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ORIGINAL PAPER

Isolation and partial characterization of antimicrobial compounds from a new strain *Nonomuraea* sp. NM94

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Abstract An actinomycete strain NM94 was isolated from a Saharan soil sample by a dilution agar plating method using chitin-vitamins B medium supplemented with penicillin. The strain presented the morphological and chemical characteristics of the genus *Nonomuraea*. On the basis of 16S rDNA analysis and physiological tests, this isolate was found to be quite different from the known species of *Nonomuraea* and might be new. The strain NM94 secreted several antibiotics on yeast extract malt extract glucose medium that were active against some Gram-positive bacteria, yeast, and fungi. The antibiotics were extracted with dichloromethane and detected by bioautography on silica gel plates using *Mucor ramannianus* and *Bacillus subtilis* as the test organisms. Among these antibiotics, a complex called 94A showed interesting antifungal activity. It was selected and purified by reverse-phase HPLC. This complex was composed of five compounds. Spectroscopic studies by infrared, mass, and ^{1}H NMR of the compounds were carried out. Initial results showed that these molecules differed from the known antibiotics produced by other *Nonomuraea* species.

Keywords Actinomycetes · *Nonomuraea* · Taxonomy · Antifungals · Saharan soil

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Introduction

Fungal infections are caused by eukaryotic organisms, and for that reason, they generally present more difficult therapeutic problems than do bacterial infections. In fact, there are relatively few agents that can be used to treat fungal infections. New fungicides are needed in agriculture, food protection, and medicine due to the increase in resistant pathogens, appearance of new infectious diseases, and the toxicity of currently used compounds. Four strategies have been employed to find new antifungal drugs. First, the search for molecules derived from known antifungal drugs, such as azoles [15]. Second, the search for novel targets within the fungi [1]. Third, the use of formulations, which can decrease the toxicity of known antifungals [10]. Fourth, an intensified search of new bioactive drugs from natural sources [4].

The actinomycetes are one of the most attractive sources of new bioactive metabolites. However, the rate of discovery of new compounds has decreased, since the ubiquitous species have been studied extensively. Recently, rare actinomycetes have been shown to be an important source of novel and useful antibiotics [25]. Several studies have been oriented towards isolation of new uncommon actinomycetes from unexplored environments [37].

The ecological distribution of actinomycetes from palm groves has been studied in Algeria. These studies showed that non-*Streptomyces* genera were abundant [32]. Selective methods have also been developed for isolation of rare actinomycete genera [31]. Screening for antibiotic metabolites showed that numbers of these actinomycetes are producers of antibiotics [23, 31, 39].

The aim of the present study was to isolate and identify antifungal non-polyenic metabolites produced by a novel actinomycete isolated from a Saharan soil. In this report, we describe the taxonomy of the producing strain NM94 and the fermentation conditions for production of the active metabolites. In addition we report on isolation and partial characterization of five antifungal compounds.

Materials and methods

Isolation of the actinomycete strain

The strain NM94 was isolated from Saharan soil collected in Beni-Abbes (South-West of Algeria). The soil sample was subjected to serial dilutions in sterile deionized water and plated on chitin vitamins B agar medium, recommended by Hayakawa and Nonomura [16] for isolation of rare actinomycetes genera. The culture medium was supplemented with penicillin (10 mg l^{-1}) and actidione (50 mg l^{-1}) as selective antibiotics to prevent growth of bacteria and fungi, respectively. The plates were incubated at 30°C for 3 weeks and then examined by light microscopy. After isolation, strain NM94 was stored on agar slant of ISP2 medium [34] at 4°C.

Morphological and chemical characterization of the isolate

The identification of the genus of the collected isolate was determined by morphological features and chemical analysis of cellular constituents. The morphological and cultural characters were observed on the media yeast extract malt extract agar (ISP2), oatmeal agar (ISP3), and inorganic salts starch agar (ISP4) [34], and on glucose yeast extract agar [2]. Spore shape and surface were observed under a scanning electron microscope (Cambridge Stereoscan, model 240).

The Chemical analysis of the cell components was investigated by the methods of Becker et al. [5] and Lechevalier and Lechevalier [26] for determination of the isomeric form of diaminopimelic acid and the whole cell sugars pattern. Phospholipids and mycolic acids were analyzed by using procedures of Minnikin et al. [29, 30].

Physiological properties of strain NM94

Seventy-six physiological tests commonly used in numerical taxonomy of actinomycetes were applied. Production of melanoid pigments was tested on peptone yeast extract iron agar (ISP6) and tyrosine agar (ISP7) [34]. Carbohydrates and organic acids assimilation as carbon source and utilization of testosterone were determined as described by Gordon et al. [14] and Goodfellow et al. [12]. Decomposition of adenine, guanine, hypoxanthine, tyrosine, and xanthine were determined by the methods of Gordon et al. [14], and arbutin and aesculin decomposition, gelatin liquefaction, starch, and tween 80 hydrolysis and nitrate reductase production were determined as described by Marchal and Bourdon [28]. The sensitivity to lysozyme was studied by using the method of Gordon and Barnett [13]. The sensitivity to phenol, potassium tellurite, sodium azide, sodium chloride, and violet crystal were determined on glucose yeast extract agar [3]. Growth at different temperatures and pH and in the presence of various antibiotics was tested in the same medium.

DNA extraction, 16S rDNA sequencing and phylogenetic analysis

The isolate NM94 was grown for 3 days at 28°C with agitation in 500-ml flasks containing 100 ml of ISP2 medium. Biomass was harvested by centrifugation at 7,000 *g* for 10 min and washed twice with double-distilled water. About 200 mg of mycelia was used for DNA extraction as follows: the sample was dispersed in 800 µl of the aqueous lysis solution (100 mM Tris–HCl, pH 7; 20 mM EDTA; 250 mM NaCl; 2% SDS; 1 mg ml^{-1} lysozyme). About 5 µl of a 50 mg ml^{-1} RNase solution was added, and the suspension incubated at 37°C for 60 min. About 10 µl of a proteinase K solution (20 mg ml^{-1}) was added, and the lysis solution was reincubated at 65°C for 30 min. The lysate was extracted with an equal volume of phenol and

centrifuged at 7,000 *g* for 10 min. The aqueous layer was re-extracted with phenol (50–50%, v/v), then by chloroform (50–50%, v/v). DNA was recovered from the aqueous phase by the addition of NaCl (150 mM final concentration) and two volumes of cool 95% (v/v) ethanol prior to centrifugation. The precipitated DNA was cleaned with 50 µl of 70% (v/v) ethanol, centrifuged at 7,000 *g* for 10 min, resuspended in 50 µl of TE buffer (10 mM Tris–HCl, pH 7.4; 1 mM EDTA, pH 8) and stored at −20°C. The purity of DNA solutions was checked spectrophotometrically at 260 and 280 nm, and the quantities of DNA were measured at 260 nm.

The 16S rDNA was amplified by using the PCR method with *Taq* DNA polymerase and primers 27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492r (5′-GGTTACCTTGTTACGACTT-3′). The conditions for thermal cycling were as follows: denaturation of the target DNA at 98°C for 3 min followed by 30 cycles at 94°C for 1 min, primer annealing at 53°C for 1 min and primer extension at 72°C for 5 min. At the end of the cycling, the reaction mixture was held at 72°C for 5 min and then cooled to 4°C. PCR amplification was detected by agarose gel electrophoresis and was visualized by ultraviolet (UV) fluorescence after ethidium bromide staining.

The PCR product obtained was submitted to Genom Express (Meylan, France) for sequence determination. The same primers as above and an automated sequencer were used for this purpose. The sequence was compared for similarity with the reference species of bacteria contained in genomic database banks, using the NCBI BLAST available at http://www.ncbi-nlm-nih.gov/.

Phylogenetic analyses were conducted using software included in MEGA Version 3.0 [21] package. The 16S rDNA sequence of the strain NM94 was aligned using the CLUSTAL W program [35] against corresponding nucleotide sequences of representatives of the genus *Nonomuraea* retrieved from GenBank. Evolutionary distance matrices were generated as described by Jukes and Cantor [19] and a phylogenetic tree was inferred by the neighbor joining method [33]. Tree topologies were evaluated by bootstrap analysis [11] based on 1,000 resamplings of the neighbor joining dataset.

Antimicrobial activity of the strain NM94

The spectrum of action of the strain NM94 was determined against several Gram-positive and Gram-negative bacteria, filamentous fungi, and yeasts. The microorganisms are listed in Table 2. The experiments were done by streaking a straight line of the NM94 inoculum across the surface of glucose yeast extract malt extract agar (ISP2) medium on plate. After incubation for 7 days at 30°C, the bioassay organisms were streaked at right angles to the strain NM94 (straight line). The plates were incubated at 30°C and observed for antibiosis on the first and the second day. The extent of growth inhibition of the bioassay organisms was recorded by measuring the length of the inhibition range away from the strain NM94.

Fermentation studies on several media

In order to select the culture medium that will enable optimal production of antimicrobial activities, several liquid culture media were tested: glucose yeast extract malt extract broth (ISP2), oatmeal broth (ISP3), inorganic salts starch broth (ISP4), glycerol asparagine broth (ISP5) [34], glucose yeast extract broth (GYEA) [3], glucose peptone yeast extract meat extract (Bennett medium) [18], starch asparagine (SAS) and starch casein (SCA) [22]. The pH of each medium was adjusted to 7.2 prior to autoclaving. Each 500-ml Xask containing 100 ml of medium was inoculated with 5% (v/v) of actinomycete culture grown in ISP2 medium for 72 h at 28°C. The Xasks were incubated at 28°C for 10 days with shaking at 250 rpm. About 0.5 ml aliquots were collected regularly to estimate antibiotic activity by the agar well method against Bacillus subtilis (ATCC 6633) and Mucor ramannianus (NRRL 1829). Wells (10 mm in diameter) made in the ISP2 agar plates were Wlled with 0.2 ml of the supernatant samples.

Time course of antibiotic production in fermentor

A loopful of strain NM94 from a mature slant culture was inoculated into a 250-ml Erlenmeyer flask containing 50 ml of sterile seed medium ISP2 broth. The culture was incubated on a rotary shaker (250 rpm) at 30°C for 3 days. All the seed culture was transferred into a 7-l fermenter (model Set, Setric, Paris, France) containing 5 l of the same medium (pH 7.2). The fermentation was carried out at 30°C for 10 days under aeration of 3 l min^{-1} and agitation rate of 300 rpm.

Extraction of antimicrobial compounds

The 5 l of culture broth was centrifuged at 3,000 *g* for 15 min and the supernatant was extracted three times with an equal volume of dichloromethane and the

dichloromethane layer was dehydrated with Na_2SO_4 and concentrated using the Rotavapor (Laborata 4000). The crude extract was resuspended in methanol and checked for activity in the bioassay described below.

Biological and chemical detection of antimicrobial compounds

Crude extract samples were subsequently subjected to thin-layer chromatography (TLC). Samples were spotted onto 20 × 20-cm^2 silica gel plates (Merck Art 5735, kieselgel 60F254), and then developed with ethyl acetate/methanol (100:15) as the solvent mixture. The antibiotics were detected by bioautography [7] on silica gel plates seeded with *M. ramannianus* or *B. subtilis*. Clear halos due to growth inhibition of the microorganisms, indicated the location of antimicrobial compounds on the TLC plates, and the retention factor (Rf) values were recorded. The antimicrobial compounds were also detected under ultraviolet irradiations at 254 and 365 nm and by spraying with some chemical reagents such as napthoresorcinol-H_2SO_4, ninhydrin, and $FeCl_3$.

Purification of antifungal metabolites

Concentrated extracts were purified on preparative TLC silica gel plates (Merck 60 F-254; 0.5 mm thick). The plates were developed with ethyl acetate/methanol (100:15, v/v), and then air dried. The active bands were visualized in ultraviolet light at 254 and 365 nm, scraped from the plates, eluted with methanol and concentrated using the Rotavapor. The antibiotic activity of each band against *B. subtilis* and *M. ramannianus* was measured by a paper disc method.

Final purification of the antimicrobial compounds was carried out by reverse phase high performance liquid chromatography (HPLC) (Waters, Milford, MA, USA) under the following conditions: flow = 2.0 ml min^{-1}; stationary phase = Delta Pak C18 column (7.8 × 300 mm^2; 15 μm); mobile phase = 80% methanol in water; detector wavelength = 220 nm.

Spectroscopic studies

The UV-visible spectrum of the antifungal antibiotics was determined in methanol solution with a Shimadzu UV-270 spectrophotometer and infrared spectra with a Perkin–Elmer FT-IR, model 1760x spectrometer. Mass spectra were recorded on a Finnigan LCQ spectrometer equipped with an electro spray ion source (negative ion mode) "ESI." ^1H NMR spectra were recorded at 300 MHz, using a Brucker AC-300 spectrometer.

Results and discussion

Morphological characteristics, chemotaxonomy, and identification of the genus

Isolate NM94 grew well on all media used (8 days at 28°C) and had a white to cream white aerial mycelium and light to dark yellow vegetative mycelium. Diffusible pigments were not observed. The isolate produced moderately branching, non-fragmenting substrate hyphae with ~0.4–0.6 μm in diameter. Aerial hyphae were formed on all media and were 0.6–1.0 μm in diameter. Straight spore chains of 5–15 non-motile spores were produced longitudinally on short sporophores (1–3 μm long) on the aerial mycelium only. The spores were ovoid to ellipsoidal (0.8–1.0 by 1.2–1.8 μm). The spore surface observed in electron microscopy, appeared folded (Fig. 1). Endospores, sclerotia, sporangia, synnemata, and whirls were not observed.

Cell-wall hydrolysate of isolate NM94 contained the *meso*-diaminopimelic acid but glycine was not found. Madurose was the characteristic sugar of the whole-cell extract in addition to galactose, glucose, mannose, and ribose. Thus, isolate NM94 had cell-wall of type III and sugar pattern type B [26]. The diagnostic phospholipids detected were phosphatidyl-ethanolamine and phospholipids containing glucosamine. This pattern corresponds to phospholipids type P IV [27]. Mycolic acids were not present. Based on the morphological and chemical characteristics, NM94 is tentatively attached to the genus *Nonomuraea* described by Zhang et al. [38]. This genus contains all species previously assigned to the "*Microtetraspora pusilla* group," initially transferred from

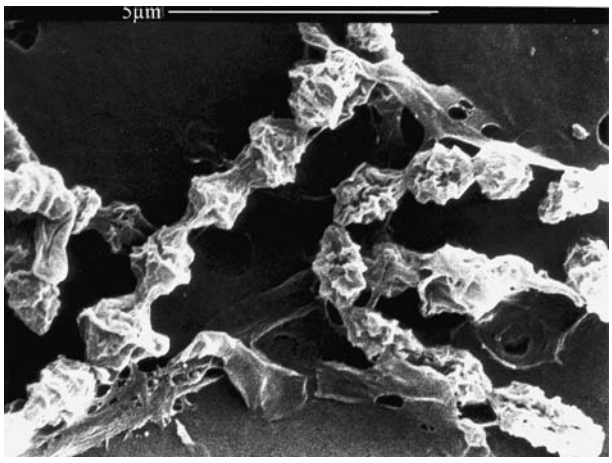

Fig. 1 Scanning electron micrography of *Nonomuraea* sp. NM94 showing chain with folded spores on the aerial mycelium. NM94 was grown on ISP2 medium for 15 days at 30°C. *Bar*, 5 μm

Actinomadura [20]. Analysis of 16S rRNA gene sequences by Zhang et al. showed that the genus *Nonomuraea* belongs to the family *Streptosporangiaceae* [38].

Physiological characteristics

Table 1 shows the physiological properties of isolate NM94. This strain readily utilized most of the carbon sources tested and was resistant to several antibiotics of which some belong to the β-lactam, aminosides and macrolides families.

Phylogenetic studies and species identification

The phylogenetic relationship of strain NM94 to the other described species in the genus *Nonomuraea* is seen in the neighbor-joining dendrogram in Fig. 2. Phylogenetic analysis using 16S rDNA sequence confirmed that the isolate NM94 belonged to the genus *Nonomuraea*. The similarity level was only 97.8% with *Nonomuraea roseola* DSM 43767^T [24], the most closely related species. Much higher 16S rDNA similarities have been found between representatives of validly described *Nonomuraea* species, such as the type strains of *N. roseola* and *N. recticatena* (99.4%), *N. roseola* and *N. africana* (98.7%), and *N. recticatena* and *N. africana* (98.6%). Strain NM94 could also be distinguished from the *N. roseola* type strain by several characters such as the color of aerial and substrate mycelia, the degradation of starch, xanthine, arabinose, cellobiose, fructose, mannitol, mannose, sucrose, xylose, and sodium pyruvate, the production of nitrate reductase

Table 1 Physiological properties of the strain NM94

Test	Results	Test	Results
Hydrolysis of		Decarboxylation of sodium salts	
Adenine	–	Acetate	+
Aesculin	+	Benzoate	–
Arbutin	+	Butyrate	+
Casein	+	Citrate	–
Gelatin	+	Oxalate	–
Guanine	+	Propionate	–
Hypoxanthine	+	Pyruvate	+
Starch	+	Succinate	+
Testosterone	+	Tartrate	–
Tween 80	+	Growth in presence of (g l^{-1})	
Tyrosine	+	Crystal violet (0.1)	+
Xanthine	+	NaCl (30)	–
		Lysozyme (0.05)	–
Utilisation of carbohydrates and derivatives		Phenol (0.5)	+
Adonitol	+	Phenol (1)	–
Arabinose	+	Potassium tellurite (0.1)	+
Cellobiose	+	Sodium azide (0.05)	+
Dextrin	+	Sodium azide (0.1)	–
Dulcitol	–		
Erythritol	–	Growth at	
Fructose	+	45°C	+
Galactose	+	48°C	–
Glucose	+	pH 5	–
α-Methyl-D-glucoside	–	pH 9	+
Glycerol	+		
Inositol	+	Growth in the presence of antibiotics (mg l^{-1})	
Lactose	+	Ampicillin (75)	+
Maltose	+	Benzyl-penicillin (10)	+
Mannitol	–	Chloramphenicol (25)	–
Mannose	+	Cycloserin (5)	+
Melibiose	+	Erythromycin (10)	+
Melezitose	–	Gentamicin (5)	+
Raffinose	–	Kanamycin (25)	+
Rhamnose	+	Novobiocin (10)	–
Ribose	+	Oleandomycin (75)	+
Sorbitol	–	Oxytetracycline (5)	+
Sucrose	–	Rifamycin (5)	–
Trehalose	+	Streptomycin (10)	+
D-Xylose	+	Sulfanylamide (75)	+
Nitrate reduction	+	Vancomycin (5)	+

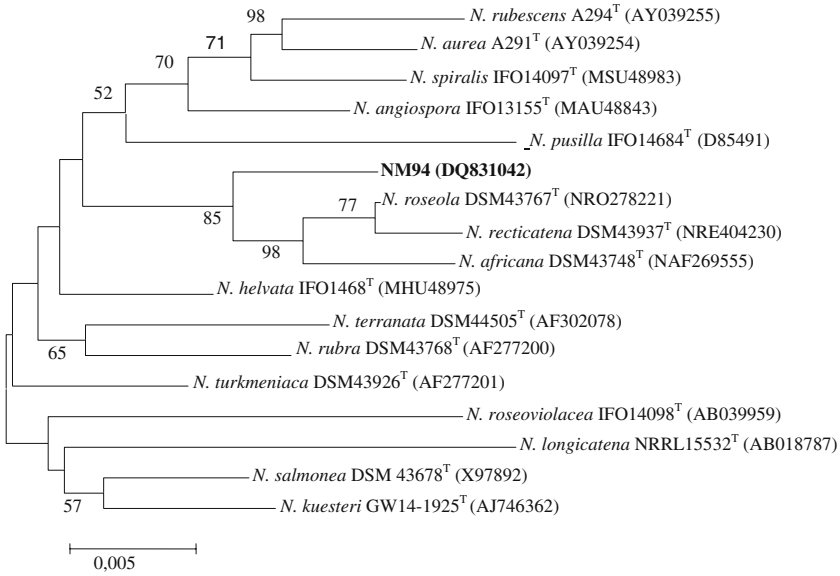

Fig. 2 Phylogenic tree based on 16S rDNA gene sequences showing relationships among strain NM94 and the most close type strain species of *Nonomuraea*. Numbers at nodes indicate percentages of bootstrap support based on a neighbor-joining analysis of 1,000 resampled datasets; only values above 50% are given. *Bar*, 0.005 substitutions per nucleotide position

Table 2 Antimicrobial activities of the strain NM94

Bioassay organisms	Inhibition zone (mm)[a]
Alternaria sp.	0
Botrytis cinerea	5
Fusarium oxysporum f. sp. *albedinis*	10
Geotrichum sp.	0
Mucor ramannianus NRRL 1829	12
Penicillium sp.	0
Pythium irregulare	12
Sclerotium sclerotiorum	0
Candida albicans IPA 200	0
Kluyveromyces lactis	12
Rhodotorula mucilaginosa	0
Saccharomyces cerevisiae ATCC 4227	12
Bacillus coagulans CIP 6625	14
Bacillus subtilis ATCC 66	18
Micrococcus luteus ATCC 9314	34
Staphylococcus aureus CIP 7625	10

The strain NM94 is non-active against the Gram-negative bacteria, *Agrobacterium tumefasciens, Escherichia coli* ATCC 10536, *Klebsiella pneumoniae* CIP 82.91, *Pseudomonas aeruginosa* CIP A22, *P. syringae* No. 1,882 and *Salmonella enterica* CIP 81.3

[a] Each value represents the average of three measurements

and the susceptibility to benzylpenicillin (10 mg l^{-1}), gentamicin (4 mg l^{-1}), oleandomycin (16 mg l^{-1}), rifampicin (0.5 mg l^{-1}), and vancomycin (2 mg l^{-1}).

These results strongly suggest that strain NM94 is a new species. The 16S rDNA sequence of strain NM94 has been deposited in GenBank under the accession Number DQ831042.

Antimicrobial activity of the strain NM94

The strain NM94 showed high activity against some Gram-positive bacteria, yeasts, and phytopathogenic fungi such as *Fusarium oxysporum* f. sp. *albedinis*, and *Pythium irregulare* (Table 2).

Fermentation studies

In liquid media, antibacterial activity was obtained on all tested media (Fig. 3). However, antifungal activity was observed only in ISP2, ISP4, Bennett and GYEA media (Fig. 4). Thus, the ISP2 medium was chosen as the production medium for the antifungal compounds.

A time course of growth and antibiotic production in ISP2 broth are shown in Fig. 5. The antibacterial activity started at the beginning of the exponential phase of growth (2 days) and reached maximum after 6 days. Antifungal activity was detected 4 days after incubation and reached a maximum at 6 days. The pH varied little (between 7.2 and 7.6) during the incubation. Maximum dry mycelial weight (15 g l^{-1}) was reached at 7 days of fermentation.

Isolation and purification of active compounds

On silica gel thin-layer chromatogram, dichloromethane extract was separated into eight bioautographic compounds with different Rf values, which were active against *B. subtilis*, *M. ramannianus* and/or *S. cerevisiae* (Table 3). The compound named 94A (Rf = 0.57) showed most interesting antimicrobial activity. It was selected and purified by HPLC. The active fractions were eluted with 80% methanol in water. The complex 94A was composed of five compounds (data not shown) designated 94A1, 94A2, 94A3, 94A4, and 94A5 eluted at retention times of 13, 13.30, 14, 14.30, and 14.50 min, respectively. These compounds have been

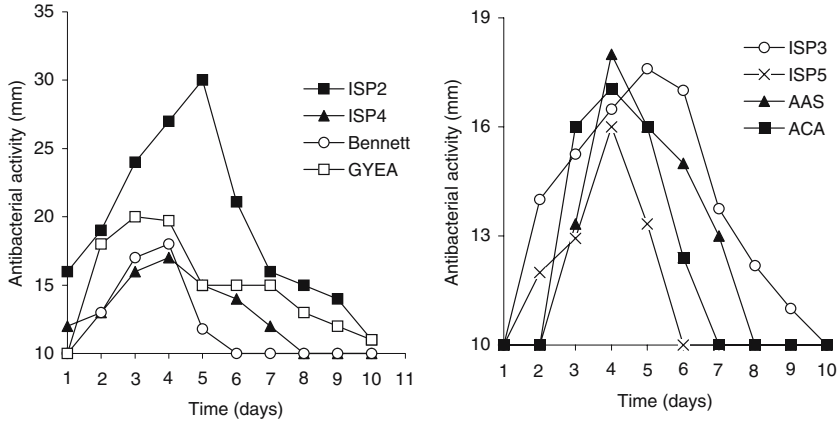

Fig. 3 Effect of various culture media on production of antibacterial activity in 500-ml flasks. Activity against *Bacillus subtilis*. Values include the diameter of wells (10 mm)

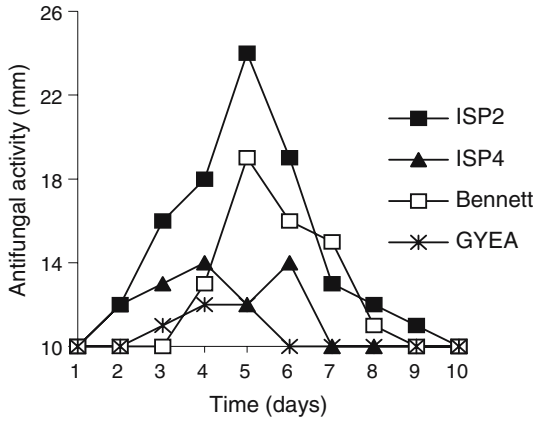

Fig. 4 Effect of various culture media on production of antifungal activity in 500-ml flasks. Activity against *Mucor ramannianus*. Values include the diameter of wells (10 mm)

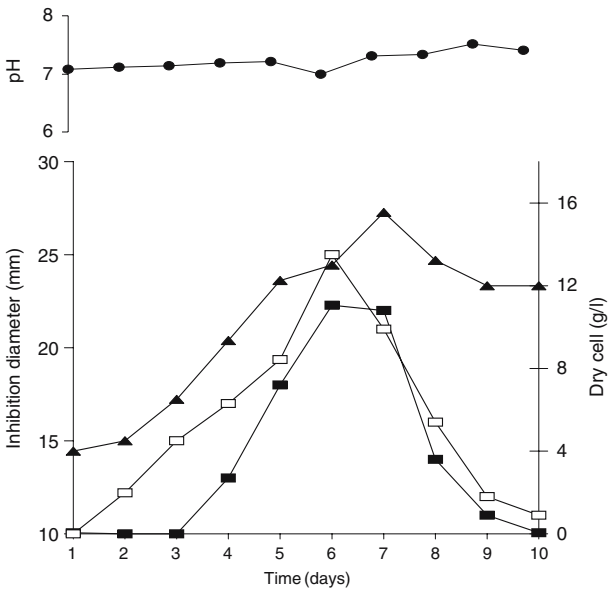

Fig. 5 Time course of antibiotic production by strain NM94 in fermentor on ISP2 broth. Antifungal activity (*filled square*); antibacterial activity (*open square*); dry cell (g l^{-1}) (*filled triangle*); pH (*filled circle*)

Table 3 Crude compounds produced by the strain NM94 on ISP2 medium and extracted with dichloromethane

Antibiotic	Rf[a]	Color under UV	Activity (mm)[b]		
			B.s.	*M.r.*	*S.c.*
94 T	0.86	Pale blue	18	7	0
94 B	0.73	Pale blue	25	18	10
94 A	0.57	Vivid blue	38	40	20
94 W	0.40	Blue purple	20	7	0
94 R	0.27	Vivid blue	10	0	0
94 C	0.20	Yellow	20	16	0
94 E	0.08	Blue purple	24	13	0
94 P	0	Green	17	0	10

B.s. Bacillus subtilis, M.r. Mucor ramannianus, S.c. Saccharomyces cerevisiae

[a] The Rf of antimicrobial compounds were calculated after bioautography

[b] The activity was measured by a paper disc method (6 mm in diameter)

recovered separately and have been re-injected several times until complete purification. The purified substances were whitish and presented a greasy appearance. They possess both antibacterial and antifungal properties. The chromogenic reactions were negative with $FeCl_3$, naphtoresorcinol-H_2SO_4 and ninhydrin reagents, suggesting the absence of phenol, osidic residues, and free amine groups.

Spectroscopic studies

The UV-visible spectra (data not shown) of the five pure products 94A1–A5 exhibited the same maxima at 227 and 272 nm, suggesting the presence of aromatic ring and the absence of polyenic structure. Infrared spectra showed a primary imine function (3,469–3,451 cm^{-1}), amine function (3,040, 673 cm^{-1}), alkane groups (2,958–2,853 cm^{-1}, 1,466–1,461 cm^{-1}) (C = C) of aromatic ring (1,639–1,495 cm^{-1}), p-disubstituted benzene (831 and 801 cm^{-1}) and secondary alcohol

function (3,469–3,451, 1,370–1,408, 1,192–1,198, 1,040–1,111 cm^{-1}). The ^1H NMR, showed some common main signals in the 0.5–2 and 2–2.7 ppm regions indicating the presence of alkyl groups. The signals at 6.9 ppm (doublet) and 7.7 ppm (doublet) indicate the presence of p-disubstituted ring aromatic. Molecular weights are 298, 312, 326, 340, and 340 for 94A1, 94A2, 94A3, 94A4, and 94A5, respectively. The difference among these four molecules is a value corresponding to CH_2 succession. 94A4 and 94A5 were probably isomeric forms. The Figs. 6, 7, 8 show the IR, ^1H NMR and mass spectra of the compound 94A4.

First results suggest that all these antibiotics belong to the same chemical family containing a benzenic ring substituted by aliphatic chains. *Nonomuraea* genus is reported to contain 15 species [38], which are known to

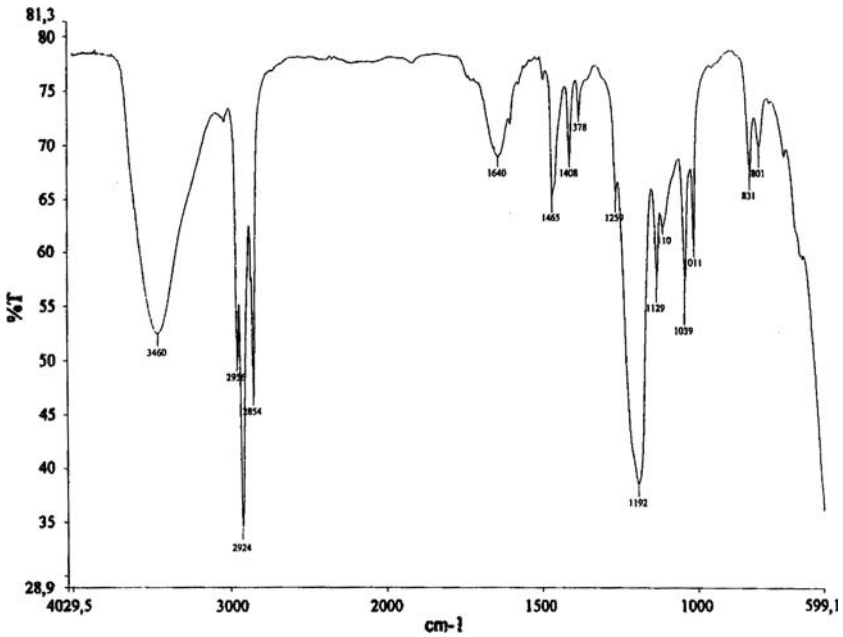

Fig. 6 IR spectrum of the 94A4 molecule in KBr

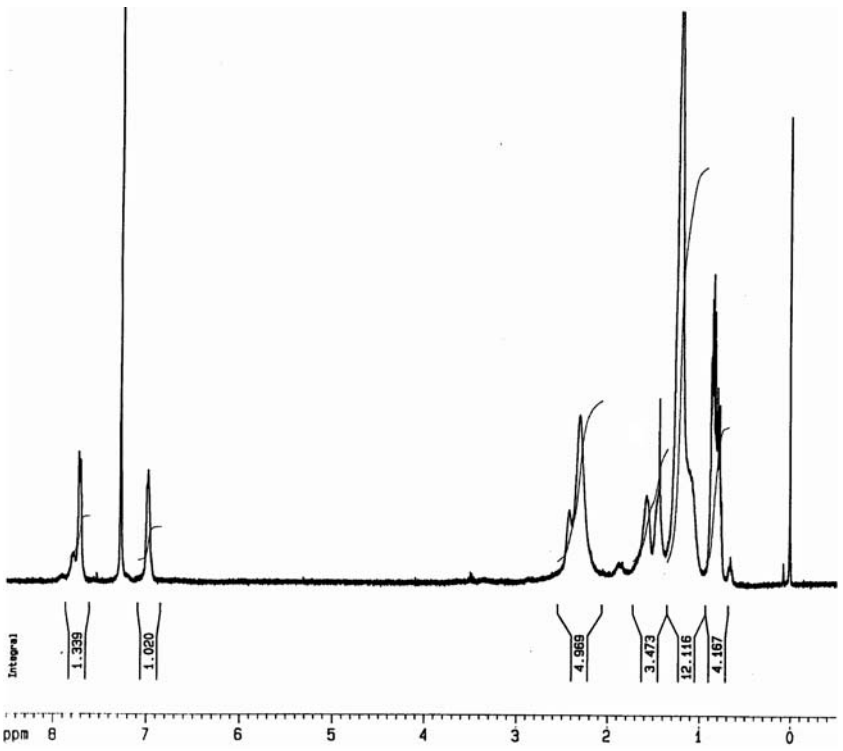

Fig. 7 ^1H NMR spectrum of the 94A4 molecule in $CDCl_3$

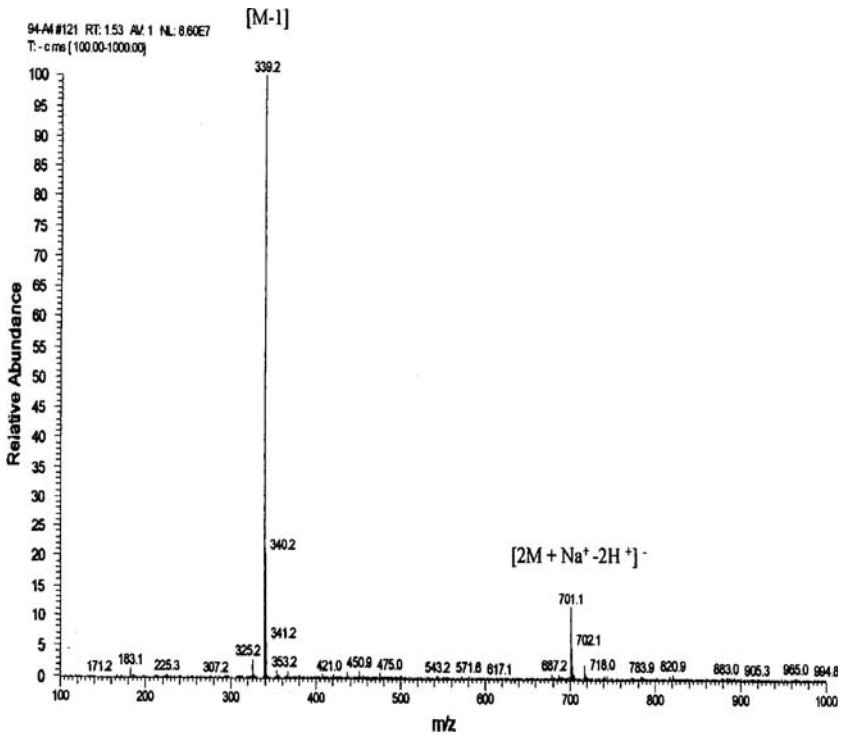

Fig. 8 Mass spectrum of the 94A4 molecule (electro spray ion source, negative mode)

produce antibiotics belonged to anthracyclin family: antibiotic AB 64 [36], akrobomycin [6], carminomycin, and maduromycin [9], napthoquinone family: rubromycin [6] and macrocyclic lactone family: madurahydroxylactone [17]. Our results indicate that the five antibiotics produced by *Nonomuraea* sp. NM94 differ from known antibiotics produced by *Nonomuraea* species and also the antibiotics described in Dictionary of antibiotics and related substances [9] and in Dictionary of natural products [8]. However, further studies will be necessary to determine the structures of the active compounds.

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