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Bioactive secondary metabolites from new terrestrial *Streptomyces* sp. TN82 strain: Isolation, structure elucidation and biological activity

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Abstract

An actinomycete strain designated TN82 was isolated from Tunisian Sahara soil and selected for its interesting antimicrobial activity against Gram positive and Gram negative bacteria and fungi. Based on the results of cultural characteristic, the 16S rRNA gene nucleotide sequence (1434 bp, accession n° LT608133) and the phylogenetic analysis, this new isolate was assigned as *Streptomyces* sp. TN82 strain. Two active compounds, 3-phenylpyrazin-2(1H)-one (**1**) and 3-O-methylviridicatin (**2**), described for the first time as natural product and bacterial compound respectively, together with four known bacterial metabolites; cyclo-(Leu, Pro) (**3**), anthranilic acid (**4**), indole-3-carbaldehyde (**5**) and *p*-hydroxybenzoic acid (**6**) were isolated from the culture broth of this strain. Structure of the natural compound (**1**) was confirmed by extensive 1D and 2D NMR and EI MS and ESI HR mass measurements, and by comparison with literature data. Minimum inhibitory concentrations of the two compounds (**1**) and (**2**) against the tested human pathogenic bacteria *S. aureus*, *L. monocytogenes* and *S. typhimurium* were significantly lower than those of kanamycin. For the natural compound 3-phenylpyrazin-2(1H)-one (**1**), MICs values were very interesting especially that against *L. monocytogenes* (1.0 μg/mL) which is 2 and 12 times lower than those of ampicillin (2.0 μg/mL) and kanamycin (12.50 μg/mL), respectively.

Keywords *Streptomyces* sp. TN82 strain · Natural compound · Bacterial compound · Antimicrobial activity · Minimum inhibitory concentrations

Introduction

Due to the misuse and multi-resistance of bacteria and the appearance of new diseases there is an urgent need for new antibiotics. In addition, in agriculture domain, plant diseases need to be controlled to maintain the quality and abundance of food and feed. The environmental pollution caused by excessive use of agrochemicals, has led to considerable changes in people's attitudes towards the use of biopesticides in agriculture as well. Consequently, the present trend is directed towards the use of safe biological products to control plant pathogens (Harris 1999; Salman and Abuamsha 2012).

Actinomycetes 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 et al. 2001). *Streptomyces* are characterised by the production of numerous extracellular enzymes as well as, many kinds of bioactive secondary metabolites having great structural and functional diversity including antimicrobial, antiviral, anticancer, immunosuppressant, herbicides, enzyme inhibitors, and other physiologically active substances (Williams et al. 1983; Bedry 2005).

During our screening programme for the isolation of novel actinomycetes bacteria producing bioactive compounds, an interesting bacterium, TN82 was isolated from

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Tunisian Sahara soil sample and selected due to its potent antimicrobial activity against Gram-positive and Gram-negative bacteria, and plant pathogenic microorganisms. The present paper describes the selection and the identification of *Streptomyces* sp.TN82 strain, the extraction, the purification and the structure elucidation of six bioactive compounds from a liquid culture broth of this strain. Minimum inhibitory concentrations of the two compounds, 3-phenylpyrazin-2(1H)-one (**1**) and 3-O-methylviridicatin (**2**), against three human pathogenic bacteria were also addressed.

Materials and methods

General experimental procedure

NMR spectra (^{1}H NMR, ^{13}C NMR, DEPT, correlated spectroscopy (COSY), heteronuclear multiple-quantum correlation (HMQC) and heteronuclear multiple bond correlation (HMBC)) were measured on Bruker Avance DRX 500 and DRX 600 MHz spectrometers using standard pulse sequences and referenced to residual solvent signals. High-resolution electron ionisation mass spectrometry was determined using GCT Premier Spectrometer. Column chromatography was carried out on silica gel 60 (0.040–0.063 mm, Merck) and Sephadex LH-20 as the stationary phases. Preparative thin layer chromatography (PTLC) (0.5 mm thick) and analytical TLC were performed with pre-coated Merck silica gel 60 $PF_{254+366}$. R_f values visualisation of the strain components and their chromatograms was carried out under UV light (254 and 366 nm) and further by spraying with anisaldehyde/sulphuric acid and heating.

Microorganisms and culture conditions

For isolation of actinomycete strains, soil samples collected from Sahara of Tunisia were spread on solid boiled bran barley medium (Mellouli et al. 1996): 0.2% yeast extract and 2% agar were added to a supernatant of a 4% boiled bran barley. The pH was adjusted to 7. After incubation at three different temperatures; 30, 35 and 40 °C for 7 days, colonies showing sporulating and filamentous aspect were picked and propagated on the same solid medium. Some actinomycetes strains were isolated and then selected in solid medium for their capacity to produce antimicrobial activity. TN82 was one strain among the selected isolates. The cultural characteristics and the colours of mature sporulating aerial and substrate mycelia of this strain were monitored on 7, 14 and 21 day-old cultures. These characteristics were tested on the bases of the observations made on yeast malt agar, nutrient agar and Bennett agar media

(Elleuch et al. 2010). TN82 strain was grown on Tryptic Soy Broth (TSB) at 30 g/L for the preparation of genomic DNA (Hopwood et al. 1985).

To investigate the antimicrobial activity of the supernatant culture of the strain TN82, spores at 10^7 spores/mL were used to inoculate 500 mL Erlenmeyer baffled flasks containing 100 mL of TSB (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 TSB (30 g/L). After incubation at 30 °C for 72 h in an orbital incubator with shaking at 200 rpm, the culture broth was filtered; centrifuged, concentrated to 1/10 volume by Rotary evaporator and then assayed for its biological activity. Antibacterial activity was realised against seven bacteria: three Gram positive bacteria; *Micrococcus luteus* (*M. luteus*) LB 14110, *Staphylococcus aureus* (*S. aureus*) ATCC 6538 and *Listeria monocytogenes* (*L. monocytogenes*) ATCC 19117 and four Gram negative bacteria; *Agrobacterium tumefaciens* (*A. tumefaciens*) ATCC 33970, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 49189, *Salmonella typhimurium* (*S. typhimurium*) ATCC 14028 and *Escherichia coli* (*E. coli*) ATCC 8739. Antifungal activity was performed against three phytopathogenic fungi obtained from the Institute of Olive of Sfax-Tunisia: *Fusarium* sp., *Pythium ultimum* (*P. ultimum*) and *Rhizoctonia solani* (*R. solani*).

Antimicrobial activity and minimum inhibitory concentration

For the selection of the isolated actinomycetes, we have used the antagonistic test in solid medium against *M. luteus* LB 14110, *E. coli* ATCC 8739 and *Fusarium* sp. After incubation of the isolated strains for 7 days on solid boiled bran barley medium at the appropriate temperature, an agar disk was recuperated and placed in LB plates covered by 3 mL of top agar containing 50 μL of a 5 h culture of *M. luteus* LB 14110 or *E. coli* ATCC 8739, then incubated overnight at 30 °C for *M. luteus* and at 37 °C for *E. coli*. For antifungal activity against *Fusarium* sp., an agar disk was recuperated and placed in potato dextrose agar (PDA) plates covered by 3 mL of top agar containing 100 μL of spore suspension at 10^4 spores/mL of *Fusarium* sp., then incubated at 30 °C.

For antimicrobial activity determination of the concentrated supernatant culture of the TN82 strain, indicator bacteria were grown overnight in LB medium at 30 °C for *M. luteus* LB 14110, *A. tumefaciens* ATCC 33970, *P. aeruginosa* ATCC 49189 and *L. monocytogenes* ATCC 19117 and at 37 °C for *S. aureus* ATCC 6538, *S. typhimurium* ATCC 14028 and *E. coli* ATCC 8739, then diluted 1:100 in LB medium and incubated for 5 h under constant agitation on 200 rpm at the appropriate temperature.

Antibacterial activity was determined by the agar diffusion method (Elleuch et al. 2010): a paper disk (8 mm Ø) was impregnated with 50 µL of the concentrated supernatant culture of the TN82 strain and then laid on the surface of an agar plate containing 3 mL of top agar in-seeded with 40 µL of a 5-h-old culture of the corresponding indicator bacterium. After 2 h at 4 °C, plates were incubated at the appropriate temperature of the indicator microorganism. Concerning antifungal activity against *Fusarium* sp., this fungus was grown on PDA medium for 7 days at 30 °C, spores were collected in sterile distilled water and then adjusted to a spore density of 10^4 spores/mL. A total of 100 µL of spore suspension were added to 3 mL of top agar and after 2 h at 4 °C, plates were incubated at 30 °C. Antagonistic activity against *P. ultimum* and *R. solani* was evaluated by dual culture assay (Gopalakrishnan et al. 2011): A one day old 5 mm agar disk containing grown mycelium of *P. ultimum* or *R. solani* was placed on the center of PDA plates and the TN82 strain was streaked in both sides on the edge of the plate (1 cm from the corner). After 3 days of incubation at 27 °C, the inhibition zone was measured.

Minimal inhibitory concentrations (MICs) of the two pure compounds **1** and **2** against *S. aureus* ATCC 6538, *L. monocytogenes* ATCC 19117 and *S. Typhimurium* ATCC 14028 were determined in Mueller – Hinton broth. The test was performed in sterile 96-well microplates with a final volume in each microplate well of 100 µL. A stock solution of each compound was two-fold serially diluted with dimethyl sulfoxide (DMSO). To each test well, cell suspension was added to final inoculum concentration of 10^6 CFU/mL of indicator bacterium (Lian and Zhang 2013). The plates were then incubated at appropriate growth conditions of the corresponding indicator bacterium. Ampicillin and kanamycin were used as standards. The MIC was defined as the lowest concentration of the pure compounds and standards at which the microorganism does not demonstrate visible growth after incubation. Twenty five microlitre of Thiazolyl Blue Tetrazolium Bromide (MTT) at 0.5 mg/mL were added to the wells and incubated at room temperature for 30 min. The colourless tetrazolium salt acts as an electron acceptor and was reduced to a red-coloured formazan product by the indicator microorganisms. When microbial growth was inhibited, the solution in the well remained clear after incubation with MTT.

DNA sequencing and analysis

DNA isolation and manipulation and PCR amplification of TN82 16S rRNA gene were performed as described by (Elleuch et al. 2010). Nucleotide sequence of the 16S rRNA gene of TN82 strain 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 (1434 bp) of TN82 strain has been assigned GenBank (EMBL) under accession number LT608133. 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 algorithm (Felsenstein 1989) using Kimura 2-parameter distance. The robustness of the inferred tree was evaluated by bootstrap (100 replications).

Fermentation, working up and isolation of the bioactive compounds

Spores at 10^7/mL of *Streptomyces* strain TN82 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 on an orbital incubator with shaking at 200 rpm; the pre-culture was used to inoculate 15 L of TSB medium. For this realisation, we have used three fermenters having each one a total capacity of 7 L (working capacity of 5 L). After 3 days fermentation at 30 °C, culture broth was subjected to filtration over celite using a filter press. The filtrate was exhaustively extracted by ethyl acetate (2×) and the obtained organic extract was concentrated in vacuo to dryness, affording 3.0 g of a brown crude extract.

The strain crude extract (3.0 g) was subjected to fractionation on silica gel column chromatography eluted with cyclohexane-DCM-MeOH gradients. After TLC monitoring and visualisation by UV (254 and 366 nm), staining with anisaldehyde/sulphuric acid and heating, four fractions have been yielded: Fraction I (0.5 g), fraction II (0.8 g), fraction III (0.6 g), and fraction IV (0.7 g). The fast fraction I was mainly containing a mixture of fats, Niax and phthalate and discarded. Purification of fraction II using two Sephadex LH-20 columns (DCM/40% MeOH) afforded colourless solid of cis-cyclo (Leu, Pro) (**3**, 6 mg) and a faint yellow solid of anthranilic acid (**4**, 5 mg). An application of fraction III to a further sub-fractionation on silica gel column eluted with DCM-MeOH gradient followed by PTLC (DCM/7% MeOH) and then Sephadex LH-20 (MeOH) led to a colourless solid of 3-phenylpyrazin-2(1H)-one (**1**, 2 mg) and indole-3-carbaldehyde as faint yellow solid (**5**, 3 mg). A final purification of the polar fraction IV on Sephadex LH-20 (MeOH), then PTLC (DCM/7% MeOH), followed by Sephadex LH-20 (MeOH) delivered 3-O-methylviridicatin (**2**, 5 mg) and *p*-hydroxy-benzoic acid (**6**, 6 mg) as two colourless solids.

Results and discussion

Isolation, selection and biological activity of TN82 strain

A new actinomycete strain called TN82 was isolated from Sahara soil of Tunisia and selected, in solid medium, due its important biological activity against the three used indicator microorganisms *M. luteus* LB 14110, *E. coli* ATCC 8739 and *Fusarium* sp. The concentrated supernatant culture of the TN82 strain exhibits strong inhibitory activity against all tested bacteria, *M. luteus* LB 14110, *S. aureus* ATCC 6538, *L. monocytogenes* ATCC 19117, *A. tumefaciens* ATCC 33970, *P. aeruginosa* ATCC 49189, *S. typhimurium* ATCC 14028 and *E. coli* ATCC 8739 and the fungus *Fusarium* sp., (Table 1). In addition and according to dual culture assay, TN82 strain shows an important antagonistic activity against *P. ultimum* and *R. solani* (Table 1). Obtained results indicate that TN82 strain secretes active metabolites able to control the tested plant pathogens (*A. tumefaciens*, *Fusarium* sp., *P. ultimum* and *R. solani*) and human pathogens (*S. aureus*, *L. monocytogenes* and *S. typhimurium*).

A. tumefaciens is the causal agent of crown gall disease on various plant species (Escobar and Abhaya 2003). *Fusarium* sp. is the most important pathogens that cause root and stem rots in cucurbit plants (Boughalleb et al. 2005). *P. ultimum* it causes the damping-off and root rot diseases of hundreds of diverse plant hosts including corn, soybean, potato, wheat, fir, and many ornamental species (Farr and Rossman 2014). *R. solani* is known to attacks 188 species of higher plants in 32 families, including various staple crops, ornamentals and turf grasses (Anderson 1982).

S. aureus is both a commensal and an extremely versatile pathogen in humans, causing three basic syndromes: (i) superficial lesions such as skin abscesses and wound infections; (ii) deep-seated and systemic infections such as osteomyelitis, endocarditis, pneumonia, and bacteremia; and (iii) toxemic syndromes such as toxic shock syndrome and staphylococcal scarlet fever (Jarraud et al. 2002). *L. monocytogenes*, a Gram-positive facultative intracellular bacterium causes invasive diseases in humans, especially central nervous system infections (Vázquez–Boland et al. 2001). *S. typhimurium* the causative agent of typhoid fever, is a major public health threat and an estimated 26.9 million cases resulting in approximately 217,000 deaths occur annually by this pathogenic bacterium (Crump et al. 2004).

Identification and phylogenetic determination of TN82 strain

Strain TN82 grew very well on yeast malt agar medium and the colours of vegetative and aerial mycelia were whitish

Table 1 Antimicrobial activity of concentrated supernatant culture of *Streptomyces* sp. TN82 strain against used indicator microorganisms

| Test microorganism	Diameter of inhibition zone (mm)
A. tumefaciens ATCC 33970	40
M. luteus LB14110	50
E. coli ATCC8739	29
S. typhimurium ATCC 14028	24
S. aureus ATCC6538	25
L. monocytogenes ATCC 19117	23
P. aeruginosa ATCC 49189	21
Fusarium sp.	40
P. ultimum	33
R.solani	32

For each indicator microorganism, the experiment was carried out simultaneously three times under same conditions. The obtained diameters of inhibition zones were quite similar and the reported inhibition zones (mm) are the average of the three experiments

and greyish respectively. This strain grew well on Bennett and nutrient agar media and the colours of vegetative and aerial mycelia were respectively clear red and whitish for Bennett medium and yellowish and whitish for nutrient medium. The spore chains were abundant and greyish on yeast malt agar medium. Comparison of these characteristics with those of actinomycete species described in Bergey's manual of systematic bacteriology (Lechevalier et al. 1989) suggested that TN82 strain belongs to the genus *Streptomyces*. Total nucleotide sequence of 1434 bp (accession no. LT608133), of the whole 16S rRNA gene of the TN82 strain was determined in both strands. The alignment of this sequence through matching with reported 16S rRNA gene sequences in gene bank showed high similarity (97–99 %) to the *Streptomyces* 16S rRNA genes. The microorganism is mostly similar to the new isolate TN82 strain was *Streptomyces werraensis* NBRC13404[T] (NR11239). Based on the cultural characteristics of TN82 strain, the nucleotide sequence of the corresponding 16S rRNA gene and the phylogenetic analysis (Fig. 1), we propose the assignment of our new isolate bacterium as *Streptomyces* sp. TN82 strain.

Structural elucidation of the produced compounds

Compounds 1 and 2

3-Phenylpyrazin-2(1H)-one (**1**) $C_{10}H_8N_2O$ (172): Colourless solid, UV absorbing (254 nm), $R_f = 0.53$ (DCM/10% MeOH). ^1H NMR (500 MHz, CDCl$_3$), ^{13}C NMR (125 MHz, CDCl$_3$): See Table 2. ±ESI MS: *m/z* (%) 195 ([M + Na], 20), 367 ([M + Na], 49). (−)−ESI MS: *m/z* (%) 171 ([M-H]). ±HRESI MS: 195.05290 (calcd. 195.05289 for $C_{10}H_8N_2ONa$ [M + Na]). (−)−HRESI MS: 171.05528
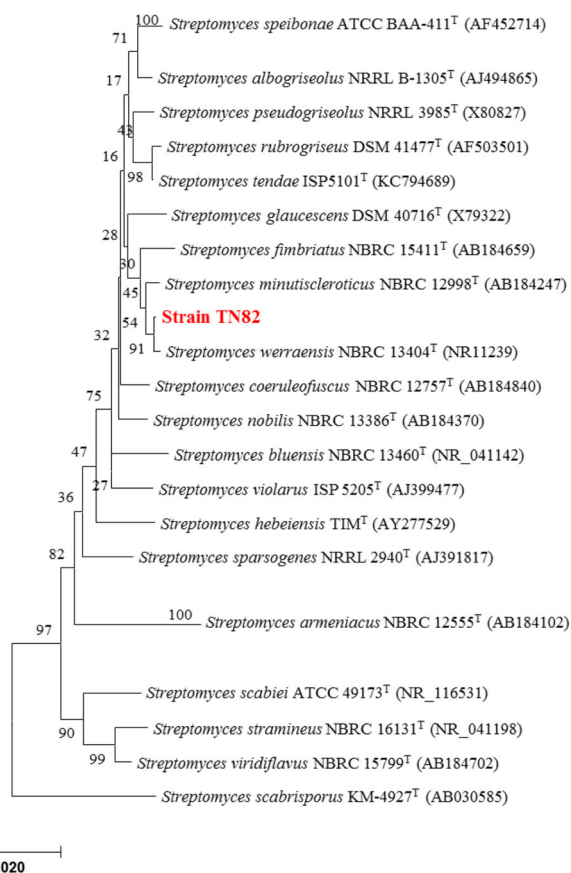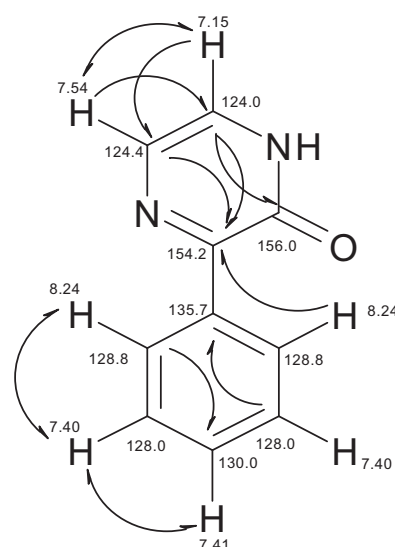
Fig. 1 Phylogenetic tree of *Streptomyces* sp.TN 82 strain

Table 2 ^{1}H and ^{13}C (125 MHz, CDCl$_3$) NMR data of 3-phenylpyrazin-2(1H)-one (**1**)

Nr.	δ_C	δ_H
2	156.0	
3	154.2	
5	124.4	7.54 (d, 4.0)
6	124.0	7.15 (d, 4.0)
7	135.7	
8, 12	128.8	8.24 (m)
9, 11	128.0	7.40 (m)
10	130.0	7.41 (m)

Fig. 2 H,H COSY (▬, ↔) and HMBC (→) connectivities of 3-phenylpyrazin-2(1H)-one (**1**)

(calcd. 171.05530 for C$_{10}$H$_7$N$_2$O [M-H]). As middle polar colourless solid, compound **1** was isolated, displaying a strong UV absorbance, while, it showed no colour staining with anisaldehyde/sulphuric acid. The molecular weight of **1** was determined as 172 Dalton according to positive and negative modes of ESI MS; and the corresponding molecular formula was established as C$_{10}$H$_8$N$_2$O, bearing eight double bond equivalents (DBE).

The ^{1}H NMR/H,H COSY spectra (Fig. 2) of **1** were concentrated in the aromatic region, such that five multiplet protons being for a mono-substituted aromatic residue were displayed at δ 8.24 (2H) and 7.41-7.40 (3H). Additionally, two doublet signals each of 1H were shown at δ 7.54 and 7.15 with a coupling constant of J~4.0, pointing to their existence in a heterocyclic six-membered ring. Based on the ^{13}C/HMQC spectra (Table 2), eight carbon signals, representing ten carbons, were found among them seven sp^2 methine carbons were in the region of δ 130.0~124.0, and the remaining carbons (sp^2 quaternary) were at δ 156.0, 154.2 and 135.7.

According to HMBC connectivities (Fig. 2), H-8/H-12 protons (8.24, δ_C: 128.8) of the mono-substituted phenyl ring displayed a ^3J correlation at C-3 (δ_C: 154.2), while the H-9/H-11 protons displayed a ^3J connectivity at C-7 (δ_C: 135.7) confirming the direct attachment between C-7 of the phenyl residue with C-3. Alternatively, the two vicinal protons, H-5 (7.54, δ_C: 124.4) and H-6 (7.15, δ_C: 124.0) exhibited ^3J and ^4J correlations at C-3 (154.2), respectively, while H-6 solely showed a $^3J_{H-C}$ versus C-2 (156.0). According to the revealed molecular formula, DBE and 2D NMR spectra, structure **1** was definitely assigned as 3-phenylpyrazin-2(1H)-one; 2-hydroxy-3-phenyl-pyrazine.
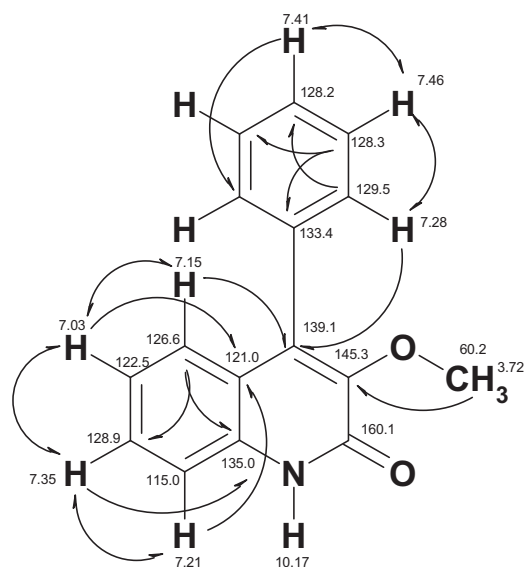
A search in different Databases AntiBase (Laatsch 2014) Dictionary of Natural Products [DNP] (Chapman and Hall 2016) and Scifinder (https://scifinder.cas.org/scifinder) confirmed that it is the first time that compound **1** is produced naturally. However, 2-hydroxy-3-Phenyl pyrazine was reported as degradative product from the antibiotic cephalexin when is exposed to some specific bacterial strains, e.g., *Pseudomonas* and *Shewanella* strains (Lin et al. 2015; Liu et al. 2016). This natural production involves that the biosynthetic pathway of this bioactive molecule is functionally and not cryptic in *Streptomyces* sp.

Table 3 ^{1}H and ^{13}C (125 MHz, CDCl$_3$) NMR data of 3-O-methylviridicatin (2)

Nr.	δ_C	δ_H
1-NH		10.17 (s)
2	160.1	
3	145.3	
3-OCH$_3$	60.2	3.72 (s)
4	139.1	
4a	121.0	
5	126.6	7.15 (dd, 8.1, 1.4)
6	122.5	7.03 (ddd, 8.1, 7.1, 1.2)
7	128.9	7.35 (ddd, 8.4, 7.1, 1.4)
8	115.0	7.21 (dd, 8.1, 1.4)
8a	135.0	
1′	133.4	
2′, 6′	129.5	7.28 (m)
3′, 5′	128.3	7.46 (dd, 7.0, 1.2)
4′	128.2	7.41 (m)

Fig. 3 H,H COSY (■, ↔) and HMBC (→) connectivities of 3-O-methylviridicatin (2)

TN82 strain. The structural elucidation and analysis of genes cluster of compound **1** from this new isolated *Streptomyces* bacterium, will constitute a real contribution for the understanding of its natural biosynthesis phenomena and will permit subsequently, the production of new hybrid derivatives with interesting biological proprieties.

3-O-Methylviridicatin (**2**) C$_{16}$H$_{13}$NO$_2$ (251): Colourless solid, UV absorbing (254 nm), $R_f = 0.48$ (DCM/10% MeOH). ^{1}H NMR (500 MHz, CDCl$_3$), ^{13}C NMR (125 MHz, CDCl$_3$): See Table 3. ±ESI MS: m/z (%) 252 ([M + H], 25), 274 ([M + Na], 100), 525 ([2M + Na], 50). (−) −ESI MS: m/z (%) 250 ([M−H]). ±HRESI MS: 252.1021 (calcd. 252.1019 for C$_{16}$H$_{14}$NO$_2$ [M + H]), 274.0842 (calcd. 274.0838 for C$_{16}$H$_{13}$NNaO$_2$ [M + Na]). (−) −HRESI MS: 250.0873 (calcd. 250.0874 for C$_{16}$H$_{12}$NO$_2$ [M−H]). As colourless solid, compound **2** was obtained displaying a strong UV absorbance, but no colour staining with anisaldehyde/sulphuric acid was observed. The molecular weight of **2** was determined as 251 Dalton; and the corresponding molecular formula as C$_{16}$H$_{13}$NO$_2$ according to HR-ESIMS data, bearing eleven DBE. The ^{1}H NMR/H,H COSY spectra of **2** (Fig. 3) displayed an amide proton signal at δ 10.17, nine aromatic proton signals (δ 7.46~7.03), arising the presence of 1,2-disubstituted aromatic ring fused with a heterocyclic residue; along with a mono-substituted phenyl group. In the aliphatic zone, a methoxy signal was observed at δ 3.72.

Based on ^{13}C and HMQC spectra (Table 3), fourteen carbon signals were visible, which are classified into five sp^2 quaternary carbons (160.0~121.0), among them an amide carbonyl (160.1), and one oxygenated carbon (145.3), nine sp^2 methines (129.5~115.0), and one methoxy (60.2). A further study of structure **2** was carried out using long range correlations of 2D NMR (H,H COSY and HMBC, Fig. 3) confirming it as 3-O-methylviridicatin. This compound was previously isolated from *Penicillium* spp. (Austin and Meyers 1964; Heguy et al. 1998) and in our knowledge, is reported herein to first time as bacterial metabolite so far. 3-O-methylviridicatin was described as blocking agent of cytokine tumour necrosis factor alpha activation of the HIV LTR expression in the HeLa-based system, with an IC$_{50}$ of 5 μM, and inhibited virus production in a cellular model of chronic infection responsive to induction by TNF-α in the OM-10.1 cell line with an IC$_{50}$ of 2.5 μM (Heguy et al. 1998).

Known compounds (3–6)

Structures of the well known metabolites, cis-cyclo (Leucyl-Prolyl) (**3**) (Elleuch et al. 2010; Ben ameur-Mehdi et al. 2006; Rhee 2002), anthranilic acid (**4**) (Shaaban 2004), indole-3-carbaldehyde (**5**) (Shaaban et al. 2008) and *p*-hydroxy-benzoic acid (**6**) (Pugazhendhi et al. 2005; Kamaya et al. 2006) were deduced by different spectroscopic means and by comparison with the corresponding literatures.

Cyclo-(Leu, Pro) (**3**) C$_{11}$H$_{18}$N$_2$O$_2$ (210.27): Colourless solid, UV absorbing turned to violet by anisaldehyde/sulphuric acid. The $R_f = 0.58$ (CHCl$_3$/MeOH 95:5). ^{1}H NMR (CDCl$_3$, 300 MHz): δ (ppm) = 6.39 (brs, 1 H, NH), 4.15

(t, $J = 8.1$ Hz, 1 H, 9-H), 4.04 (dd, $J = 10.2$, 6.2 Hz, 1 H, 6-H), 3.64–3.54 (m, 2 H, 3-CH$_2$, 10-Hb), 2.37 (m, 1 H, 10-Ha), 2.22-1.98 (m, 3 H, 4-H$_2$, 5-Ha), 1.75 (m, 1 H, 5-Hb), 1.55 (m, 1 H, 11-H), 1.03 (d, $J = 8.1$ Hz, 3 H, 12-CH$_3$), 0.96 (d, $J = 8.1$ Hz, 3 H, 13-CH$_3$). ^{13}C/APT NMR (CDCl$_3$, 75,487 MHz): $\delta = 170.3$ (Cq-1), 166.1 (Cq-7), 59.0 (CH-6), 53.4 (CH-9), 45.5 (CH$_2$-3), 38.6 (CH$_2$-5), 28.1 (CH$_2$-10), 24.7 (CH-11), 23.3 (CH$_3$-12), 22.7 (CH$_2$-4), 21.2 (CH$_3$-13). CI MS (NH$_3$): m/z (%) = 438 ([2M + NH$_4$]$^+$, 7), 421 ([2M + H]$^+$, 4), 245 ([M + NH$_3$ + NH$_4$]$^+$, 64), 228 ([M + NH$_4$]$^+$, 100), 211 ([M + H]$^+$, 40). EI/MS (70 eV): m/z (%) = 195 (M–CH3, 16), 154 (100), 125 (14), 86 (36), 70 (65), 41 (12).

Anthranilic acid (**4**) C$_7$H$_8$N$_2$O$_2$ (137.136): Yellow solid, UV blue fluorescent substance, coloured to yellow by anisaldehyde/sulphuric acid and/or Ehrlich's reagent. The $R_f = 0.22$ (CHCl$_3$ /MeOH 95:5). ^{1}HNMR ([D$_6$]DMSO, 300 MHz): $\delta = 7.68$ (dd, $^3J = 8.0$ Hz, $^4J = 1.0$, 1 H, 6-H), 7.18 (td, $^3J = 8.0$ Hz, $^4J = 1.0$, 1 H, 4-H), 6.68 (dd, $^3J = 8.0$ Hz, $^4J = 1.1$ Hz, 1H, 3-H), 6.56 (td, $^3J = 8.2$ Hz, $^4J = 1.1$ Hz 1 H, 5-H). − EI MS (70 eV): m/z (%) = 137 ([M]$^+$, 72), 119 ([M-NH2]$^+$, 100), 92 (52), 65 (17).

Indole-3-carbaldehyde (**5**) C$_9$H$_7$NO (145.16). Light yellow powder. ^{1}H NMR (600 MHz, DMSO-d$_6$, δ, ppm, J/Hz): 12.1 (1H, br.s, NH), 9.91 (1H, s, 3-CHO), 8.26 (1H, d, $J = 2.3$, H-2), 8.07 (1H, d, $J = 7.6$, H-4), 7.19 (1H, dd, $J = 7.8$, 7.6, H-5), 7.24 (1H, dd, $J = 8.0$, 7.8, H-6), 7.49 (1H, d, J = 8.0, H-7). ^{13}C NMR (150 MHz, DMSO-d$_6$, δ, ppm): 185.5 (3-CHO), 139.4 (C-2), 118.4 (C-3), 124.7 (C-3a), 121.5 (C-4), 123.1 (C-5), 124.4 (C-6), 113.2 (C-7), 137.6 (C-7a). EI-MS (m/z, I_{rel}, %): 146 (100), 116 (45), 77 (17).

p-hydroxybenzoic acid (**6**) C7H6O3 (138.122). White. ^{1}H NMR (300 MHs, DMSO-d6, TMS), δ_H : 7.73 (2H, d, $j = 7.95$) Hz, H-2. 6), 6.71 (2H, d, $J = 7.95$ Hz, H-3, 5). ^{13}C NMR (75.5 MHz, DMSO-d$_6$, TM) δ_C : 169.4 (-COOH), 159.9 (C-4), 131.1(C-2,6), 127.5 (C-1), 114.4(C-3. 5). EI-MS m/z: 138 (72), 121 (100), 105 (7.3), 93 (24.6), 79 (22.1).

MIC of compounds 1 and 2

The biological activity of the two compounds, 3-phenylpyrazin-2(1H)-one (**1**) and 3-O-methylviridicatin (**2**) was estimated as MIC against *S. aureus*, *L. monocytogenes* and *S. typhimurium*. As shown in Table 4, MICs of these compounds against the three tested human pathogenic bacteria were significantly lower than those of kanamycin. For the natural compound 3-phenylpyrazin-2(1H)-one (**1**), MICs values were very interesting especially that against *L. monocytogenes* (1.0 μg/mL) which is two times lower than that of ampicillin (2.0 μg/mL). The compound **2** showed no apparent toxic effects in mice even at high doses (Hodge et al. 1988). In accordance, 3-O-methylviridicatin is a very

Table 4 Determination of minimum inhibitory concentrations (MICs) expressed in μg/mL of compounds phenylpyrazin-2(1H)-one (1) and 3-O-methylviridicatin (2)

Test microorganism	Compound			
	1	**2**	Ampicillin	Kanamycin
S. aureus ATCC 6538	4.0	4.50	0.25	6.25
L. monocytogenes ATCC 19117	1.0	9.0	2.0	12.50
S. typhimurium ATCC 14028	2.0	4.50	4.0	12.50

promising compound capable of inhibiting replication of the human immunodeficiency virus induced by tumour necrosis or as promising lead compounds for the development of new anti-inflammatory agents (Paterna et al. 2013).

Conclusions

A new *Streptomyces* sp. TN82 strain was isolated from Sahara soil of Tunisia and selected due to its potent antimicrobial activity. The concentrated supernatant culture of the TN82 strain exhibits strong inhibitory activity against human and plant pathogens. According to the cultural characteristics, analysis of the nucleotide sequence of the whole 16S rRNA gene (accession no. LT608133) and the phylogenetic study, this new bacterium has been assigned as *Streptomyces* sp. TN82 strain. Working up of extract of 15 L liquid culture of the TN82 strain lead to the isolation of six bioactive compounds: 3-phenylpyrazin-2(1H)-one (**1**) described the first time in this work as natural product and 3-O-methylviridicatin (**2**), reported herein to first time as bacterial metabolite, and four known metabolites; cyclo-(Leu, Pro) (**3**), anthranilic acid (**4**), indole-3- carbaldehyde (**5**) and p-hydroxybenzoic acid (**6**). Structures of the isolated compounds were determined by an intensive study of their chromatographic properties and spectroscopic means. MICs of compounds **1** and **2** against the three tested human pathogenic bacteria (*S. aureus*, *L. monocytogenes* and *S. typhimurium*) were significantly lower than those of kanamycin. Concerning compound 3-phenylpyrazin-2(1H)-one (**1**), MICs values were very interesting especially that against *L. monocytogenes* (1.0 μg/mL), which is two times lower than that of ampicillin (2.0 μg/mL).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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