude extract was applied to hyphenated analytical HPLC coupled with mass spectrometry (HPLC/MS). In accordance, HPLC analysis of the strain crude extract yielded enormous signal peaks of two major confused component signals located in two regions of R_t 23.88-25.15 and 29.54 to 29.89 min with UV non-absorbance. Based on HPLC-MS, these unpolar components were recognized as lipopeptides isomers with different molecular weights. The first major band of signals was in the region of R_t 23.51 to 25.12, which were recognized by (+) ESI MS to entail three homologues of lipopeptides, the first of them was of R_t 23.88, 24.11 and 24.19 min, having the molecular weight *m/z* 1035. On the basis of a search in AntiBase (Laatsch, 2009), Dictionary of Natural Products (DNP) (Chapman and Hall, 2004), five isomeric hepta cyclo-lipopeptides ($C_{53}H_{94}N_8O_{12}$, 1035.359), belonging to halobacillin family were revealed. The whole cyclic lipopeptides reported here are cyclized *via* a lactone ring. These five isomers entail seven amino acids (3 leucin; 1 isoleucin; 1 asparagin; 1 asparatyl; 1 valin) and long fatty acid chain containing 12 carbons, which located in different forms. The second region (of R_t 24.27 and 24.35 min) was corresponded to molecular weight of 1049 Dalton. Based on search in the different data basis, four isomeric lichenysin family cyclo-lipopeptides ($C_{54}H_{96}N_8O_{12}$, 1049.386) were recognized (Grangemard et al., 1999): lichenysin-G9a (16_1), lichenysin-G9b (16_2), lichenysin-G8b (16_3) and lichenysin-G8a (16_4). These lipopeptides are constructed similarly from 7 amino acids (3 isoleucin; 2 leucin; 1 asparagin and 1 asparatyl), and



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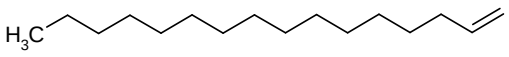

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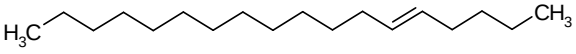

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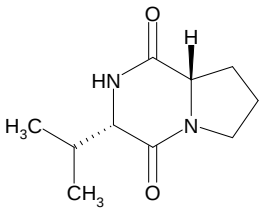

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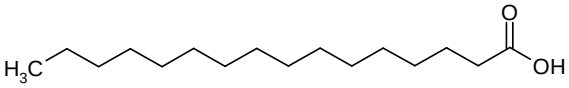

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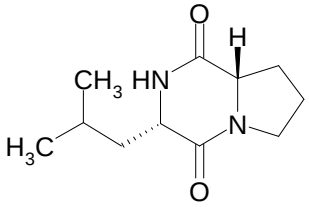

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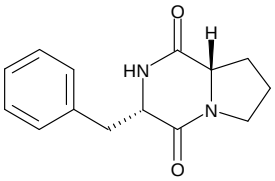

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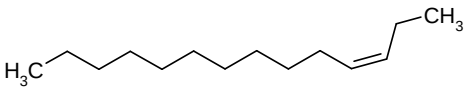

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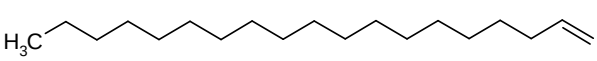

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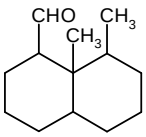

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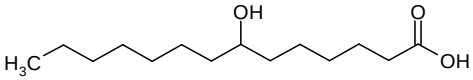

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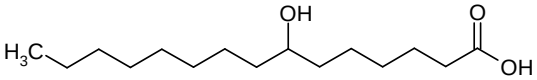
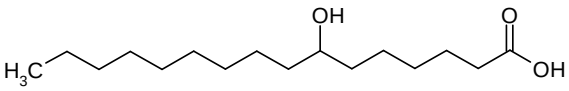
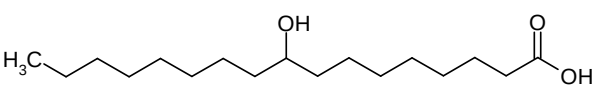


12



13



14

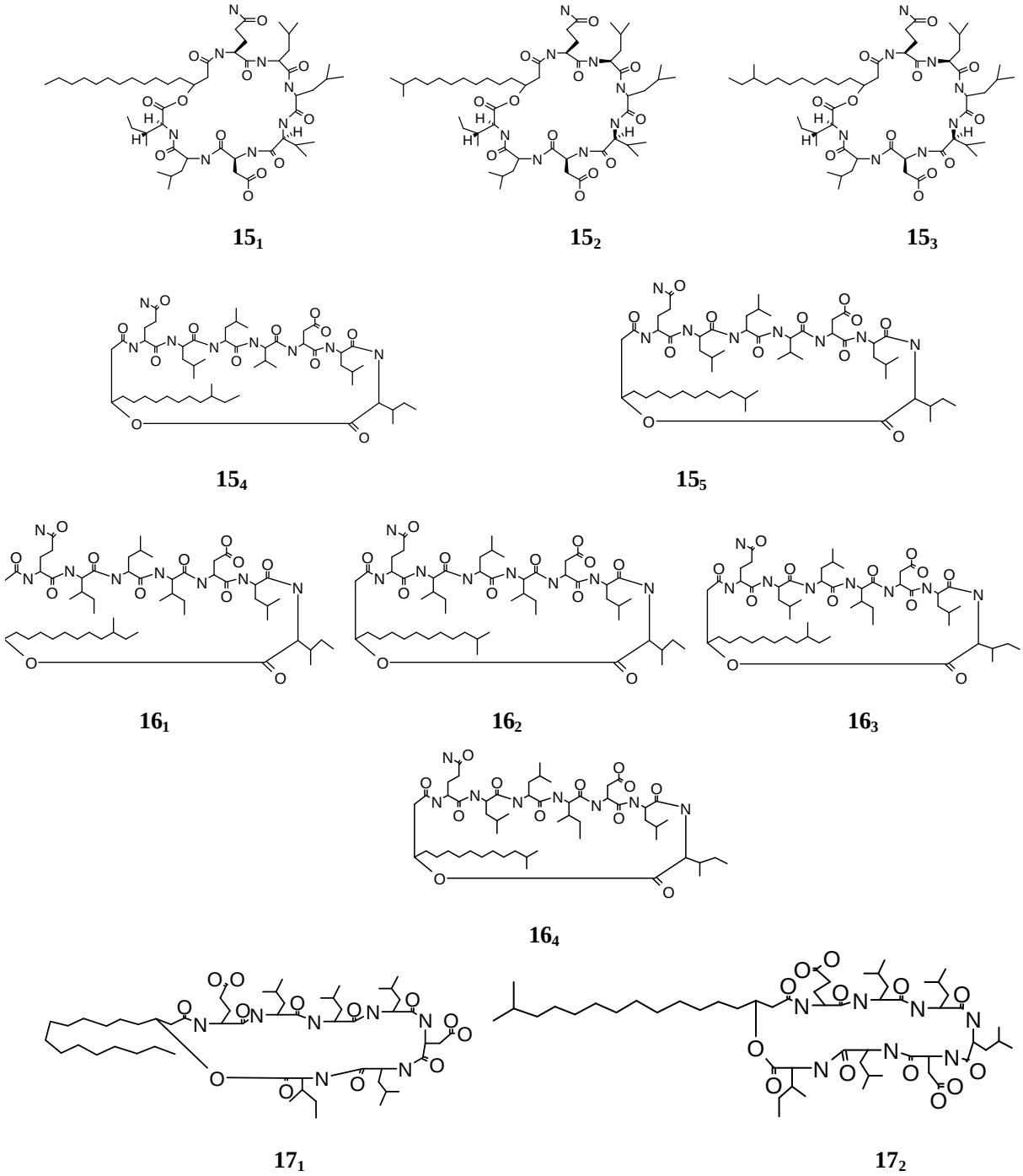


Figure 3. Structure formulas of compounds (1-14) and (15_1-15_5; 16_1-16_4 and 17_1-17_2).

are different only in the fatty acid chain, containing also 12 carbons, are present in different forms. The third region (of R_t 25.07 and 25.15 min) was corresponded to a molecular weight of 1078 Dalton. According to the search in the databases, only two cyclic lipopeptides, surfactin family, ($C_{56}H_{99}N_7O_{13}$; 1078.42) were revealed; daitocidin B3 (17_1) (Kozasa et al., 1988) and pumilacidin C (17_2) (Naruse et al., 1990), which are constructed similarly from seven amino acids (1 isoleucin; 4 leucin; and 2 asparatyl) and are different only in the fatty acid chain, containing 14 carbons, are present in n- and iso-chains, respectively. The second major band of signals (R_t 29.54 to 29.97 min) was present and HPLC chromatogram was recognized by (+) ESI MS to bear one homologue of

lipopeptides, with a molecular weights of 662 Dalton. According to the search in the different databases, no consistent cyclic or linear lipopeptides have been detected. The last unpolar multiple and confused isomers ofof cyclic lipopeptides were successfully isolated during the working up of the strain extract using silica gel column chromatography, giving fraction 1. Fixing the structures of the afforded unknown cyclic lipopeptides, with a molecular weight of 662 Dalton, are in progress as they require rather high techniques for example, "Tandem mass spectrometry coupled with ESI MS high resolution" and HPLC/ESI-TOF-MS techniques (Han et al., 2006) to assign each fragment and hence to fix the amino acids sequencing in each cyclic lipopeptide. According to our microbiological tests, these unknown cyclic lipopeptides (18) showed effective antifungal activities against the two tested fungi, *Fusarium* sp. and *Candida tropicalis* R2 CIP203 from a concentration of 35 µg/disk (Figure 2).

Conclusions

A new *Streptomyces* sp. TN272 bacterium, isolated from south Tunisian soil, was selected for its antimicrobial activity against Gram-positive and Gram-negative bacteria and fungi. Based on the results of the cultural characteristics, analysis of the nucleotide sequence of the whole 16S rRNA gene (accession no. FN687756), and the phylogenetic study, the isolate has been assigned as *Streptomyces* sp. TN272 strain. Glycerol (at 0.75%, w/v) as carbon source, magnesium and potassium at 3.5 and 1 mM respectively as chemical additives and trace mineral oligoelements, in modified Bennett liquid medium (72 h, 30°C, 250 rpm), was the most optimum condition for the production of the largely antimicrobial activity by the *Streptomyces* sp. TN272. Working up of the strain extract after large-scale fermentation, led to isolation of at least eighteen active compounds, among them three diketopiperazine derivatives, three known cyclic lipopeptides homologous [151 to 155 (m/z 1035); 16_1 to 16_4 (*m/z* 1049) and 17_1 to 17_2 (*m/z* 1078)] and unknown cyclic lipopeptides (18)(*m/z* 662) which exhibit antifungal activities against the two tested fungi, *Fusarium* sp. and *C. tropicalis* R2 CIP203 from a concentration of 35 µg/disk. Structures of the purified compounds were established by our different spectroscopic tools and comparison with corresponding literatures (Figure 3).

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