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Original article

Antimicrobial activities of novel bipyridine compounds produced by a new strain of *Saccharothrix* isolated from Saharan soil

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ABSTRACT

The actinobacterium strain ABH26 closely related to *Saccharothrix xinjiangensis*, isolated from an Algerian Saharan soil sample, exhibited highly antagonist activity against Gram-positive bacteria, yeasts and filamentous fungi. Its ability to produce antimicrobial compounds was investigated using several solid culture media. The highest antimicrobial activity was obtained on Bennett medium. The antibiotics secreted by strain ABH26 on Bennett medium were extracted by methanol and purified by reverse-phase HPLC using a C18 column. The chemical structures of the compounds were determined after spectroscopic (^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMBC spectra), and spectrometric (mass spectrum) analyses. Two new cyanogriside antibiotics named cyanogriside I (1) and cyanogriside J (2), were characterized along with three known caerulomycins, caerulomycin A (3), caerulomycin F (4) and caerulomycinonitrile (5). This is the first report of cyanogrisides and caerulomycins production by a member of the *Saccharothrix* genus. The minimum inhibitory concentrations (MIC) of these antibiotics were determined against pathogenic microorganisms.

1. Introduction

Cyanogrisides and caerulomycins are bioactive secondary metabolites containing a 2,2′-bipyridine skeleton and produced by two mycelial actinobacteria species, *Streptomyces caeruleus* (Funk and Divekar, 1959) and *Actinoalloteichus cyanogriseus* (Fu et al., 2011). Cyanogrisides differ to caerulomycins by the presence of one or more sugars bonded to the bipyridine skeleton. At the time of writing, 9 cyanogrisides and 13 caerulomycins have been isolated and characterized. These compounds have been shown to have antibacterial, antifungal, antiparasitic and antitumor activities (Funk and Divekar, 1959; Chatterjee et al., 1984; Ambavane et al., 2014; Kujur et al., 2015).

Actinobacteria are Gram-positive bacteria with high (≥55%) guanine and cytosine content in their genomes. This group of microorganisms represents one of the most important sources of natural bioactive compounds. Berdy (2012) estimated that 62% of known bioactive microbial compounds are originally from actinobacteria. *Saccharothrix* spp. are relatively rare actinobacteria known to produce several antibiotics with different structures and biological activities, such as the antibacterial agent tianchimycins from *Saccharothrix xinjiangensis* NRRL B-24321 (Wang et al., 2013) and the antiviral agent fluvirucin from *Saccharothrix mutabilis* R516-16 (Naruse et al., 1991).

The arid soils of the Algerian Sahara have been shown to be rich in members of the *Saccharothrix* genus (Sabaou et al., 1998; Zitouni et al., 2005) including new species, such as *Saccharothrix algeriensis* NRRL B-24137^{T} (Zitouni et al., 2004a), *Saccharothrix hoggarensis* DSM 45457^{T}, *Saccharothrix saharensis* DSM 45456^{T}, *Saccharothrix tamanrassetensis* DSM 45947^{T} (Boubetra et al., 2013a, 2013b, 2015), *Saccharothrix isguenensis* DSM 46885^{T} and *Saccharothrix*

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ghardaiensis DSM 46886^T (Bouznada et al., 2016, 2017). In addition, many studies have shown the ability of *Saccharothrix* strains isolated from these soils to produce new or known antibiotics, such as dithiolopyrrolones (Lamari et al., 2002; Bouras et al., 2008; Merrouche et al., 2011), anthracyclines (Zitouni et al., 2004b) and chloramphenicol (Aouiche at al., 2012). These promising results emphasise the need to continue the research into *Saccharothrix* antimicrobial production.

In Algeria, we isolated a mycelial actinobacterium strain with strong antibacterial and antifungal activities designated as ABH26. Based on morphological, physiological and molecular characterization the strain was found to be related to *Saccharothrix xinjiangensis* (Lahoum et al., 2015). Initially, this strain had antimicrobial activity on solid and liquid ISP (International *Streptomyces* Project) 2 culture medium (Shirling and Gottlieb, 1966). However, the strain has subsequently lost its activity in liquid medium while this activity was conserved on solid medium (data not shown).

The aim of this study was to characterize the bioactive compounds secreted by strain ABH26 on various solid culture media, and to test the antimicrobial activity of these molecules against some representative pathogenic microorganisms.

2. Materials and methods

2.1. Microbial strains

The actinobacterial strain ABH26 was isolated from a Saharan soil sample collected in the Adrar region (Southern Algeria). It was found to be most closely related to *Saccharothrix xinjiangensis* (Lahoum et al., 2015). The target microorganisms used for antimicrobial testing are from our laboratory collection and are listed in Table 1. This list includes Gram-positive and Gram-negative bacteria, yeasts and filamentous fungi, most of which are pathogens for humans or plants.

Table 1
Antimicrobial activity of the strain ABH26 on Bennett, ISP2, NA and ISP1 culture media.

Target microorganism	Distance of inhibition (mm)			
	Bennett	ISP2	NA	ISP1
Aspergillus brasiliensis AB1	25	24	18	16
A. carbonarius AC1	35	33	19	15
A. carbonarius AC2	28	22	16	15
A. flavus NRRL 3251	23	21	17	17
A. parasiticus CBS 100,926	22	17	16	15
A. westerdijkiae ATCC 3174	22	20	17	13
Fusarium culmorum FC1	31	28	19	16
F. equiseti FE1	25	24	16	14
F. graminearum FG1	27	24	19	15
F. oxysporum f. sp. *albedinis* Foa1	27	27	17	18
F. o. f. sp. *radicis-lycopersici* Forl	23	22	17	17
F. solani Fsol	28	27	18	17
F. sporotrichioides FS1	27	26	18	15
Rhizoctonia solani AG3	31	28	20	18
Umbelopsis ramanniana NRRL 1829	38	32	21	19
Candida albicans M2	25	22	12	4
C. albicans M3	31	30	15	12
C. albicans IPA200	24	23	13	5
Saccharomyces cerevisiae ATCC 4226	34	32	16	14
Bacillus subtilis ATCC 6633	22	20	19	19
Corynebacterium diphtheriae CD1	28	31	22	20
Enterococcus faecalis EF1	21	25	18	17
Listeria monocytogenes ATCC 13,932	27	31	21	19
Micrococcus luteus ATCC 9314	30	33	18	18
Staphylococcus aureus ATCC 29,213	21	24	16	15
S. aureus ATCC 43,300	19	20	14	11
S. aureus MRSA 639c	13	15	13	12
Escherichia coli E52	0	0	0	0
E. coli E195	0	0	0	0
Klebsiella pneumoniae E40	0	0	0	0
Pseudomonas aeruginosa IPA1	0	0	0	0

2.2. Antimicrobial activity of strain ABH26 on solid culture media

Antimicrobial activity was evaluated on ISP1 (tryptone-yeast extract agar), ISP2 (yeast extract-malt extract agar) (Shirling and Gottlieb, 1966), NA (nutrient agar) and Bennett (Waksman, 1961) media by the cross streak method. The actinobacterial strain was inoculated in a straight line on solid culture media and then incubated for 10 days at 30 °C. After the incubation period, target microorganisms were seeded in perpendicular streaks that cross the ABH26 streak. After further incubation at 30 °C for 24 h for bacteria and yeasts, and at 25 °C for 48 h for filamentous fungi, the antimicrobial activity was determined by measuring distance of inhibition between target microorganisms and the actinobacterial strain.

2.3. Kinetics of bioactive compound production on solid culture media

The spores of strain ABH26 were dislodged from the pre-culture media with a sterile loop and transferred to 10 ml of sterile distilled water. Spores were counted using a counting chamber and a 10^6 spores/ml solution was prepared. In Petri dishes (170 mm in diameter) filled with ISP1, ISP2, NA, or Bennett media, 1 ml of spore suspension was spread on the surface of each medium. The plates were incubated for 12 days at 30 °C. Agar plugs (10 mm in diameter) were regularly taken each day and used to determine antagonistic activity against *Bacillus subtilis* ATCC 6633, *Candida albicans* M3 and *Umbelopsis ramanniana* NRRL 1829. All the experiments were performed in duplicate.

2.4. Extraction and purification of antimicrobial products

Strain ABH26 was cultured on ISP1, ISP2, NA and Bennett solid media (150 ml of each medium in 170 mm diameter Petri dishes) as described above for 7 days at 30 °C. At the end of the incubation period, the medium was cut into small pieces and extracted with 100 ml of methanol on a magnetic stirrer set at 250 rpm for 2 h. The extracts were concentrated to dryness by a rotary evaporator under vacuum at 40 °C. The residues were dissolved in 1 ml of methanol. A 80 µl volume of each sample was injected into the HPLC system (Agilent 1260) using a reverse phase C18 column (200 × 10 mm; 5 µm) with a continuous linear gradient solvent system from 20 to 100% methanol in water. A total run time of 60 min was maintained at a flow rate of 1.2 ml/min under ambient temperature. The detection of products was carried out at 220 and 254 nm. The fractions corresponding to peaks were collected, concentrated then tested (6 mm paper disk diffusion method) against *Bacillus subtilis* ATCC 6633 and *Umbelopsis ramanniana* NRRL 1829 to detect the active fractions and distinguish them from the non-active fractions. Final purification of the antibiotics produced on Bennett medium was achieved after the second re-injection in the HPLC under the same conditions.

2.5. Spectroscopic and spectrometric analysis of antibiotics

These analyses were made with the pure bioactive compounds isolated from Bennett medium. The UV–visible spectra in methanol were determined with a Shimadzu UV 1605 spectrophotometer. The mass spectra were recorded on LCQ ion-trap mass spectrometer (Finnigan MAT, San Jose, CA) equipped with a nanospray ion electro-spray ionization source (positive and negative ion mode). ^{1}H and ^{13}C NMR spectroscopy were used for the final characterization of bioactive compounds. NMR samples were prepared by dissolving 3 mg of each compound in 600 µl of CD_3OD. All spectra

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were recorded on a Bruker Avance 500 spectrometer equipped with a 5 mm triple resonance inverse Z-gradient probe (TBI 1H, 31P, BB). All chemical shifts for ^{1}H and ^{13}C are relative to TMS using ^{1}H (residual) or ^{13}C chemical shifts of the solvent as a secondary standard. The temperature was set at 298 K. All the ^{1}H and ^{13}C signals were assigned on the basis of chemical shifts, spin-spin coupling constants, splitting patterns and signal intensities, and by using ^{1}H-^{1}H COSY45, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC experiments. Gradient-enhanced ^{1}H COSY45 was performed and included 36 scans per increment. ^{1}H-^{13}C correlation spectra using a gradient-enhanced HSQC sequence (delay was optimised for 1JCH of 145 Hz) was obtained with 120 scans per increment. A gradient-enhanced HMBC experiment was performed allowing 62.5 ms for long-range coupling evolution (240 scans were accumulated). Typically, 2048 t2 data points were collected for 256 t1 increments.

2.6. Determination of minimum inhibitory concentrations

Minimum inhibitory concentrations (MIC) of pure antibiotics were determined using the conventional agar dilution method (Oki et al., 1990) on the target microorganisms listed in Table 1. The target strains were inoculated onto Mueller Hinton medium for bacteria and Sabouraud medium for fungi, containing different concentrations of each active compound (0.5, 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90 and 100 μg/ml). The same concentration of two standard antibiotics were used for comparison: nystatin (antifungal compound) and amoxicillin (antibacterial compound). After a growth period at 30 °C for 24 h for bacteria and yeasts and 48 h for filamentous fungi, the plates were examined for growth and the lowest antibiotic concentration that inhibited the growth of each organism was determined. Mueller Hinton and Sabouraud media inoculated with target organisms, but without active compounds were used as control treatments. All the experiments were performed in duplicate.

3. Results

3.1. Antimicrobial activity of strain ABH26 on solid media

The antimicrobial activity of strain ABH26 against various target microorganisms is shown in Table 1. The strain exhibited a strong ability to inhibit growth of Gram-positive bacteria, yeasts and filamentous fungi. However, this strain did not show any activity against Gram-negative bacteria. The highest antifungal activity was observed on Bennett medium, while the highest antibacterial activity was obtained on ISP2 medium. The strain showed less antimicrobial activity on ISP1 and NA media.

3.2. Kinetics of bioactive compound production on solid media

Antimicrobial activity was detected on all tested media, ISP1, ISP2, NA and Bennett (Fig. 1). The antibacterial activity started on the second or third day and reached a maximum after 7 or 8 days of incubation. The highest activity was observed in ISP2 medium then Bennett medium. The antifungal activity against *Umbelopsis ramanniana* started after 2 or 3 days of incubation and after 4 days for *Candida albicans*. Antifungal activity reached a maximum at 7 days on all tested media. The highest antifungal activity was observed in Bennett medium. Thus, Bennett medium was chosen as the production medium for purification of antimicrobial compounds.

3.3. Detection of antimicrobial compounds by HPLC

The HPLC profiles of methanolic extracts obtained after 7 days of incubation on ISP1, ISP2, NA and Bennett solid media are shown in Fig. 2. Five antimicrobial compounds (1–5) were collected from Bennett medium. The retention times of these compounds were 46.4 min (compound 1), 48.5 min (compound 2), 42.8 min (compound 3), 49.9 min (compound 4) and 54.3 min (compound 5). These compounds were also detected in the ISP2 methanolic extract. However, compounds 1–5 were not detected or produced in very small amounts in NA and ISP1 media. Compounds 1, 2, 3 and 5 were active only against Gram-positive bacteria. However, compound 4 showed antibacterial and antifungal activities. In addition of these five compounds, a hydrophilic unseparated complex (named X) was detected in all tested media. This complex has antibacterial and antifungal activity. The HPLC profiles from different days of incubation on Bennett medium (Fig. 3) showed that after 4 days the strain produced the five compounds. The major products were compounds 3, 4 and 5. After 7 days, the strain produced higher amounts of the five antimicrobial compounds. After 10 and 12 days, the amounts of the products were slightly reduced. We continued our study with products 1–5.

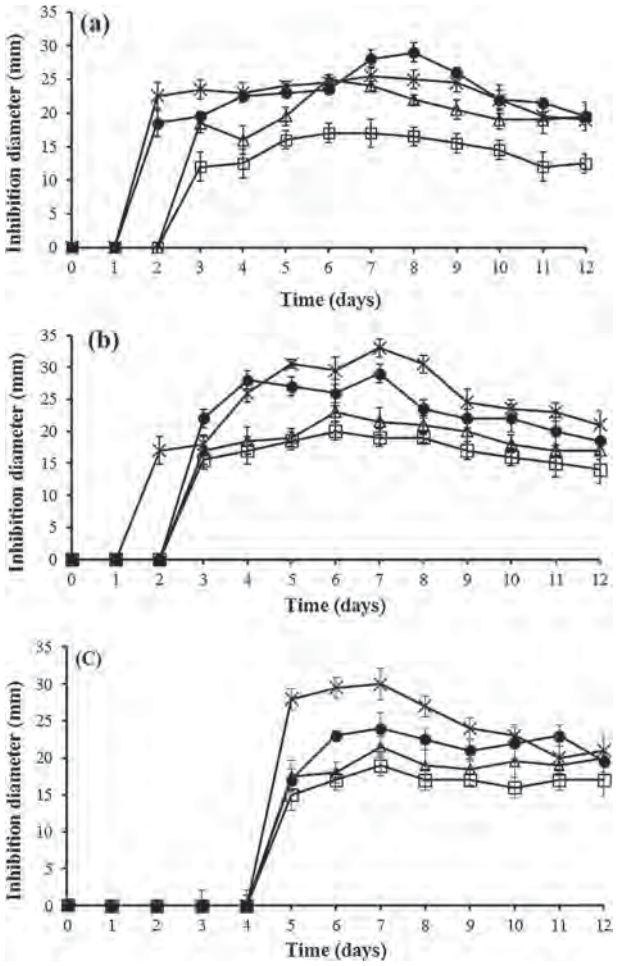

Fig. 1. Time course of antimicrobial activity of strain ABH26 on ISP1 (□), ISP2 (●), Bennett (×) and NA (△) solid culture media against *Bacillus subtilis* ATCC 6633 (a), *Umbelopsis ramanniana* NRRL 1829 (b) and *Candida albicans* M3 (c).

3.4. Spectroscopy and spectrometry analyses of antimicrobial products

Strain ABH26 was cultivated in 16 Petri dishes (170 mm in diameter), each containing 150 ml of Bennett solid medium at 30 °C for 7 days. The total volume of medium was 2.4 L.

Compound 1 was obtained as a light purple oil. The UV spectrum showed the maximal absorbance at 234 and 266 nm. The

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ESIMS spectrum contained an ion peak at m/z 579.09 for $[M-H]^+$. The ^1H and ^{13}C chemical shifts are given in Table 2. The ^{13}C and HSQC spectra show 27 carbon signals. From the ^{13}C data, it was possible to discern one amide group (δ_c 173.25), five hydroxyl groups (δ_c 61.0 to 74.56), one methoxy group (δ_c 54.90), 11 sp2–hybridized carbons (δ_c from 106.15 to 167.44) and 7 sp^3-hybridized

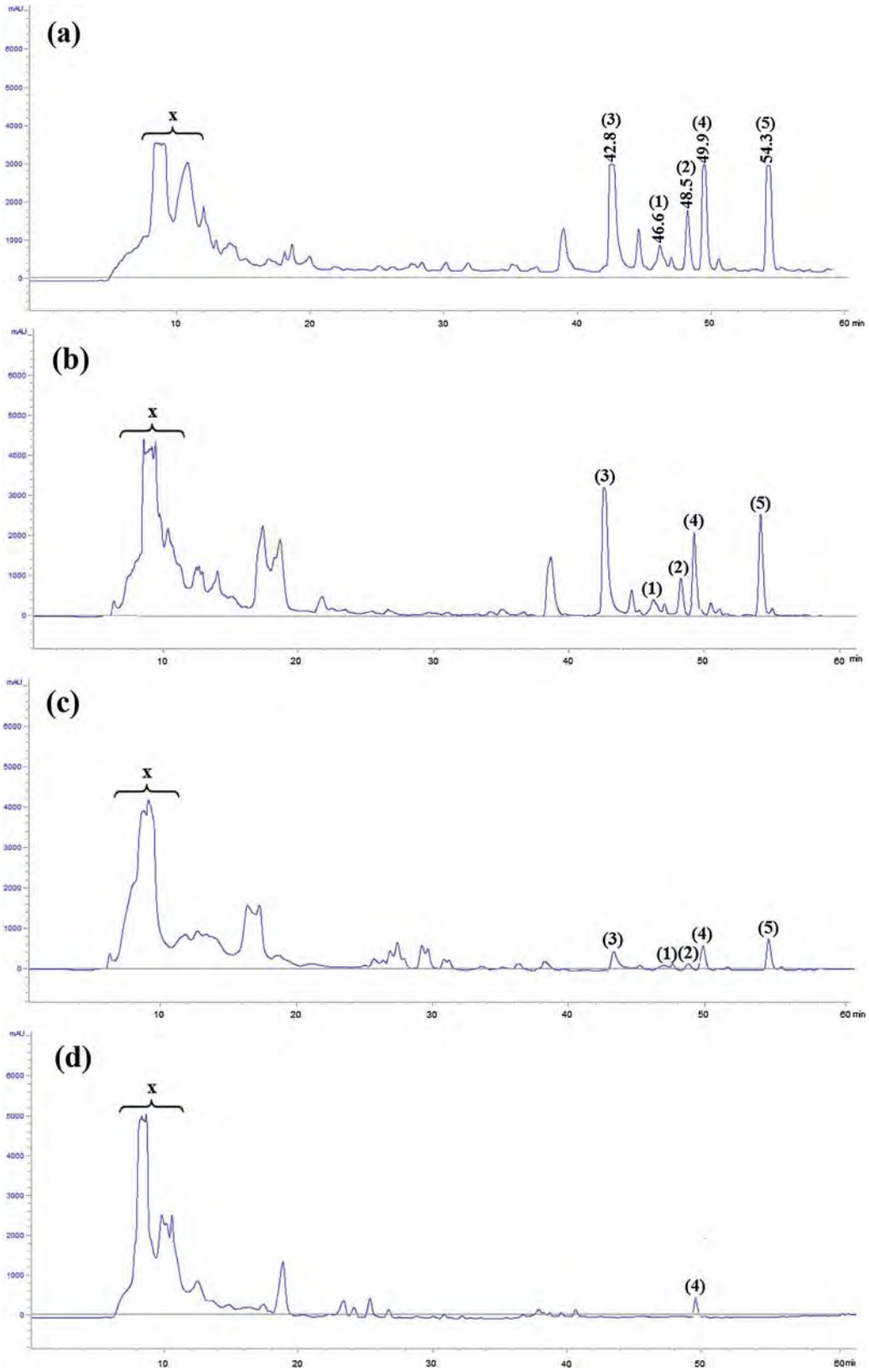

Fig. 2. HPLC analysis of methanolic extracts of strain ABH26 cultured on Bennett (a), ISP2 (b), NA (c) and ISP1 (d) solid culture media. Numbers in brackets indicate the antimicrobial compounds secreted by the strain. Retention times were also indicated in Bennett medium.

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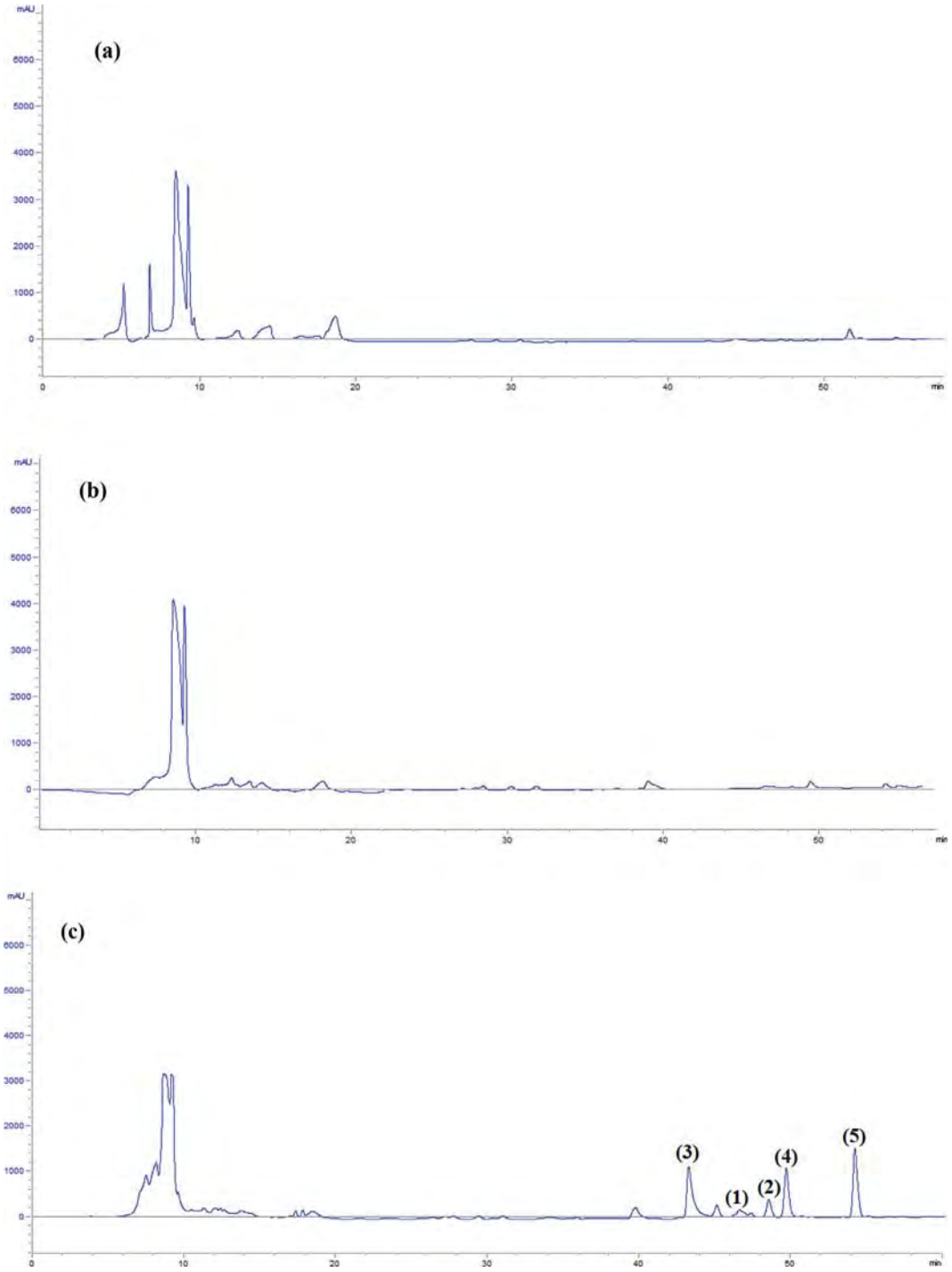

Fig. 3. HPLC analysis of methanolic extract of strain ABH26 cultured on Bennett medium for 0 day (a), 2 days (b), 4 days (c), 7 days (d), 10 days (e) and 12 days (f).

carbons (δ_c 16.63–102.74). The hydrogens of the hydroxyl and amide groups are not observed due to rapid exchange with CD_3OD. The 2D ^1H-^1H and ^1H-^{13}C experiments and especially the long range ^1H-^{13}C couplings observed in the HMBC spectrum permitted to establish the connectivity between all the groups of the compound. The HMBC and COSY correlations of compound 1 are shown in Fig. 4, and the structure in Fig. 5. The NMR data suggested that compound 1 represents a new antibiotic belonging to the family of cyanogrisides, for which the name cyanogriside I was proposed. This compound is a methoxy-bipyridine linked (through a cyano radical C═N) to two osidic derivatives, 2,4-dimethyl-α-L-mannose and N-acetylglucosamine. It differs from compound 4 by the presence of these sugars.

Compound 2 was also obtained as a light purple oil. The UV spectrum showed the maximal absorbance at 238 and 272 nm. The ESIMS spectrum contained an ion peak at *m*/*z* 376.11 for $[M\text{-}H]^+$. The ^1H and ^{13}C chemical shifts are given in Table 2. Eighteen carbon signals were present in the ^{13}C and HSQC spectra. From the ^{13}C data, it was possible to discern three hydroxyl groups (δ_c 69.27–72.00), one methoxy group (δ_c 54.93), 11 sp^2–hybridized carbons (δ_c from 105.83 to 167.30) and 3 sp^3-hybridized carbons (δ_c 16.32–102.79). Due to rapid exchange with CD_3OD the

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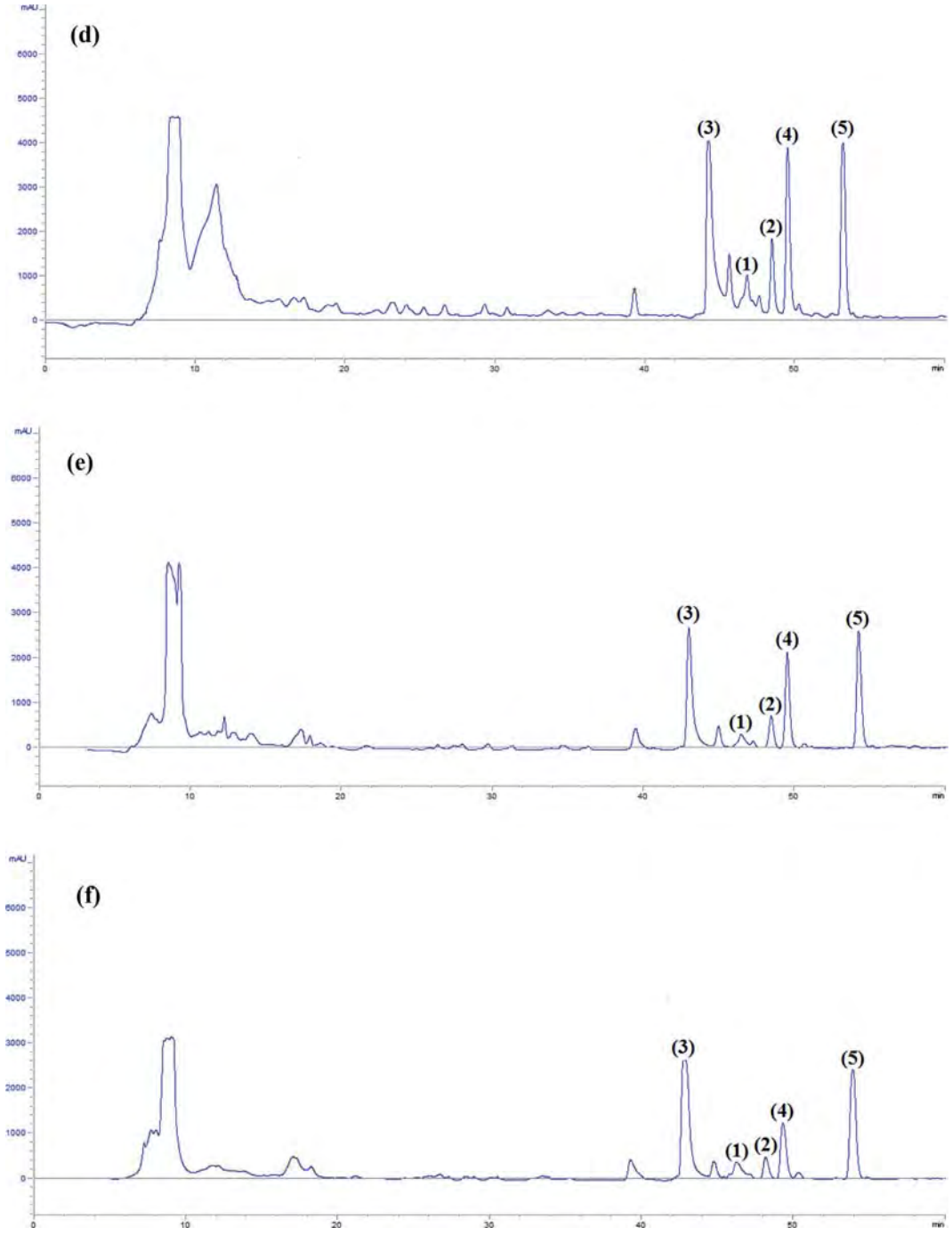

Fig. 3 (*continued*)

hydrogens of the hydroxyl group are not observed. The 2D ^{1}H-^{1}H and ^{1}H-^{13}C experiments and especially the long range ^{1}H-^{13}C couplings observed in the HMBC spectrum permitted to establish the connectivity between all the groups of the compound. The HMBC and COSY correlations of compound 2 are shown in Fig. 4, and the structure in Fig. 5. The NMR data suggested that compound 2 represents a new antibiotic belonging to the family of cyanogrisides, for which the name cyanogriside J was proposed. This compound is a methoxy-bipyridine linked (through a cyano radical C═N) to 2,4-dimethyl-α-L-mannose. It differs from the compound 4 by the presence of this sugar, and from the compound 1 by the absence of N-acetylglucosamine.

Compound 3 was obtained as white amorphous powder. The UV spectrum showed the maximal absorbance at 222 and 280 nm. The ESIMS spectrum contained an ion peak at *m*/*z* 217.16 for $[M + H]^{+}$. The NMR data (Table 3) led to identify compound 3 as caerulomycin F (Fu et al., 2011). The structure of compound 3 is shown in Fig. 4. This compound is a methoxy-bipyridine containing a hydroxymethyl group CH_2OH.

Compound 4 was obtained as white powder. The UV spectrum showed the maximal absorbance at 225, 245 and 283 nm. The ESIMS spectrum contained an ion peak at *m*/*z* 230.11 for $[M + H]^{+}$. The NMR data (Table 3) led to identify compound 4 as caerulomycin A (Funk and Divekar, 1959). The structure of compound

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Table 2
^{1}H and ^{13}C NMR data assignments of compounds 1 and 2 in CD_3OD at 298 K. See Figs. 4 and 5 for numbering of hydrogen and carbon atoms.

^{1}H and ^{13}C number	^{1}H chemical shift, ppm		^{13}C chemical shift, ppm	
	1	2	1	2
1	1.04	4.04	21.67	69.27
2	–	3.71	173.25	71.00
3	–	3.48	–	72.00
4	3.72	3.71	56.41	69.00
5	3.51	1.31	74.56	16.32
6	3.43	5.50	70.33	102.79
7	3.34	8.32	76.37	151.20
8	3.76–3.89	–	61.00	152.42
9	4.71	7.53	102.74	105.83
10	3.79	–	80.40	167.30
11	3.58	4.02	71.17	54.93
12	3.73	7.93	69.50	107.65
13	1.31	–	16.63	157.49
14	5.50	–	102.53	155.12
15	4.29	8.68	69.06	148.60
16	8.34	7.47	151.64	124.11
17	–	7.96	157.42	137.20
18	7.54	8.43	106.15	121.36
19	–	–	167.44	–
20	4.02	–	54.90	–
21	7.93	–	108.02	–
22	–	–	157.49	–
23	–	–	155.12	–
24	8.66	–	148.77	–
25	7.48	–	124.21	–
26	7.96	–	137.27	–
27	8.43	–	121.26	–

4 is shown in Fig. 4. This compound is a methoxy-bipyridine containing an oxime group CH═N—OH.

Compound 5 was obtained as a light purple oil. The UV spectrum showed the maximal absorbance at 222, 246 and 280 nm. The ESIMS spectrum contained an ion peak at *m*/*z* 212.23 for $[M+H]^+$. The NMR data (Table 3) led to identify compound 5 as caerulomycinonitrile (Fu et al., 2011). The structure of compound 5 is shown in Fig. 4. This compound is a methoxy-bipyridine linked to a nitrile group C≡N.

3.5. Minimum inhibitory concentrations of antimicrobial compounds

Minimum inhibitory concentrations of compounds 1–5 are summarized in Table 4. The most active product was compound 4. The MIC values of this compound were between 1 and 20 μg/ml for filamentous fungi, 2–10 μg/ml for yeasts and 3–50 μg/ml for Gram-positive bacteria. The strains of Gram-negative bacteria were resistant. The comparison of MIC values of compound 4 with those of the standard antibiotics used in this study indicated that the compound 4 showed a better activity than amoxicillin against the Gram positive bacteria *Micrococcus luteus* ATCC 9314, *S. aureus* ATCC 43,300 and *S. aureus* MRSA 639c, a same activity against four other Gram-positive bacteria, and a lower activity against all the Gram negative bacteria tested. For the antifungal activity, the compound 4 showed a better activity against nine filamentous fungi and yeasts by comparison with nystatin, a same activity against five fungi and a lower activity against five other fungi. Both the new compounds (1 and 2) and the known compounds (3 and 5) showed activity only against Gram-positive bacteria and this activity was less than that of compound 4. For these compounds, the most sensitive strains were *Micrococcus luteus* ATCC 9314, *Corynebacterium diphtheriae* CD1 and *Listeria monocytogenes* ATCC 13,932 (25 to 30 μg/ml), and the most resistant strain was *Staphylococcus aureus* MRSA 639c (100 μg/ml). The compounds 1, 2, 3 and 5 showed a better activity than amoxicillin against *S. aureus* ATCC 43,300 and *S. aureus* MRSA 639c, a same activity against *Corynebacterium diphtheriae* CD1, *Enterococcus faecalis* EF1 and *Listeria monocytogenes* ATCC 13,932 (except for the compound 5) and a lower activity against *Micrococcus luteus* ATCC 9314 and *Staphylococcus aureus* ATCC 29213.

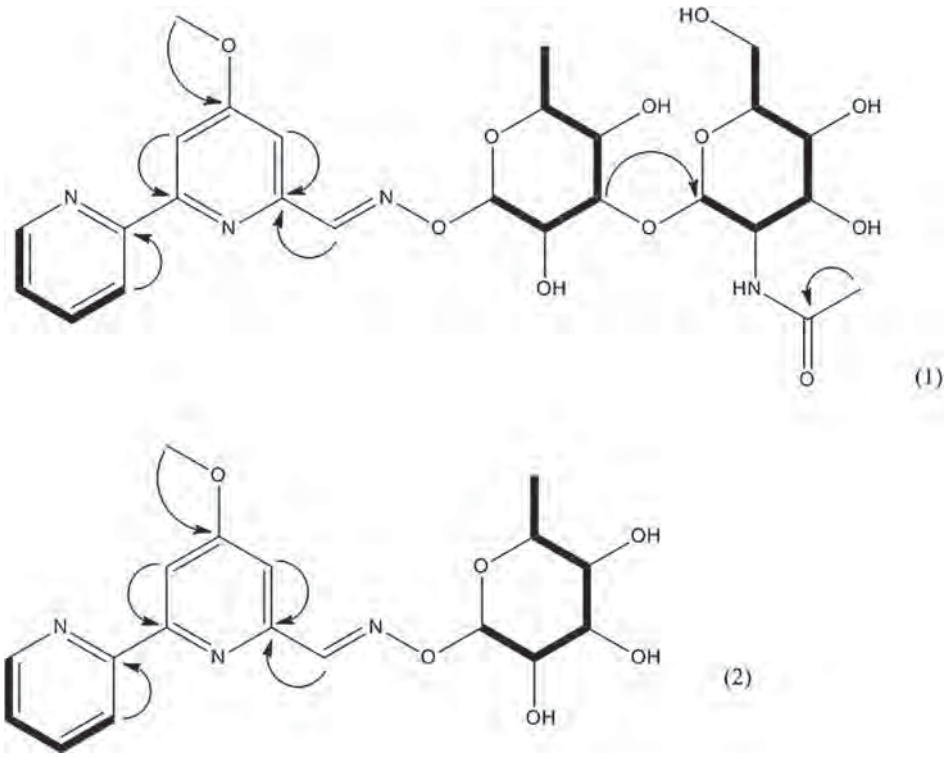

Fig. 4. HMBC and COSY correlations of compounds 1 (1) and 2 (2).

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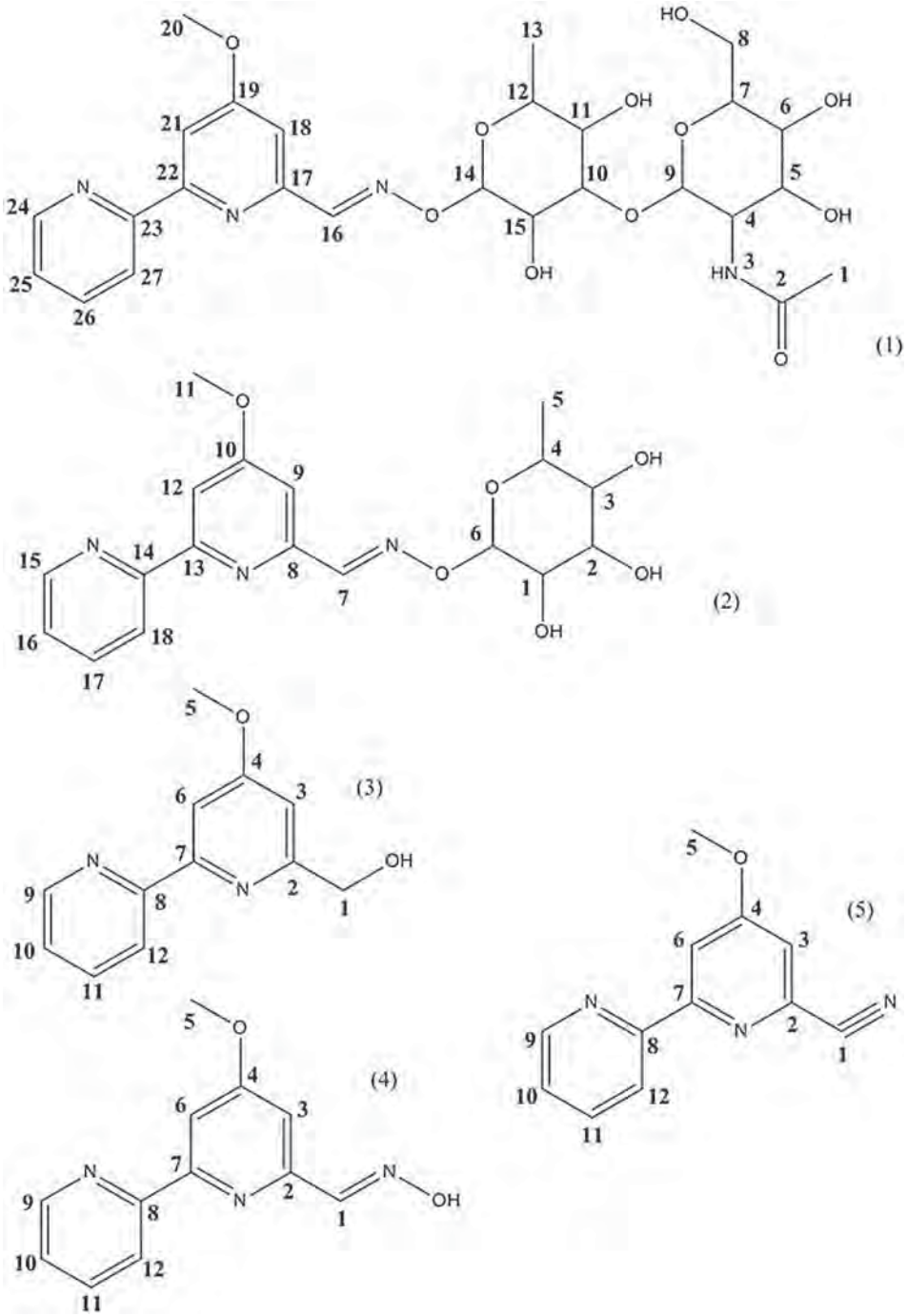

Fig. 5. Structures of compounds 1 (1), 2 (2), 3 (3), 4 (4) and 5 (5).

4. Discussion

Strain ABH26 is related to *Saccharothrix xinjiangensis* (Lahoum et al., 2015). It has not been indicated in the literature that this species produces cyanogrisides and caerulomycins, but rather that it is a producer of tianchimycins, which are macrolides (Wang et al., 2013). This is the first report of cyanogrisides and caerulomycins production by a member of the *Saccharothrix* genus. The antibiotics belonging to these two families are known to be produced by *Streptomyces caeruleus* (Funk and Divekar, 1959) and *Actinoalloteichus cyanogriseus* (Fu et al., 2011), but are also manufactured synthetically because their important biological activity (Bobrov and Tyvorskii, 2010). The new cyanogrisides obtained in this study (products 1 and 2) only showed inhibitory activity against Gram-positive bacteria. In the literature, antimicrobial activity of cyanogrisides has never been reported. However, many works have described the antitumor activity of these products (Fu et al., 2011, 2014). In addition, Fu et al. (2014) reported that the cytotoxicity of cyanogrisides was lower than that of caerulomycin A. The previously identified caerulomycin A (product 4) exhibited an important activity. We noted that its antifungal activity was stronger than its antibacterial activity. The broad antifungal activity of caerulomycin A has been extensively reported (Chatterjee et al., 1984; Ambavane et al., 2014). Other studies have suggested the important role of caerulomycin A in the regulation of immune homeostasis (Gurram et al., 2014; Kujur et al., 2015). Product 3

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Table 3
^{1}H and ^{13}C NMR data assignments of compounds 3, 4 and 5 in CD_3OD at 298 K.

^{1}H and ^{13}C number	^{1}H chemical shift, ppm			^{13}C chemical shift, ppm		
	3	4	5	3	4	5
1	4.75	8.21	–	64.34	149.02	117.44
2	–	–	–	162.85	153.90	
3	7.14	7.45	7.53	106.28	105.35	115.81
4	–	–	–	167.86	167.31	167.50
5	4.00	4.00	4.05	54.62	54.58	55.80
6	7.75	7.87	8.21	105.33	107.15	109.47
7	–	–	–	156.84	156.82	156.80
8	–	–	–	155.82	155.38	155.65
9	8.65	8.66	8.73	148.65	148.54	149.22
10	7.46	7.47	7.53	123.94	123.96	125.00
11	7.95	7.96	8.00	137.27	137.20	137.63
12	8.35	8.41	8.43	121.63	121.60	121.49

Table 4
Minimum inhibitory concentration (MIC) of compounds 1–5 produced by the strain ABH26.

Target microorganism	Minimum inhibitory concentration (μg/ml)				
	1	2	3	4	5
Bacillus subtilis ATCC 6633	50	50	50	10	60
Corynebacterium diphtheriae CD1	25	25	25	25	30
Enterococcus faecalis EF1	50	50	50	50	60
Listeria monocytogenes ATCC 13,932	25	25	25	25	30
Micrococcus luteus ATCC 9314	25	25	25	3	30
Staphylococcus aureus ATCC 29,213	50	50	50	50	60
S. aureus ATCC 43,300	50	50	50	50	60
S. aureus MRSA 639c	100	100	100	20	100
Escherichia coli E52	>100	>100	>100	>100	>100
E. coli E195	>100	>100	>100	>100	>100
Klebsiella pneumoniae E40	>100	>100	>100	>100	>100
Pseudomonas aeruginosa IPA1	>100	>100	>100	>100	>100
Aspergillus brasiliensis AB1	>100	>100	>100	10	>100
A. carbonarius AC1	>100	>100	>100	1	>100
A. carbonarius AC2	>100	>100	>100	10	>100
A. flavus NRRL 3251	>100	>100	>100	15	>100
A. parasiticus CBS 100,926	>100	>100	>100	20	>100
A. westerdijkiae ATCC 3174	>100	>100	>100	15	>100
Fusarium culmorum FC1	>100	>100	>100	3	>100
F. equiseti FE1	>100	>100	>100	10	>100
F. graminearum FG1	>100	>100	>100	10	>100
F. oxysporum f. sp. *albedinis* Foa1	>100	>100	>100	3	>100
F. o. f. sp. *radicis-lycopersici* Forl	>100	>100	>100	10	>100
F. solani Fsol	>100	>100	>100	3	>100
F. sporotrichioides FS1	>100	>100	>100	5	>100
Rhizoctonia solani AG3	>100	>100	>100	3	>100
Umbelopsis ramanniana NRRL 1829	>100	>100	>100	2	>100
Candida albicans M2	>100	>100	>100	10	>100
C. albicans M3	>100	>100	>100	2	>100
C. albicans IPA200	>100	>100	>100	10	>100
Saccharomyces cerevisiae ATCC 4226	>100	>100	>100	2	>100

(caerulomycin F) presented the same activity spectrum as the two new cyanogrisides, while product 5 (caerulomycinonitrile) has the lowest activity. In our study, we noted that the isolated products were not active against Gram-negative bacteria. The same observation was found by Boubetra et al. (2013c), who reported that the new antibiotics produced by *Saccharothrix* SA198 (isolated from Saharan soil) showed no activity against Gram-negative bacteria.

Compounds 1, 2, 3, 4 and 5 were secreted by strain ABH26 only on solid media, especially those containing glucose, such as Bennett medium (glucose, peptone, yeast extract and beef extract) and ISP2 medium (glucose, yeast extract and malt extract), while very small amounts were obtained on NA (peptone, yeast extract and beef extract) and ISP1 (tryptone and yeast extract) media. Thus, glucose seems to have an important role in the synthesis of these products. The production of compounds (1–5) started at 4 days in Bennett medium and reached a maximum after 7 days. Caerulomycins F (product 3) and A (product 4) and caerulomycinonitrile (product 5) are the major compounds. The new cyanogrisides (products 1 and 2) are produced in smaller quantities. The structure of the active product contained in the hydrophilic complex X could not be determined because of the difficulties encountered during its purification. However, this product appears to be the cause of activity observed in ISP1 and NA media (where the compounds 1–5 are not produced or produced in small amounts) and also the cause of activity observed during the second day of the kinetics study (compounds 1–5 being produced until the 4th day).

Moreover, we noted a correlation between the structure and antimicrobial activity of the compounds. Caerulomycin A (methoxy-bipyridine containing an oxime group CH=N—OH) showed potent activity against Gram-positive bacteria, yeasts and filamentous fungi. The linkage of a sugar (2,4-dimethyl-α-L-

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mannose) or two sugars (2,4-dimethyl-α-L-mannose and N-acetylglucosamine) for formation of the new cyanogrisides (products 2 and 1 respectively), led to a considerable decrease in activity. This activity becomes weaker against Gram-positive bacteria and absent against filamentous fungi and yeasts. However, the presence of sugars may be also lead to decreased cytotoxicity to animal cells as reported by Fu et al. (2014). Similarly, we noted that the oxime group CH═N—OH of product 4 had a very important role in the high activity of this product. Indeed, when this group is replaced by a hydroxymethyl group CH_2OH (product 3) or by a nitrile group (product 5) the activity decreased considerably against Gram-positive bacteria and becomes absent against filamentous fungi and yeasts.

5. Conclusion

Strain ABH26, related to *Saccharothrix xinjiangensis*, produced five bipyridine antibiotics belonging to the caruleomycins and cyanogrisides families. Two new cyanogriside antibiotics, named cyanogriside I and cyanogriside J, were characterized along with three known caerulomycins: caerulomycin A, caerulomycin F and caerulomycinonitrile. The five compounds showed activity against several pathogenic Gram-positive bacteria and one of them also exhibited an interesting antifungal activity. The activity of the compounds was related to their chemical structure. The oxime group bonded to the bipyridine ring strongly increased activity while the presence of sugars decreased this activity. This is the first report of cyanogrisides and caerulomycins production by a member of the *Saccharothrix* genus.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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