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ORIGINAL PAPER

Rare actinobacteria: a potential source of bioactive polyketides and peptides

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Abstract

Polyketides and peptides obtained from actinobacteria are important therapeutic compounds which include front line antibiotics and anticancer drugs. Many screening programs are directed towards isolation of bioactive compounds from these organisms but the chances of finding novel antimicrobial leads among common actinobacteria are fast dwindling. As a result, the focus has shifted to the members of less exploited genera of rare actinobacteria. Three isolates, MMS8, MMS16 and KCR3 found to be potent polyketide and peptide producers were identified by 16S rRNA gene sequencing and their sequences deposited in the GenBank under the accession numbers MG407702, MG372012 and MG430204 respectively. MMS8 identified as *Micromonospora auratinigra*, yielded one potent compound determined to be chloroanthraquinone with an minimum inhibitory concentration (MIC) of 8 µg/ml against *Bacillus subtilis* and an IC_{50} value of 10 µg/ml and 4 µg/ml against HeLa and IMR cell lines respectively. This is the first report of the production of chloroanthraquinone by *M. auratinigra*. MMS16, identified as a member of the family *Micromonosporaceae*, yielded a potent compound MMS16B analyzed to be a novel bafilomycin analogue. The MIC of the compound was found to be 7 µg/ml against *B.subtilis* and IC_{50} value against HeLa and IMR was observed to be 9 µg/ml and 14 µg/ml respectively. MMS16B was also found to exhibit anti-quorum sensing (AQS) activity at sublethal concentrations. KCR3 identified as *Kocuria kristinae* yielded a novel antimicrobial peptide with antibacterial, antifungal and AQS activity. To the best of our knowledge, no antimicrobial activity has ever been reported from *K. kristinae*.

Keywords Anti-quorum sensing · Peptides · Polyketides · Rare actinobacteria

Introduction

Actinobacteria are aerobic, gram-positive, filamentous, soil dwelling bacteria (Anderson and Wellington 2001) with exceptional metabolic diversity and are a rich source of several useful bioactive natural products, such as polyketides and peptides (Bundale et al. 2018a; Zhao et al. 2018). Polyketides, which contain repeating (–CH_2–CO–) groups, represent 20% of pharmaceutical drugs in the market (Tiwari and Gupta 2012).

Based on the diversity in structure and function, polyketides can be divided into three classes. The type I polyketides include macrolides like erythromycin, azithromycin and rapamycin and polyenes like amphotericin B and nystatin. The type II polyketides are aromatic polyketides such as tetracycline, doxorubicin, daunorubicin, rhodomycin, actinorhodin etc. The type III polyketides include chalcones and stilbenes in plants and polyhydroxy phenols in bacteria (Shen 2003).

Antimicrobial peptides (AMPs) are a well-known group of therapeutic agents, including tyrocidin, gramicidins, cyclosporine, polymyxins, daptomycin and surfactin. Non-ribosomal peptides, synthesized by non-ribosomal peptide synthetases are known to exhibit a wide range of biological activities including, antiviral, antiprotozoal, hypocholesterolemic, antifungal, siderophore, antimicrobial, antitumor,

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antioxidant, anti-hypertensive and immunomodulatory activities (Hamedi et al. 2015; Rajanbabu and Chen 2011).

Most of these antibiotics in clinical use today have been developed from compounds isolated from actinobacteria, *Streptomyces* being the dominant genus (Barka et al. 2016). However, the recent search for the novel compounds from *Streptomyces* species has often led to the rediscovery of known compounds. Hence, the focus of screening programs has shifted to bioactive compounds from non-*Streptomyces* group also referred as rare actinobacteria (Bundale et al. 2018b). At present more than 50 rare actinobacterial taxa are reported to produce 2500 bioactive compounds (Kurtböke 2012). Thus, it is crucial that new groups of rare actinobacteria be pursued as sources of novel pharmaceutically active metabolites. Amongst the novel metabolites, anti-quorum-sensing (AQS) agents, which can curb infection without a killing action, are gaining importance. Bacterial cell–cell communication, dubbed quorum sensing, is intricately related to virulence. An associated phenomenon is the bacterial swarming which allows the spread of disease and virulence. With this as a background and the rising incidence of resistance to extant antibiotics, a search for AQS agents as new molecules to treat infection has become logical and gathered momentum (Nashikkar et al. 2011; Kurtböke 2012). Although antimicrobial properties of actinobacteria have been extensively studied, less is known about AQS activities of rare actinobacteria which may be a rich source of active compounds that can act against bacterial quorum sensing systems.

The current study involves the purification and characterization of the bioactive polyketides and peptides from the three isolates selected from our previous study and their evaluation for antimicrobial, anticancer and AQS properties. These isolates were identified by 16S rRNA gene sequencing as *Micromonospora auratinigra*, Family *Micromonosporaceae* and *Kocuria kristinae* and their sequences deposited in the GenBank under the accession numbers MG407702, MG372012 and MG430204 respectively (Bundale et al. 2018b).

Materials and methods

Chemicals and media

All chemicals and solvents were of analytical grade and purchased from Merck, Germany and culture media from Hi-media, Mumbai, India.

Test organisms and animal cell lines

The target strains used for screening antimicrobial activity were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India and were: *Bacillus subtilis* MTCC 441, *Escherichia coli* MTCC 443, *Proteus mirabilis* 425, *Serratia marcescens* MTCC 86 and *Candida albicans* MTCC 227. HeLA and IMR cell lines were purchased from National Centre for Cell Science (NCCS), Pune, India.

Methods

Production of polyketides and peptides

The selected potent isolates were grown in potato dextrose broth and incubated in a rotary shaker incubator (REMI CIS-24 BL) at 130 rpm at 28 °C. 1 ml aliquots were withdrawn after every 24 h for a period of 12 days to optimize incubation period for maximum bioactive metabolite production. The cell free supernatant was concentrated fivefold in a vacuum concentrator and 50 µl was used to determine antimicrobial bioactivity against test organisms. The diameters of zones of inhibition were noted and correlated to the concentration of the bioactive compound in the cell free supernatant (Bundale et al. 2015).

Extraction of the bioactive compounds

For polyketides Crude antimicrobial compound was recovered from the mycelium as well as culture filtrate of both bioactive isolates by solvent extraction with ethyl acetate (1:1 v/v). The solvent was evaporated to dryness in a vacuum concentrator to obtain the crude cell and broth extracts which were stored at – 20 °C until further use (Bundale et al. 2018a).

For peptides The cell free broth was cooled overnight and acetone precipitation/ammonium sulphate precipitation was carried out. The protein precipitate was separated by centrifugation. Ammonium sulphate precipitate was resuspended in phosphate buffer and was subjected to dialysis using a dilute buffer. The dialysed peptide was used for bioactivity studies. The solvent in the acetone precipitate was allowed to evaporate completely overnight at 4 °C. This precipitate was resuspended in phosphate buffer and used for bioactivity studies. The presence of protein in the precipitate was confirmed by biuret and ninhydrin tests.

Purification of the bioactive compounds

For polyketides The dried crude extract was dissolved in ethyl acetate and 100–500 µl was loaded over the silica gel column. A stepwise gradient of chloroform/methanol was applied and the fractions thus separated were collected.

Preparative thin layer chromatography (TLC) with silica gel plate 60 F254 was used for the partial purification of antimicrobial products. The crude extracts were spotted and developed in different solvent systems.

The solvent systems used were chloroform:petroleum ether:methanol (10:10:3), chloroform:acetone:methanol (75:15:10), chloroform:methanol (8:2), petroleum ether:chloroform:methanol (7:2:1), chloroform:methanol (9:1), benzene:acetone:methanol (100:10:1). The developed plates were air dried and the separated bands were detected by observations of the color of the bands. The TLC was repeated several times and the mean R_f of the bands was calculated. The fractions were physically separated from each other by scraping the bands from the plates, extracting with methanol, concentrating the extracts and again subjecting each concentrate to TLC using the same solvent system, thereby confirming the purity of each fraction (Bundale et al. 2018a; Johdo et al. 1991; Kim et al. 1996).

For peptides The protein precipitate was purified by preparative TLC using the solvent system butanol:acetic acid:water (3:1:1) (Dharmaraj 2011). The developed plate was air dried and the band was visualized by spraying ninhydrin and the R_f was noted. The TLC was repeated in parallel and the band calculated by R_f was scraped and later extracted in the same solvent system. The purified extract was concentrated and again subjected to TLC using the same solvent system to confirm its purity. The peptide thus purified was sent for LC–MS analysis to confirm its purity and to assess its molecular weight.

Spectral studies The UV–vis absorption spectra (190–1100 nm) of the purified fractions were determined to identify the chromophores present in the metabolites by using a double beam bio-spectrophotometer (BL-198, Elico Ltd.) (Silverstein et al. 2014). Furthermore, Fourier transform infra red (FT-IR) spectrum of each active extract was obtained (as KBr discs) between 400 and 4000 cm^{-1} on Perkin Elmer 2000 FT-IR spectrophotometer and plotted as intensity versus wave number (Augustine et al. 2005). ^1H NMR spectra of the purified bioactive compounds was measured using a Bruker AMX 300 Coupling constants (*J*) in Hz. The mass spectra were obtained on a Bruker micro TOF-Q II 10,330 between 50 and 3000 m/z.

Bioactivity studies

Antimicrobial activity The antimicrobial activity of the pure compounds was assessed by the agar well diffusion method using Mueller–Hinton agar for the antibacterial and potato dextrose agar for anti fungal assays.

15 µl of 1 mg/ml stock were used for the tests. The diameter of the inhibition zones was determined after 24 h of incubation at 37 °C for bacteria and 28 °C for *C. albicans*. The minimum inhibitory concentrations (MICs) of the bioactive compounds were determined via a microdilution method using sterile 24-well plates with tetracycline as a standard (Arthington-Skaggs et al. 2002).

MTT-based cytotoxicity assay The cytotoxicity of bioactive fractions on established cell lines like HeLa and IMR was determined in vitro by the MTT based cytotoxicity assay (Mosmann 1983; Begde et al. 2011). The adherent cells were exposed to a concentration gradient of 1–40 µg/ml of the purified compounds.

Anti-quorum sensing activity *Pigment quenching assay* Quorum sensing inhibition (QSI) by the bioactive compounds was determined by studying pigment quenching using agar well diffusion method with *S. marcescens* as the indicator organism. A positive QSI result was indicated by a lack of pigmentation of the indicator strain around the vicinity of the well. Negative results were indicated by no pigmentation inhibition (Kanagasabhapathy et al. 2009; Bundale et al. 2018b).

Swarming motility assay To study the effect of bioactive compounds from rare actinobacteria on the swarming motility of *P. mirabilis*, 5 µl of an overnight culture was centrally inoculated on swarm agar plates containing various concentrations of the compounds. Two control plates were also set up for each study; one containing no additives and termed positive control and the other containing 20% DMSO and termed solvent control. All the plates were incubated at 37 °C for 20 h. Thereafter, the diameter of the swarm zone was measured and compared to the control (Nashikkar et al. 2011).

Statistical analysis

The MIC values were expressed as average of four independent replicates ± SD and IC_{50} values in the MTT based cytotoxicity assay as an average of eight replicates ± SD. Student's *t*-test was performed using SYSTAT Software (Systat Software, Inc., Chicago, IL, USA). *P* value ≤ 0.05 was considered significant unless otherwise mentioned.

Results

Three rare actinobacterial strains, MMS8, MMS16 and KCR3, which exhibited the ability to produce bioactive polyketides and peptides on the basis of pre-screening results were selected for this study.

Production and extraction of bioactive compounds

The bioactive compound production by the three isolates was monitored over a period of 12 days and was found to start only after 48 h for all the three isolates. It reached a maximum (24 mm) for KCR3 on the 4th day, and remained almost stable till the 12th day. A similar

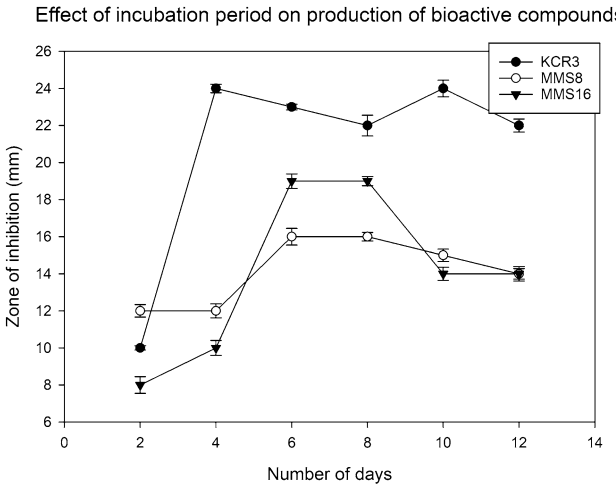
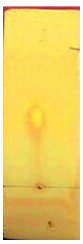


Fig. 1 Effect of incubation time on production of polyketides by MMS8 and MMS16 and antimicrobial peptide by KCR3. Each point represents mean of three independent observations ± SD

pattern was observed for MMS8 where, maximum bioactive metabolite production was observed on the sixth day (16 mm). But for MMS16, maximum production reached on 6th day (19 mm) and then showed a sharp decline from day 10 (Fig. 1).

The polyketide complexes extracted from MMS8 and MMS16 were purified using silica gel adsorption column chromatography followed by preparative TLC. The purified fractions were named MMS8A, MMS8B, MMS8C and MMS8D for the isolate MMS8. A similar numbering scheme was used for MMS16 too. The AMP from KCR3 was purified by acetone precipitation followed by preparative TLC. The solvent system used, colour, and R_f's of the fractions obtained from each organism are given in the Table 1.

Table 1 R_f values and antimicrobial activity of fractions of the isolates

Isolates	Solvent system	Fractions	R_f of fractions	Color of fractions		Antimicrobial activity (zone of inhibition, cm)			
						B. subtilis	*E. coli*	*S. marcescens*	*C. albicans*
KCR3	Butanol:acetic acid:water (3:1:1)	A	0.52	Purple (after ninhydrin spray)		2.2	1.7	3.2	0.8
MMS8	Benzene:acetone:methanol (80:15:5)	D	0.23	Purple		–	–	1.1	2.0
		C	0.48	Orange		0.8	–	1.0	1.8
		B	0.79	Pale yellow		–	–	0.7	2.2
		A	0.93	Brownish yellow		1.2	–	1.5	1.2
MMS16	Chloroform:acetone:methanol (75:15:10)	C	0.93	Yellow		1.87	–	1.0	2.5
		B	0.86	Orange		1.2	–	0.96	2.6
		A	0.78	Brown		1.3	–	0.6	3.1

Characterization of the polyketides and peptides produced by rare actinobacterial isolates

This section describes the characterization of the most potent bioactive compounds produced by the respective organisms.

Identification of bioactive compound from MMS8

The UV–vis spectra of MMS8B, which was the most potent compound showed the peaks at 247, 303, 379 and 410 (Fig. 2a). The IR (KBr) spectra of MMS8B showed prominent peaks at 3326, 2946, 2833, 1651, 1447, 1418, 1113, 1020, 666 (cm^{-1}) (Fig. 2b). The ^{1}H NMR spectra showed chemical shifts at δ 9.99 (aldehyde), δ 7.06–δ 7.57 (3 aromatic protons), δ 2.36 (C attached to Cl), δ 1.15–δ 1.39 (alkyl, methylene), δ 0.9 (methyl), δ 0.02–δ 0.096 (Fig. 2c). The mass spectrum of the compound showed a peak at *m/z* 242. The obtained molecular ion peak showed a further fragmentation to give a base peak at *m/z* 214 (Fig. 2d).

Identification of bioactive compound from MMS16

MMS16B, the most potent compound was found to be soluble in acetone, methanol and chloroform and showed a sharp yellow band with an R_f of 0.48 in chloroform:methanol (9:1). The UV–vis spectra of MMS16B showed the peaks at 255, 280, 318, 342, 417 nm (Fig. 3a). The IR (KBr) spectra of MMS16B showed prominent peaks at 3344, 2943, 2833, 1650, 1438, 1131, 1028 (cm^{-1}) (Fig. 3b). The chemical shifts shown by the proton NMR were at δ 7.07–δ 7.57 (3 aromatic protons), δ 2.19–δ 2.29 (C attached to N), δ 1.28–δ 1.57 (alkyl, methine, methylene), δ 0.9 (methyl), δ 0.02–δ 0.096 (Fig. 3c). The molecular weight was determined by mass spectra and by high resolution of the molecular ions to be m/z 811(Fig. 3d).

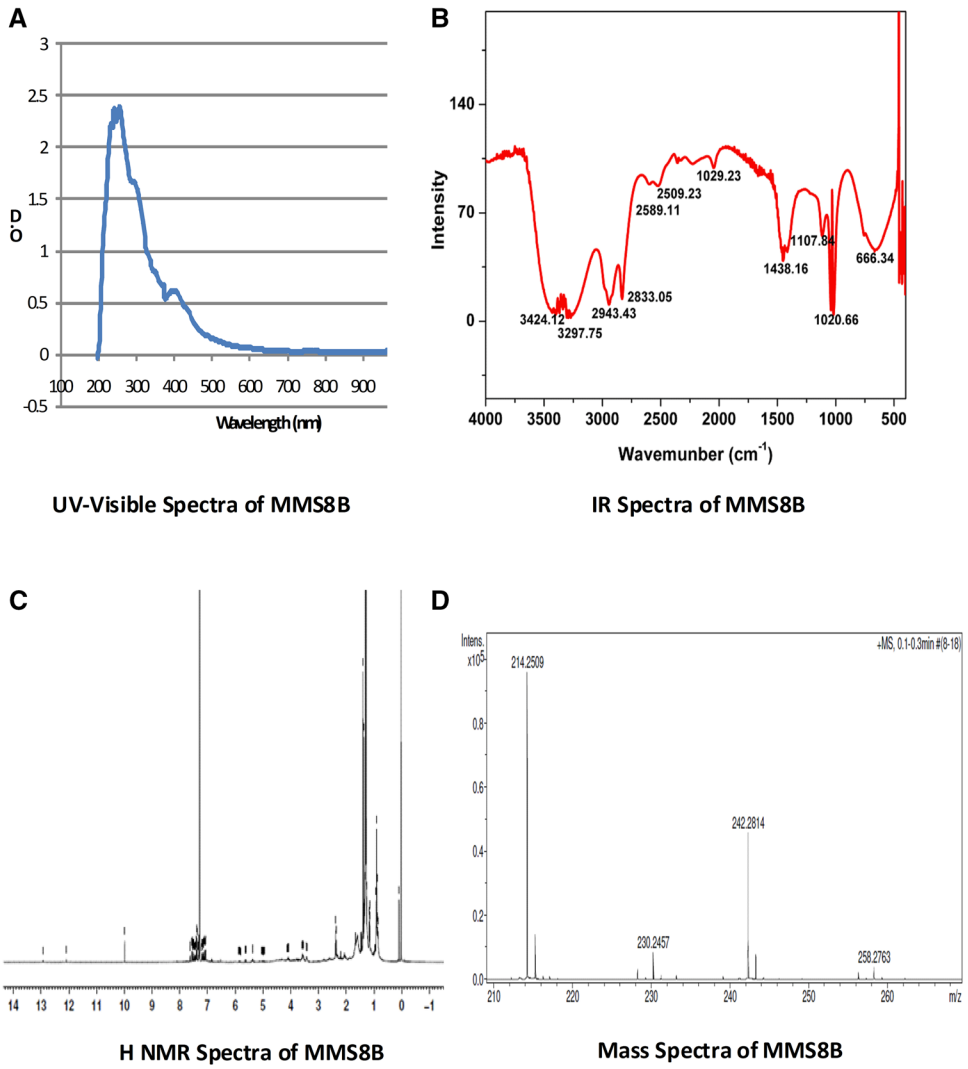


Fig. 2 Characterization of compound MMS8B obtained from MMS8: **a** UV–vis spectra of compound MMS8B, **b** IR spectra of compound MMS8B, **c** proton NMR of compound MMS8B and **d** mass spectra of compound MMS8B

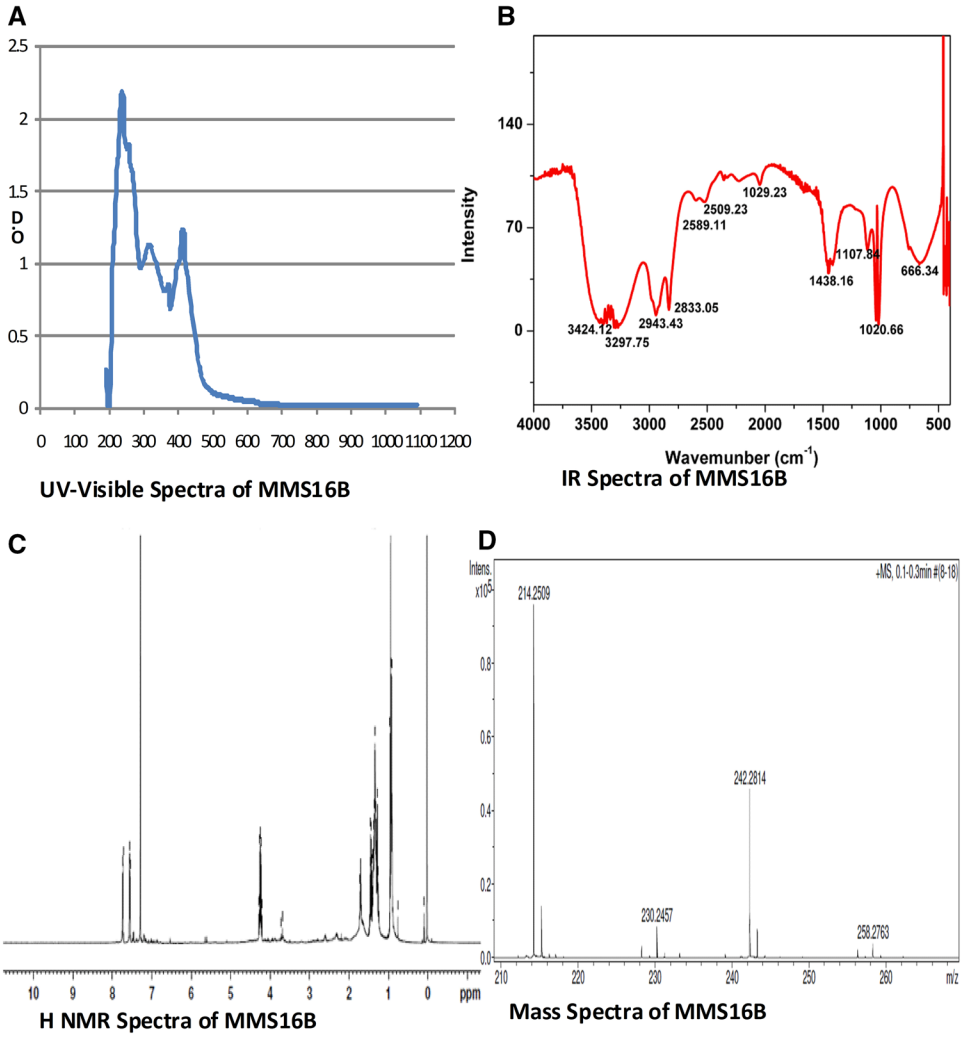


Fig. 3 Characterization of compound MMS16B obtained from MMS16: **a** UV–vis spectra of compound MMS16B, **b** IR spectra of compound MMS16B, **c** proton NMR of compound MMS16B and **d** mass spectra of compound MMS16B

Identification of AMP from KCR3

The peptide nature of the bioactive compound, KCR3A obtained from KCR3 has been established in a previous study by us. The purified compound from KCR3 resolved as one single band with an R_f of 0.52 in the solvent system butanol:acetic acid:water (3:1:1) which turned purple on spraying with ninhydrin. The UV–vis spectra showed prominent peaks at 232 and 372 nm (Fig. 4a). The IR (KBr) spectra showed prominent peaks at 2281, 1595, 1488, 1153, 1069, 1039 and 962 (cm^{-1}) (Fig. 4b). High resolution LC–MS of the KCR3A yielded highest molecular mass of 1097 with a range of several fragmentation peaks (m/z 579, 637, 695, 753, 811, 869, 927, 985) (Fig. 4c, Supplementary Fig. 1).

Table 2 summarizes the physicochemical properties of the purified compounds obtained from all the three isolates.

Bioactivity studies

Antimicrobial activity

MMS8A and MMS8C did not exhibit any activity against *B. subtilis* but MMS8B and MMS8D were active. None of the compounds of MMS8 was active against *E. coli*. All purified compounds of MMS8 were active against *C. albicans*. MIC of MMS8B against *B. subtilis* was found to be 8 µg/ml. The purified compounds from isolate MMS16 were found to be active against *B. subtilis* with zones of inhibition in the range of 14–17 mm. These compounds were not active against *E. coli*. The compounds were also antifungal exhibiting zones of inhibition against *C. albicans*. The MIC of MMS16B, the most potent compound, against *B. subtilis* was found to be 7 µg/ml. The purified peptide from KCR3 was potent against all test organisms (refer Table 1).

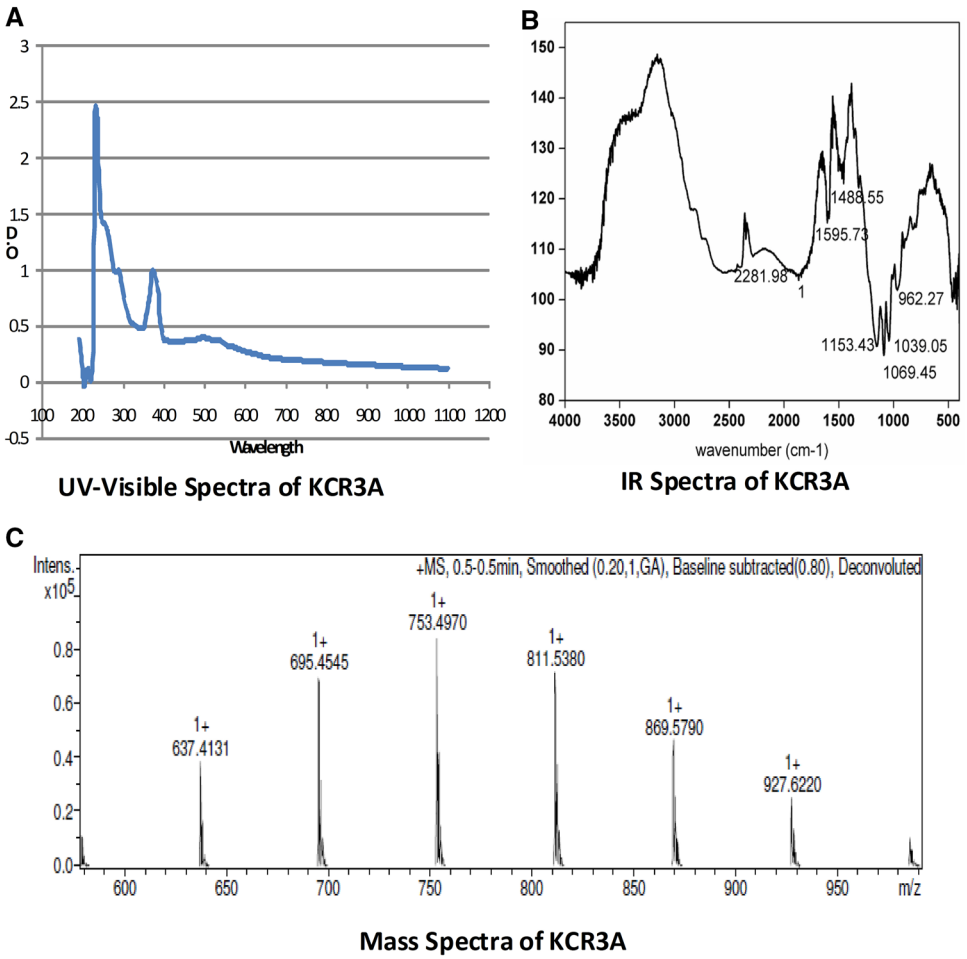


Fig. 4 Characterization of compound KCR3A obtained from KCR3: **a** UV–vis spectra of compound KCR3A, **b** IR spectra of compound KCR3A and **c** mass spectra of compound KCR3A

Table 2 Physicochemical properties of the Bioactive Compounds

Compound → properties↓	KCRA	MMS8B	MMS16B
Appearance	White amorphous powder	Yellow solid	Orange solid
UV–vis λ_{max} (nm)	232, 372	247, 303, 379, 410	255, 280, 318, 342, 417
IR_{max}	2281, 1595, 1488, 1153, 1069, 1039, 962	3326, 2946, 2833, 1651, 1447, 1418, 1113, 1020, 666	At 3344, 2943, 2833, 1650, 1438, 1131, 1028
NMR	ND	δ 9.99, δ 7.06–δ 7.57, δ 2.36, δ 1.15–δ 1.39, δ 0.027–δ 0.921	δ 7.28–δ 7.74, δ 4.22–δ 4.28, δ 1.25–δ 1.72, δ 0.027–δ 0.96
Molecular mass (m/z)	1097	242	811
Compound identified as	Non-thio peptide	Chloroanthraquinone	Bafilomycin

Anticancer activity

In vitro antitumor activity of the bioactive compounds was judged by MTT based cytotoxicity assay against established cancer cell lines, HeLa and IMR. The IC_{50} of MMS8B determined from the graph was ~ 10 µg/ml for HeLa and ~ 4 µg/ml for IMR and that of MMS16B was observed to be ~ 9 µg/ml for HeLa and ~ 14 µg/ml for IMR (Fig. 5a, b).

Anti-QS activity

All purified compounds from both the polyketide producers exhibited AQS activity. The most potent compounds, MMS8B and MMS16B showed pigment quenching zones of 10 mm and 12 mm respectively against *S. marcescens*. KCR3A showed extremely high AQS activity against *S. marcescens* with a turbid zone of inhibition of 32 mm

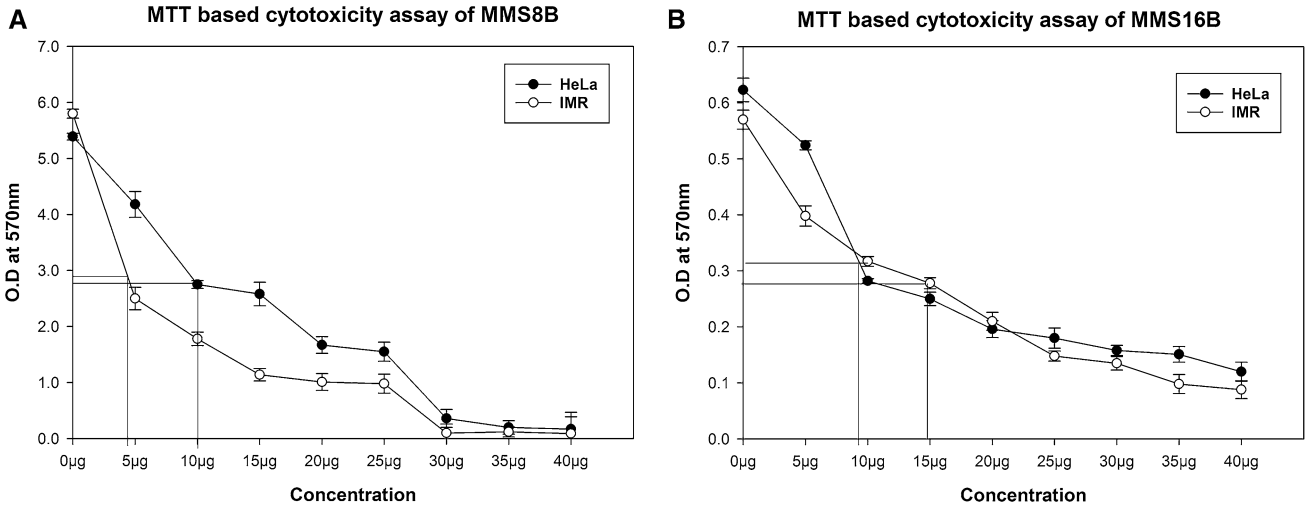


Fig. 5 **a** MTT based cytotoxicity assay of MMS8B on HeLa and IMR, IC_{50} against HeLa was found to be 10 µg and 4 µg respectively and **b** MTT based cytotoxicity assay of MMS16B on HeLa and IMR, IC_{50} against HeLa was found to be 9 µg and 14 µg respectively. Each point represents mean of three independent observations ± SD

Fig. 6 Anti-quorum sensing activity of KCR3A against *Serratia marcescens*. The compound has inhibited the pigment formation but has not inhibited the growth as can be seen by the turbid zone

(Fig. 6) However, nisin used as a standard, failed to show AQS even at concentration of 10 mg/ml. MMS16B also showed antiswarming activity against *P. mirabilis* at a concentration of 0.3 mg/ml and 0.5 mg/ml with 70–80% reduction in swarm zones as can be seen in Fig. 7.

Discussion

The current study was undertaken with the aim of obtaining novel polyketides and peptides from rare actinobacteria isolated from soil. These were purified and their antimicrobial, anticancer and anti-QS activities were assessed followed by their structure elucidation.

Compound MMS8B isolated from *M. auratinigra*, was obtained as an orange yellow compound with an R_f of 0.45 in the solvent system chloroform:methanol (9:1). The UV–vis

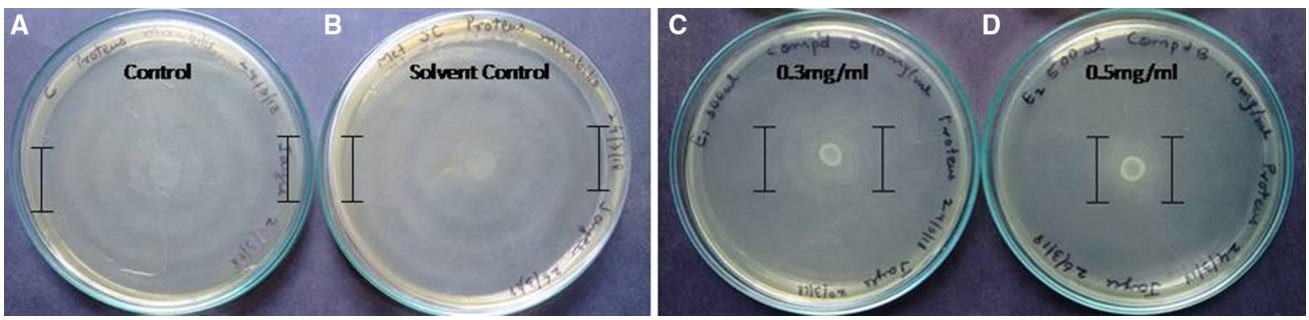


Fig. 7 Anti swarming activity of MMS16B against *Proteus mirabilis*: **a** positive control, **b** solvent control, **c** swarm zone inhibition by compound MMS16B at 0.3 mg/ml concentration (50% reduction in swarm zone) and **d** swarm zone inhibition by compound MMS16B at 0.5 mg/ml concentration (80% reduction in swarm zone)

spectrum indicates the compound to belong to anthraquinones showing bands in the wavelength range 220–350 nm and one absorption band at longer wavelengths, close to 400 nm (Osman et al. 2014). IR spectra and NMR indicate the compound MMS8B to be chloroanthraquinone, with the presence of a peak at 660 cm^{-1} (C–Cl) in IR spectrum and chemical shift at δ 2.37 (C attached to Cl) in ^{1}H NMR. The structure of the compound was further confirmed by the fragmentation pattern of the molecule in the mass spectrum showing a sharp peak at 242 and fragmentation peak at 214 as reported in Pubchem. The compound was thus identified as chloroanthraquinone, reported to have a molecular mass of m/z 242 in published data.

Anthraquinone derivatives other than chloroanthraquinones have been previously reported from *Micromonospora rhodorangea* (Xue et al. 2009) and *Micromonospora lupini* (Igarashi et al. 2007). However, to the best of our knowledge, this is the first report of chloroanthraquinone being isolated from *M. auratinigra* which is a relatively less studied species of this widely reported genus with only one report wherein, Talukdar et al. have reported the bioactive compound, 2-methylheptylisonicotinate, similar to isoniazids from this organism (Talukdar et al. 2016).

MMS8B was found to have antimicrobial, antifungal and anticancer activity. A similar range of activity was also reported in 7-chloroemodin, a novel chloroanthraquinone isolated from lichen (Rosso et al. 2003). Moreover, 2-methylheptylisonicotinate isolated from *M. auratinigra* has been reported to have very high values of MIC of 40 µg/ml against *B. subtilis* (Talukdar et al. 2016). As compared to it, chloroanthraquinone, isolated from this strain of *M. auratinigra* has been found to have a much lower MIC of 8 µg/ml. There are practically no reports on anticancer activities of chloroanthraquinone. However our compound was found to be potent against both the tested cell lines.

Isolate MMS16, identified as novel member of Family *Micromonosporaceae* sp., yielded a yellow coloured compound, MMS16B. The UV–vis spectra indicate that it may belong to bafilomycin group of macrolide antibiotics exhibiting maximum absorptions at 242, 248 and 280 nm. Bafilomycins B and C show in addition, shoulders between 340 and 360 nm (Werner et al. 1984). The additional shoulder at 343 in our compound, indicates that it may be bafilomycin B. Further the R_f also matches to that reported for setamycin which is a type of bafilomycin B_1 (Omura et al. 1981). The IR spectra too was very similar to that reported for setamycin by Omura et al. (1981), showing peaks in the range of 3300–3500 cm^{-1} and around 1650 cm^{-1} as reported for bafilomycins (Werener et al. 1981). However, the peak due to an ester group at 1710–1730 cm^{-1} and at 1220 cm^{-1}, reported for bafilomycin B_1, was missing.

High resolution LC–MS of the compound yielded molecular weights of 811 which matches the published data for bafilomycin B_1. The mass showed the same fragmentation peaks (m/z 568, 525, 399, 368, 338, 211, 169, 137, 113, 109) as reported by Otoguro for setamycin (bafilomycin B_1). Also the appearance of characteristic fragment peak at around m/z 211 in our compound is assignable to flavensomycinoic acid which has been involved in the molecules of bafilomycin B_1 (Otoguro et al. 1988). The chemical shift values shown by the ^{1}H NMR of MMS16B were very similar to that of bafilomycins. Thus R_f, UV spectra, and mass spectra of MMS16B is almost identical to setamycin (bafilomycin B_1) but IR spectra is similar to bafilomycin A. This indicates that MMS16B might be slightly different from the known bafilomycins.

MIC of MMS16B (7 µg/ml) was found to be much lower than the reported value of 25 µg/ml for setamycin against *B. subtilis* (Omura et al. 1981; Otoguro et al. 1988). As MMS16B did not show any appreciable activity against *E. coli* and *P. mirabilis* but exhibited pigment quenching against *S. marcescens*, it was explored further for its AQS properties. MMS16B showed antiswarming activity against *P. mirabilis* at a concentration of 0.3 mg/ml and 0.5 mg/ml with 70–80% reduction in swarm zones. To the best of our knowledge there are no previous reports on the AQS properties of any analogue of bafilomycins.

The IC_{50} value against cancer cell lines HeLa and IMR is comparable to that of reported values of bafilomycin B_1; 5.88 nM and 14.37 nM for other cell lines like leukemia and melanoma (Laakso et al. 2003). These studies on the antimicrobial, AQS and anti cancer properties of MMS16B, which may be a bafilomycin analogue, make it a good candidate for being studied as an AQS and anticancer drug.

KCR3 identified as *K. kristinae*, was found to produce an AMP. The IR spectra of the AMP obtained from KCR3 shows the characteristic IR bands of peptide linkage (2281-C≡N stretch, 1595-C = O amide region, 1488-NH bending and CN stretching, 1153-C–OH stretch, C–O–C stretch, 962-C–H out of plane bending) (Fabian and Mäntele 2006). However, the peak of 2500 cm^{-1} indicative of S–H stretch, was lacking in KCR3A, confirming the absence of thiol group in this AMP. The mass spectra indicated that the component with m/z 753 was present at the highest concentration and is likely to be the active compound. Our peptide showed a much broader bioactivity range unlike the AMP's produced by other species of *Kocuria* such as kocurin produced by *Kocuria palustris* and variacin produced by *Kocuria varians*. KCR3A showed activity against both gram positive as well as gram negative bacteria and was also found to be antifungal in nature. Further, it was found to have very high AQS activity at sublethal concentrations as judged by the pigment quenching capability using *S. marcescens* as the test organism. Kocurin is a thiazolyl peptide, reportedly showing in vitro activity against gram-positive bacteria (Martín et al. 2013). Similarly, variacin, a lantipeptide has also been

reported to be active against gram positive bacteria with no antifungal activity (Pridmore et al. 1996). To the best of our knowledge, *K. kristinae* has not been previously studied for production of any bioactive compounds.

Compounds like KCR3A and MMS16B, which interfere with the QS mechanism of pathogens, may prove to be highly effective in controlling their pathogenicity, especially opportunistic pathogens, that cause disease only when their population becomes quorate, and they are able to express their virulence factors. AQS compounds like these can hence attenuate the virulence of the pathogens without challenging their growth, thereby preventing the emergence of drug resistant strains.

Although screening programs do not always result in the discovery of new compounds, this study yielded two novel polyketides and an AMP which can be taken up in a drug development program wherein they may be chemically modified to further increase their activity. These can be subsequently evaluated in clinical trials rendering valuable chemotherapeutic compounds in future.

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Compliance with ethical standards

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

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