Appl Microbiol Biotechnol (2009) 81:839–845
DOI 10.1007/s00253-008-1684-y

A new strain of *Streptomyces avermitilis* produces high yield of oligomycin A with potent anti-tumor activity on human cancer cell lines in vitro

Xiuping Lin · Ying Wen · Meng Li · Zhi Chen · Jia Guo · Yuan Song · Jilun Li

Received: 11 May 2008 / Revised: 15 August 2008 / Accepted: 21 August 2008 / Published online: 20 September 2008

Abstract A new actinomycete strain, isolated from soil in China, strongly inhibited in vitro proliferation of human hepatoma, chronic myelogenous leukemia, and colonic carcinoma cell lines. The strain, designated L033, was identified as a strain of *Streptomyces avermitilis* based on cultural property, morphology, carbon source utilization, 16s rRNA gene analysis, and DNA–DNA relatedness studies. The anticancer component from L033 was purified to homogeneity by preparative positive-phase high-performance liquid chromatography and crystallization. Nuclear magnetic resonance and mass spectrometric analysis showed that this compound had the same structure as oligomycin A. Different with other reported naturally occurring strains of *S. avermitilis*, L033 produced high quantity of oligomycin A (maximal 1,461 μg/ml). Therefore, L033 was considered of great potential as an industrial oligomycin-A-producing strain.

Keywords *Streptomyces avermitilis* · Oligomycin A · Anti-tumor activity

Introduction

Cancer has now emerged as a major public health threat worldwide. World population growth and aging imply a progressive increase in the cancer burden in the future (Parkin 2001). In the USA, cancer has become the number one killer in age under 85 since 1999 (Twombly 2005). Although many anticancer drugs have been developed, most are also toxic to normal cells and tissues. Therefore, only a few of them have found a certain place in the treatment of cancer (Waksman 1966; Lokich 1980). Furthermore, intrinsic and/or acquired (multi-)drug resistance is often a major impediment to successful cancer chemotherapy (Hait 1996). To overcome these limitations and improve the effectiveness of chemotherapy for cancer, it is necessary to isolate new anticancer drugs. Fortunately, numerous anti-tumor metabolites with a variety of structures are produced by actinomycetes (Waksman 1966). These compounds are unrivaled and unmatched in medical significance.

In this work, we describe a new soil-inhabiting actinomycete strain, L033, which produces anticancer agent and exhibits potent anti-tumor activity against human hepatoma, chronic myelogenous leukemia, and colonic carcinoma cell lines. The isolate was identified as a strain of *Streptomyces avermitilis* based on taxonomic experiments. One anti-tumor component was purified, and its structure was determined to be the same as those of oligomycin A by nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). Strain L033 was distinguished from other wild-type strains of *S. avermitilis* reported (Miller et al. 1979; Ikeda et al. 1993) by high yield of oligomycin A among its fermentation products.

Materials and methods

Microbial strains

An actinomycete strain, termed L033, was isolated from a soil sample collected in Guangzhou City, China and grown on yeast extract–malt extract–soluble starch medium (YMS) agar

Electronic supplementary material The online version of this article (doi:10.1007/s00253-008-1684-y) contains supplementary material, which is available to authorized users.

X. Lin · Y. Wen · M. Li · Z. Chen · J. Guo · Y. Song · J. Li (✉)
State Key Laboratories for Agro-biotechnology and College of Biological Sciences, China Agricultural University, Beijing, People's Republic of China 100193
e-mail: lijilun@cau.edu.cn

(Ikeda et al. 1988) at 28°C. L033 was deposited at the China General Microbiological Culture Collection, as CGMCC4.5508. *S. avermitilis* ATCC 31267^T (MA-4680^T=NCIMB 12804^T= NRRL 8165^T) was purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA). The above strains were maintained on YMS agar at 4°C and as spore suspension in 20% (*v*/*v*) glycerol at −70°C.

Human tumor cell lines

Human hepatoma cell line Bel-7402 was obtained from the China Center for Type Culture Collection (Wuhan City). Human chronic myelogenous leukemia cell line K-562 and human colonic carcinoma cell line HCT-8 were from the Cell Culture Centre, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing City).

Cultural and morphological properties of strain L033

Strain L033 was inoculated onto peptone yeast extract iron agar (ISP-6) medium in order to determine melanin-producing ability. Its colony-forming and pigmentation properties were examined on inorganic salt-starch agar (ISP-4) and oatmeal agar (ISP-3) media after 14 days at 28°C. Spore chain morphology and spore surface features were examined by scanning electron microscopy of 7-day cultures grown on Bennett's agar. Using the cover technique described previously (Zhou et al. 1998; Kawato and Shinobu 1959), samples were observed with a Hitachi S-3400N scanning electron microscope, with secondary mode operating at 20 kV.

Carbon source utilization tests

Utilization of substrates as sole carbon and energy sources was tested as described by Kämpfer et al. (1991). Carbon sources were filter-sterilized, with a final concentration of 0.2% (*w*/*v*).

16S rDNA sequence and phylogenetic analysis

Genomic DNA was isolated from cells as described by Hopwood et al. (1985). The 16S rRNA gene of strain L033 was amplified by polymerase chain reaction, using two universal bacterial primers, 1492R (5′-GGTTACCTTGTTAC GACTT-3′) and Eubac27F (5′-AGAGTTTGATCCTGGCTC AG-3′; Jiang et al. 2006). The amplified products were purified using TIANgel mini purification kit (TianGen Biotech Beijing), ligated to pMD18-T simple vector (TaKaRa), and transformed into competent cells of *Escherichia coli* DH5α. 16S rRNA gene fragment was sequenced using forward primer M13F (−47) and reverse primer M13R (−48). The derived 16S rRNA gene sequence was compared to the GenBank database (NCBI), to search for similar sequences using the basic local alignment search tool algorithm. Similarity analysis was performed using ClustalW program (Thompson et al. 1994). A phylogenetic tree was constructed using the neighbor-joining method (Saitou and Nei 1987) and the Molecular Evolutionary Genetics Analysis (MEGA 3.1) software program (Kumar et al. 2004). Tree topology was evaluated by bootstrap analysis (Felsenstein 1985) based on 1,000 replicates.

DNA–DNA relatedness

Level of DNA–DNA relatedness between strain L033 and the type strain *S. avermitilis* ATCC 31267^T was determined by thermal denaturation method (De Ley et al. 1970), using a lambda 35 UV/Vis spectrophotometer (PerkinElmer) fitted with a PTP1 temperature programmer (PerkinElmer) and standard software. Results were expressed as the mean of three determinations.

Fermentation in shaken flasks

Spores of strain L033 stored in 20% glycerol at −70°C were inoculated on YMS agar plates and cultured at 28°C for 12 days. Five-hundred-milliliter Erlenmeyer flasks were filled with seed medium consisting of soluble starch (30 g), malt extract (2 g), soy peptone (2 g), and $CoCl_2.6H_2O$ (5 mg) per liter deionized water, which was inoculated with strain L033 by addition of small areas of growth cut from the agar plate. pH was adjusted to 7.0–7.2 before sterilization. Flasks were incubated at 28°C for 48 h on a rotating shaker (170 rpm).

Production medium G, also placed in 500-ml flasks, consisted of soluble starch (70 g), dried yeast (16 g), $MgSO_4.7H_2O$ (0.5 g), $K_2HPO_4.3H_2O$ (0.5 g), KCl (4 g), $CoCl_2.6H_2O$ (5 mg), and $CaCO_3$ (2 g) per liter deionized water, and was inoculated with 4-ml seed solution. pH was adjusted to 7.0–7.2 before sterilization, and flasks were incubated at 28°C on a rotating shaker at 170 rpm. After 10 days, broth from 156 flasks was combined and centrifuged, and the cell pellet was washed with deionized water and centrifuged again for isolation of substances showing anti-tumor activity.

Purification and structure determination of compound 1

The strain L033 cell mass (4,937 g) was mixed with acetone (3,800 ml), stood for 2 days, and filtered. The residue was added twice with acetone (2,000 ml, 1,500 ml), stood for 1 day, and filtered after each addition. The combined filtrates of three marinations were evaporated (55–65°C) to remove acetone. The aqueous residue was kept at 18°C overnight. A white solid appeared, which was collected, washed, with distilled water, and filtered. The resulting white solid was mixed with *n*-hexane/ethanol (96:4), stirred, and centrifuged. The clear supernatant was moved to a different container,

dried (Na_2SO_4), decolored (activated carbon), and centrifuged. The resulting supernatant was kept at 18°C overnight. A cream-colored crystalline precipitate appeared, which was collected, dissolved with *n*-hexane/ethanol (96:4), and separated by preparative positive-phase high-performance liquid chromatography (HPLC) using SiO_2 column (250× 20 mm, 10 μm) [Chuang Xin Tong Heng (Beijing City)], with *n*-hexane/ethanol mixture (95:5) as eluent, at a flow of 25 ml/min. Solutions of the same peak were combined and evaporated to remove *n*-hexane/ethanol. *n*-Hexane/ethanol (95:5) was added to the residue of fraction containing compound 1, and the mixture was stirred until pure white crystal precipitated. The crystal and supernatant were divided by centrifugation.

Structure of compound 1 was determined by ^{1}H- and ^{13}C-NMR spectroscopy, 2D NMR spectroscopy, and fast atom bombardment mass spectrometry (FAB-MS), performed at the National Center of Biomedical Analysis (Beijing City). ^{1}H- and ^{13}C-NMR, and 2D NMR spectra were recorded at ambient temperature or 300 K on a Varian Inova 600 (600 M) spectrometer. The solvent was $CDCl_3$.

HPLC analysis

Secondary metabolites were analyzed and quantified using a Waters 600 reversed-phase HPLC. Methanol (1 ml) was added to broth (1 ml), stirred for 30 min, and stood for 12 h. Cell residue was removed by centrifugation, and clear supernatant (20 μl) was injected onto a YMG-C18 column (250×4.6 mm, 10 μm). The solvent system was methanol/water (9:1) at a flow rate of 1 ml/min. Products were monitored by a Waters 2487 dual lambda absorbance detector at 246 nm.

Anti-tumor effect of compound 1 in vitro

3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay (Hansen et al. 1989) was employed to evaluate the anti-tumor effect of compound 1 on Bel-7402 hepatoma, K-562 leukemia and HCT-8 colonic carcinoma cells in vitro. Cells in a volume of 100 μl (5×10^3 cells/ml) were seeded onto 96-well plates and incubated at 37°C for 24 h in 5% CO_2 atmosphere. Various concentrations of compound 1 (each 10 μl) plus 90 μl RPMI-1640 medium were added; 100 μl RPMI-1640 medium was added to control wells. Plates were incubated at 37°C in 5% CO_2 atmosphere for 3 days. Supernatant was removed, 100 μl freshly prepared 0.5 mg/ml MTT per well was added to form formazan salt, and incubation continued at 37°C for 4 h. Supernatant was carefully removed and 200 μl/well dimethyl sulphoxide was added to dissolve the MTT formazan salt. The solution was mixed, absorbance was read on an enzyme-linked immunosorbent assay (ELISA) reader at 544 nm, and growth-inhibitory ratio was calculated using the formula $(1-A/B)\times100\%$, where A and B are mean absorbance of the treated and control wells, respectively. 5-Fluorouracil (5-FU) was used as reference compound for positive control.

Nucleotide sequence accession number

The nucleotide sequence of 16S rRNA gene reported in this article was assigned to the GenBank accession no. EU621830.

Results

Characterization and identification of isolated strain L033

A bacterial isolate from a soil sample collected in Guangzhou City, China displayed strong anti-tumor activity against human hepatoma, chronic myelogenous leukemia, and colonic carcinoma cell lines. The cultural and morphological properties suggested that the isolate, termed L033, was a strain of actinomycete. Sporulation occurred on standard media such as inorganic salt-starch agar and oatmeal agar. Aerial spore masses of light gray and gray color, respectively, were formed on these media. Melanin pigments were produced on peptone yeast extract iron agar. L033 formed an extensively branched substrate mycelium and aerial hyphae that differentiated into long, compact spiral chains, which became looser as the culture aged. The spore chains were composed of spherical to oval-shaped spores, with smooth surfaces (Fig. 1). L033 was able to use glucose, D-fructose, inositol, mannitol, L(+)-arabinose, D-xylose, L(+)-rhamnose, sodium citrate, D-galactose, glycerol, maltose, lactose, or D-mannose as sole carbon source, but was not able to use melezitose, L(−)-sorbone, inulin, or cellulose. Nearly complete 16S rRNA gene sequence (1487 bp) of L033 was obtained and was found to be most similar to those of *S. avermitilis* ATCC 31267^{T}, *S. cellostaticus* NBRC 12849^{T}, and *S. griseochromogenes* NBRC 13413^{T}, with sequence identities of 99.9%, 98.9%, and 98.9%, respectively. *S. avermitilis* ATCC 31267^{T} appeared to be the closest relative. A phylogenetic tree was constructed, using the neighbor-joining method based on similarity of a 1,461-bp consensus length of 16S rRNA gene sequence (Fig. 2), and confirmed that L033 grouped most closely with *S. avermitilis* ATCC 31267^{T}. DNA–DNA relatedness studies were conducted between L033 and *S. avermitilis* ATCC 31267^{T}. The mean DNA–DNA hybridization value of three determinations was 100%. The properties of culture, morphology, and carbon source utilization of strain L033 were consistent with those of *S. avermitilis* ATCC 31267^{T} as described by Kim and Goodfellow (2002). The 16S rRNA gene sequence identity and DNA–DNA relatedness data confirmed that strain L033 belonged to *S. avermitilis*.

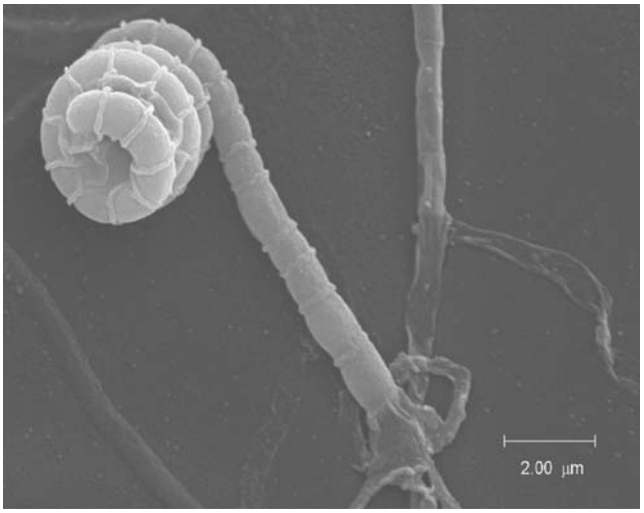

Fig. 1 Scanning electron micrograph of *Streptomyces avermitilis* L033 grown on Bennett's agar at 28°C for 7 days. The spiral spore chains consist of spores with smooth surfaces

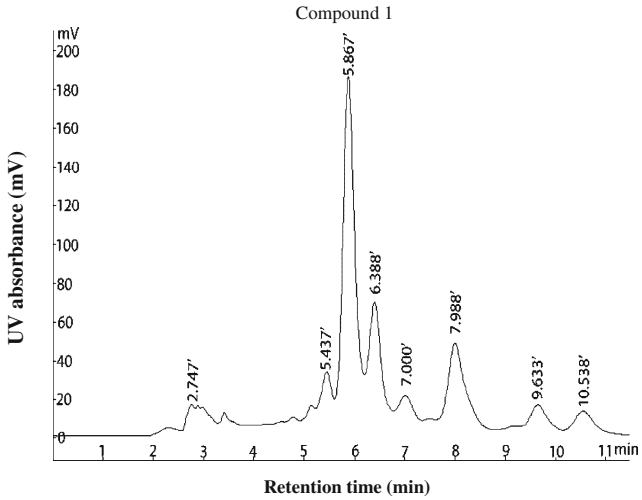

Fig. 3 High-performance liquid chromatography profile of extract from broth of *Streptomyces avermitilis* L033 at 246 nm. The solvent system was methanol/ water (9:1). Compound 1 displayed potent anti-tumor activity against human hepatoma, chronic myelogenous leukemia, and colonic carcinoma cell lines

Fermentation

Production of compound 1 by strain L033 in medium G at 28°C started after 1 day of fermentation. The maximal concentration of compound 1 was observed after 9 days of fermentation. A 4,937-g cell pellet was obtained from combined broth of 156 flasks for isolation of substances displaying anti-tumor activity. When cottonseed protein flour (16 g/l) was substituted for dried yeast as the nitrogen source in production medium G, production of compound 1 was 1,461 μg/ml.

Isolation and in vitro anti-tumor activity of compound 1

In tests against human tumor cells, six fractions prepared from crude extract by preparative positive-phase HPLC were determined. Of these, fraction 3 (containing compound 1, retention time=5.867′; Fig. 3) was found to have anti-tumor activity; 191.4 mg of compound 1 were isolated from the 4,937-g cell pellet as above and purified to homogeneity by preparative positive-phase HPLC and crystallization.

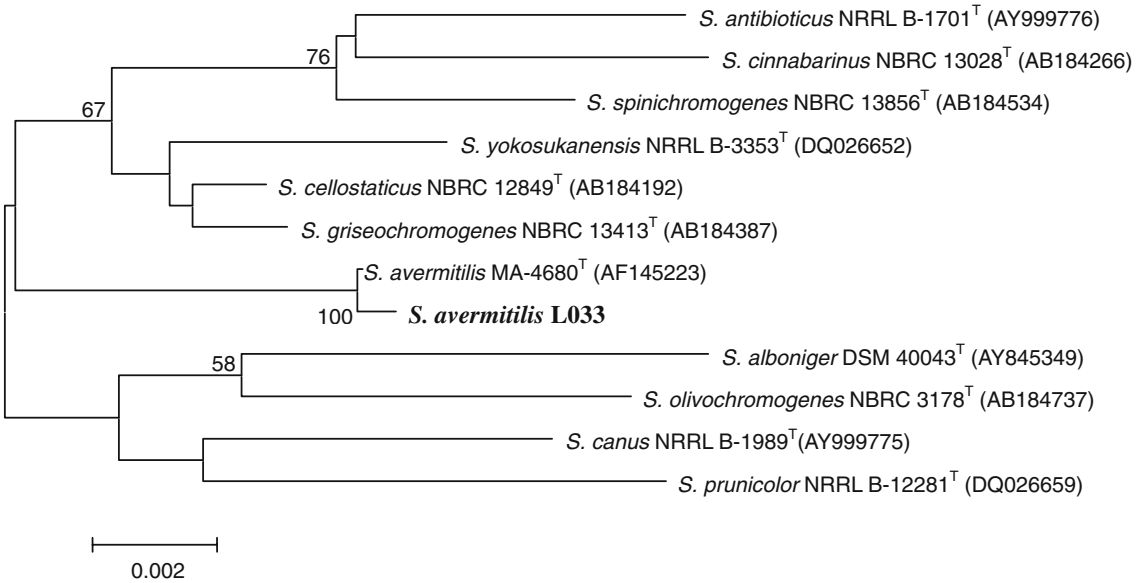

Fig. 2 Neighbor-joining tree based on nearly complete 16S rRNA gene sequences, showing phylogenetic relationships between *Streptomyces avermitilis* L033 and related *Streptomyces* species. *Numbers at nodes* indicate bootstrap values from 1,000 replicates. GenBank accession numbers are given in *parentheses*. *Bar*, 0.2% sequence divergence

The ability of compound 1 to inhibit human tumor cell proliferation was investigated in vitro using the MTT assay. 5-FU, a commonly used agent for treatment of malignant tumors of the digestive tract, was used as the reference compound for positive control. Compound 1 inhibited cell proliferation of Bel-7402, K-562, and HCT-8 in a dose-dependent manner (Table 1). For compound 1 at a concentration of 5.0×10^{-5} μg/ml (i.e., 6.3×10^{-5} μM), rates of inhibition against Bel-7402, K-562, and HCT-8 were 29.9%, 43.7%, and 53.5%, respectively. In contrast, for 5-FU at a concentration of 5.0×10^{-2} μg/ml (i.e., 3.8×10^{-1} μM), rates of inhibition against these three cell lines were near or below zero. Thus, compound 1 was much more potent than 5-FU at inhibiting proliferation of these human tumor cell lines.

Structural determination of compound 1

The structure of compound 1 was determined by ^{1}H-NMR, ^{13}C-NMR, and MS analysis and compared with those of oligomycin A. Signals corresponding to 26-membered macrolide structures were found in the ^{1}H- and ^{13}C-NMR spectra of compound 1 (see Supplemental material). ^{13}C-NMR chemical shifts of compound 1 were identical to those of oligomycin A (Carter 1986). The molecular ion of compound 1 (M+H, *m/z* 791.3) indicated a molecular weight (MW) of 790.3 (Fig. 4), consistent with the MW of oligomycin A. On the basis of the above data in combination with 2D NMR studies (data not shown), we concluded that compound 1 is oligomycin A (Fig. 5).

Discussion

A new actinomycete strain, *S. avermitilis* L033, was isolated from a soil sample during a screening program and displayed strong anti-tumor activity against human hepatoma, chronic myelogenous leukemia, and colonic carcinoma cell lines in vitro. Strain L033 was identified on the basis of cultural property, morphology, carbon source utilization, 16S rRNA gene sequence, and DNA–DNA relatedness. Compound 1, with anticancer activity, was isolated from the cells and identified as oligomycin A. L033 is the first reported naturally occurring strain of *S. avermitilis* producing a high yield of oligomycin A (maximal 1,461 μg/ml). In contrast, other reported wild-type strains of *S. avermitilis* produced low oligomycin (Miller et al. 1979; Ikeda et al. 1993).

Oligomycin and its analogues are a series of 26-membered macrocyclic lactones. Many of them display strong anti-tumor activity (Kobayashi et al. 1987; Yamazaki et al. 1992; Kim et al. 1997). For example, oligomycin SC-1 and SC-2 exhibit antiproliferative activity against mouse P388 lympholeukemia cells (IC_{50}=0.0013 and 0.00033 μg/ml, respectively; Daisuke et al. 1997). The antibiotic NK86-0279, a structural analogue, displays growth-inhibitory effect on a variety of mouse and human cancer cells (IC_{50}=0.0027~2.13 μg/ml; Nishikiori et al. 1991). In a study of 37,000 molecules tested against 60 human cancer cell lines as mitochondrial targeting agents, oligomycin was among the top 0.1% most cell line selective agents. In a study using R-HepG2 cells, oligomycin was able to bypass doxorubicin (Dox) resistance and trigger apoptosis (Li et al. 2004). Oligomycin ABC mixture reduced survival of P388 lympholeukemia cells to 54% at a concentration of 30 pg/ml, which is $>10^{3}$ times lower than the level required to inhibit respiration. Oligomycin A was more efficient than oligomycin ABC mixture in inhibiting P388 growth. The authors hypothesized that oligomycin at low concentration interferes with signaling events of apoptosis, which differ in tumor vs. normal cells (Korystov et al. 2003). Thus, oligomycin A has potential application as an anti-tumor agent. However, little is known about the effect of oligomycin A on other cancer cell lines.

When anti-tumor activity against human tumor cell lines Bel-7402, K-562, and HCT-8 in vitro was examined by MTT assay in this study, oligomycin A was found to inhibit

Table 1 *In vitro* sensitivity of cancer cell lines to compound 1, detected by MTT assay

Conc.[a]	Bel-7402		K-562		HCT-8	
	Compound 1	5-FU	Compound 1	5-FU	Compound 1	5-FU
50	87.74[b]	ND	91.55	ND	88.03	ND
5	54.17	62.93	56.47	62.98	57.17	59.13
0.5	31.17	37.99	48.10	30.77	46.34	32.76
0.05	34.59	-3.88	45.45	12.36	48.64	-5.08
0.005	32.12	-9.76	49.11	5.64	48.23	-1.76
0.0005	31.40	ND	48.14	ND	50.84	ND
0.00005	29.94	ND	43.72	ND	53.54	ND

5-FU: positive control
ND Not done
[a] Concentrations (μg/ml) of drugs in MTT assay
[b] Data shown as percentage inhibition rate

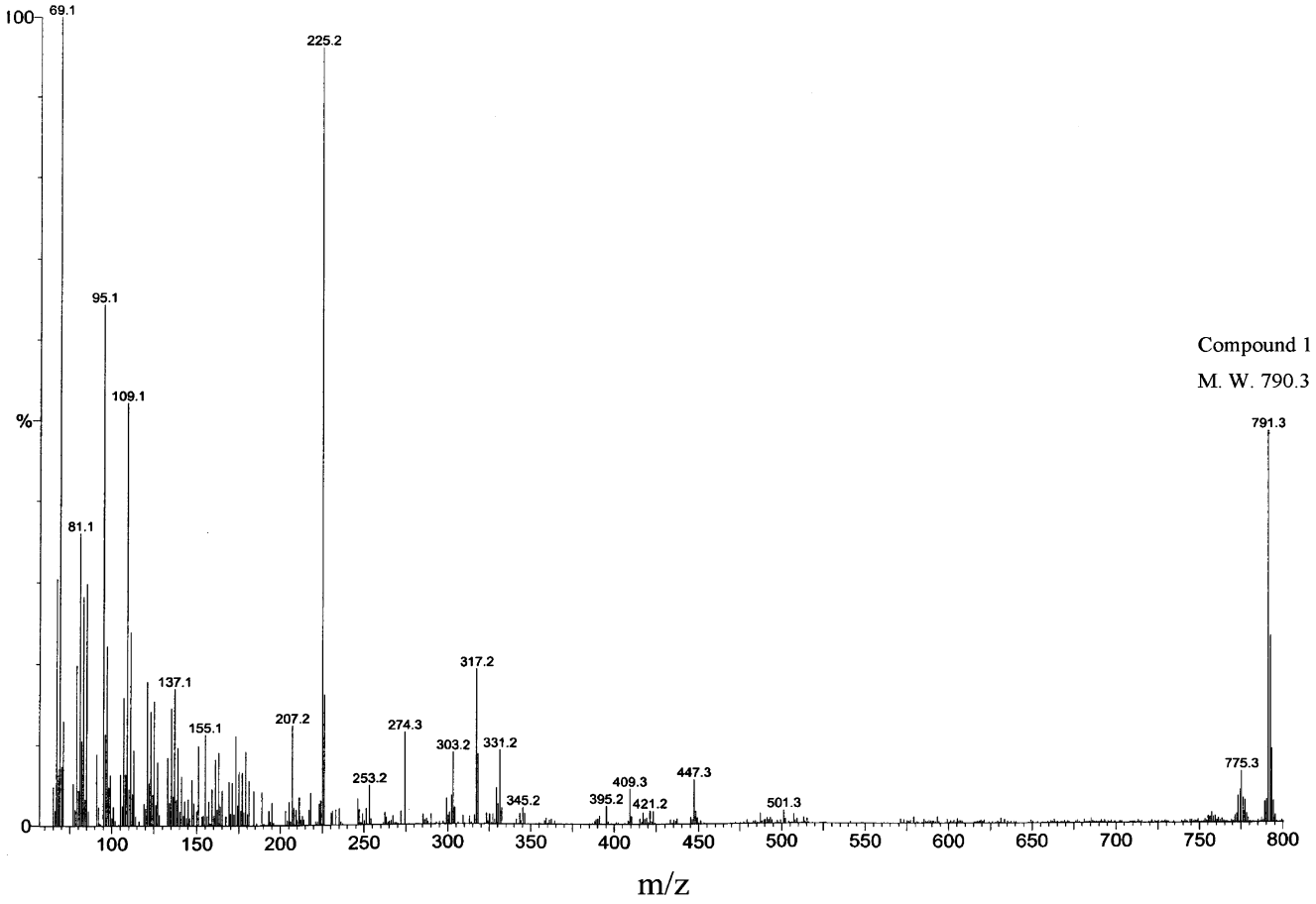

Fig. 4 Determination of molecular weight of compound 1 by FAB-MS in positive-ion mode. Spectral data showed that the molecular weight of compound 1 was 790.3

proliferation of these cell lines much more strongly than 5-FU. Inhibition rates of oligomycin A at a concentration of 5.0×10^{-5} μg/ml against Bel-7402, K-562, and HCT-8 cells were 29.9%, 43.7%, and 53.5%, respectively. This concentration was thousands of times lower than that required to inhibit respiration (Currie and Gregg 1965). These results were similar to those reported by Korystov et al. (2003).

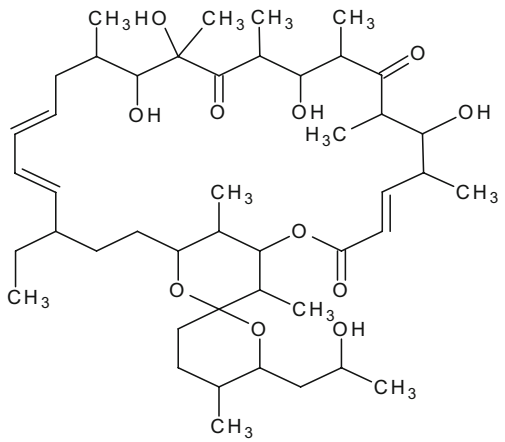

Fig. 5 Structure of compound 1 isolated from *Streptomyces avermitilis* L033

During procedures for isolation of compound 1, the aqueous residue of the marinated solution formed aqueous, oily, and solid layers (from bottom to top) when left at 18°C overnight. The solid layer, which contained most of the desired antibiotic compound, was recovered for further purification; the other two layers, which contained small quantity of the compound, were discarded. An immiscible organic solvent for second extraction (Albers-Schönberg et al. 1982; Visser et al. 1960) was therefore not required in the present study but could provide an economical method for additional isolation if applied industrially.

In summary, oligomycin A, a useful anti-tumor agent, was isolated with high yield and high purity from strain L033 cells by a relatively simple procedure. The yield can be further improved through induced breeding, optimized composition of culture medium, and production in tanks. *S. avermitilis* strain L033 should be great potential for industrial production of oligomycin A.

Acknowledgments This study was supported by grants from the National Basic Research Program of China (grant no. 2003CB114205) and the National High Technology Research and Development Program (grant no. 2006AA10A209). Anti-tumor activities of oligomycin A *in vitro* were determined by the National Center for Pharmaceutical Screening, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College. We are grateful to Han Bing (Beijing Institute of Biomedicine) for structural elucidation of the compound.

References

Albers-Schönberg G, Wallick H, Ormond RE, Miller TW, Burg RW (1982) Novel substances and process for their production. U S Patent 4,310,519

Carter GT (1986) Structure determination of oligomycin A and C. J Org Chem 51:4264–4271

Currie WD, Gregg CT (1965) Inhibition of the respiration of cultured mammalian cells by oligomycin. Biochem Biophys Res Commun 21:9–15

Daisuke K, Makoto K, Kaoru Y, Kazuyoshi Y, Mayumi K (1997) Oligomycin SC compounds and anticancer medicine. Japan Patent JP9208587

De Ley J, Cattoir H, Reynaerts A (1970) The quantitative measurement of DNA hybridization from renaturation rates. Eur J Biochem 12:133–142

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Hait WN (1996) Drug resistance. Kluwer, Boston

Hansen MB, Nielsen SE, Berg K (1989) Re-examination and further development of a precise and rapid dye methods for measuring cell growth/cell kill. J Immunol Methods 119:203–210

Hopwood DA, Bibb MJ, Chater KF, Kieser T, Bruton CJ, Kieser HM, Lydiate DJ, Smith CP, Ward JM, Schrempf H (1985) Genetic manipulation of *Streptomyces*: a laboratory manual. The John Innes Foundation, Norwich, UK

Ikeda H, Kotaki H, Tanaka H, Ōmura S (1988) Involvement of glucose catabolism in avermectin production by *Streptomyces avermitilis*. Antimicrob Agents Chemother 32:282–284

Ikeda H, Takada Y, Pang C-H, Tanaka H, Ōmura S (1993) Transposon mutagenesis by Tn*4560* and applications with avermectin-producing *Streptomyces avermitilis*. J Bacteriol 175:2077–2082

Jiang H, Dong H, Zhang G, Yu B, Chapman LR, Fields MW (2006) Microbial diversity in water and sediment of Lake Chaka, an athalassohaline lake in northwestern China. Appl Environ Microbiol 72:3832–3845

Kämpfer P, Steiof M, Dott W (1991) Microbiological characterization of a fuel-oil contaminated site including numerical identification of heterotrophic water and soil bacteria. Microb Ecol 21:227–251

Kawato M, Shinobu R (1959) On *Streptomyces herbaricolor* sp. nov., supplement: a single technique for microscopical observation. Mem Osaka Unit Lib Arts Educ B Nat Sci 8:114–119

Kim SB, Goodfellow M (2002) *Streptomyces avermitilis* sp. nov., nom. rev., a taxonomic home for the avermectin-producing streptomycetes. Int J Syst Evol Microbiol 52:2011–2014

Kim HS, Band HJ, Lee SY, Yoo OJ, Yoo JC, Kim YH, Lee JJ (1997) 44-Homooligomycin E, a new cytotoxic macrolide antibiotic from *Streptomyces ostreogriseus*. Biosci Biotechnol Biochem 61:378–380

Kobayashi K, Nishino C, Ohya J, Sato S, Mikawa T, Shiobara Y, Kodama M, Nishimoto N (1987) Oligomycin E, a new antitumor antibiotic produced by *Streptomyces* sp. MCI-2225. J Antibiot (Tokyo) 40:1053–1057

Korystov YN, Kublik LN, Kudryavtsev AA, Levitman M, Shaposhnikova VV, Drinyaev VA, Mosin VA, Kruglyak EB, Sterlina TS, Chailakhyan LM (2003) Opposite effects of low oligomycin concentrations on the apoptosis of normal and tumor cells. Dokl Biol Sci 392:475–477

Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Brief Bioinform 5:150–163

Li YC, Fung KP, Kwok TT, Lee CY, Suen YK, Kong SK (2004) Mitochondria-targeting drug oligomycin blocked P-glycoprotein activity and triggered apoptosis in doxorubicin-resistant HepG2 cells. Chemotherapy 50:55–62

Lokich JJ (1980) Clinical cancer medicine: treatment tactics. Hall, Boston, USA, pp 1–15

Miller TW, Chaiet L, Cole DJ, Cole LJ, Flor JE, Goegelman RT, Gullo VP, Joshua H, Kempf AJ, Krellwitz WR, Monaghan RL, Ormond RE, Wilson KE, Albers-Schönberg G, Putter I (1979) Avermectins, new family of potent anthelmintic agents: isolation and chromatographic properties. Antimicrob Agents Chemother 15:368–371

Nishikiori T, Yamazaki M, Saito S, Shimada N, Kurokawa T, Hirose K, Yamshita T, Tsuchiya T, Harada T (1991) Antibiotic NK86–0279, process for production of the same and application of the same. US Patent 5,003,056

Parkin DM (2001) Global cancer statistics in the year 2000. Lancet Oncol 2:533–543

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Twombly R (2005) Cancer surpasses heart disease as leading cause of death for all but the very elderly. J Natl Cancer Inst 97:330–331

Visser J, Weinauer DE, Davis RC, Peterson WH, Nazarewicz W, Ordway H (1960) Production and isolation of the antibiotic, oligomycin. J Biochem Microbiol Tech Eng 11:31–48

Waksman SA (1966) Antibiotics today. Bull N Y Acad Med 42:623–632

Yamazaki M, Yamashita T, Harada T, Nishikiori T, Saito S, Shimada N, Fujii A (1992) 44-Homooligomycins A and B, new antitumor antibiotics from *Streptomyces bottropensis*. Producing organism, fermentation, isolation, structure elucidation and biological properties. J Antibiot (Tokyo) 45:171–179

Zhou ZH, Liu ZH, Qian YD, Kim SB, Goodfellow M (1998) *Saccharopolyspora spinosporotrichia* sp. nov., a novel actinomycete from soil. Int J Syst Bacteriol 48:53–58