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Production of dihydroabikoviromycin by *Streptomyces anulatus*: production parameters and chemical characterization of genotoxicity

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J. HOLMALAHTI, O. RAATIKAINEN, A. VON WRIGHT, H. LAATSCH, A. SPOHR, O.K. LYNGBERG AND J. NIELSEN. 1998. The *Streptomyces* strain *Streptomyces anulatus* JH801 showed submerged sporulation and production of a genotoxic activity. The genotoxin production of this strain was found to be correlated to morphology and is likely to be related to sporulation. The influence of the medium on genotoxin production was investigated. Genotoxin production and sporulation were not inhibited by glucose, but were negatively affected by low ammonia concentrations. No correlation was found between genotoxin production and other morphological parameters such as total hyphal length and total average tip number. Bioassay-directed fractionation of the chloroform extract of *S. anulatus* JH801 resulted in the isolation of dihydroabikoviromycin. This compound shows selective activity towards DNA repair-deficient *Escherichia coli*. The isolation and structure of this genotoxin are reported.

INTRODUCTION

Actinomycetes are soil bacteria, which produce many kinds of secondary metabolites. Some of these metabolites suppress the growth of other microbes (Cross 1982). Actinomycetes have been isolated from different soils, plant material, waters and marine sediments (Hunter-Cevera *et al*. 1986; Pisano *et al*. 1987; Jensen *et al*. 1991) and also from buildings with moisture problems (Räty *et al*. 1995). In the present screening programme, actinomycetes were isolated from two different environments: shore sediments of a marsh pond heavily polluted by pulp mill effluent; and typical topsoil from Finnish coniferous woodland. Several antifungal, antibacterial or genotoxic isolates were obtained (Holmalahti *et al*. 1994).

Initial screening of the organic extracts from one of the isolates, JH801, showed that a chloroform extract of the cultivation medium contained potent genotoxic agents. The active components were found to be stable with little loss of activity during storage.

In this paper, the taxonomy of JH801 and its morphological characteristics during genotoxin production is described. The correlation between the morphology of filamentous microorganisms and their product formation in relation to other cultivation parameters such as medium composition, agitation rate, temperature and pH has been investigated for several other species (Trinci 1971; Miles and Trinci 1983). Elucidating the morphological properties connected with the production and secretion of desired compounds has, in several cases, led to a better understanding of the relation between morphology and productivity. This, in turn, should give some indications of the characteristics that should be expressed in new and improved strains. In this paper, the isolation and purification of one of the genotoxic compounds and its identification as dihydroabikoviromycin is also described.

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MATERIALS AND METHODS

Organism

The strain JH801 was isolated from a soil sample collected from coniferous woodland around Kuopio, Finland using the method described by Holmalahti *et al*. (1994).

Media

All batch and fed-batch cultivations were carried out using a defined medium (Table 1).

The medium composition varied in the individual cultivations with respect to the limiting substrate, which was either glucose, ammonium or phosphate. Pluronic® F68 (Sigma) was used as an antifoam agent. The feed medium for fed-batch cultivations contained 80 g l^{-1} glucose as the only carbon source. All other substrates were supplied in excess. Shake flask cultivations aimed at the chemical identification of the genotoxic compounds produced were carried out using a complex medium consisting of Glycerol-Arginine-Yeast extract (GAY) medium (glycerol (85%), 12·5 g; arginine monohydrochloride, 1·0 g; Difco yeast extract, 3·0 g; $MgSO_4$, 0·5 g in 1000 ml distilled water, pH 7·0).

Bioreactors

The cultivation experiments were carried out in a 5 l in-house-built bioreactor (equipped with four baffles and two Rushton four-blade disk turbines with a working volume of 4·5 l). Temperature, pH and agitation rate were controlled during the cultivations. The pH was kept constant by adding either 1 mol l^{-1} H_2SO_4 or 4 mol l^{-1} NaOH.

Batch and fed-batch cultivations

A spore suspension (about 10^9–10^{10} spores l^{-1} medium) was used as inoculum. Culture temperature and pH were maintained at 30 °C and 6·0, respectively. The aeration rate and agitation rate were kept constant at 1 vvm and 500 rev min^{-1}, respectively.

Table 1 Composition of the defined media

		Limiting ingredient	
Nutrients	Carbon	Ammonia	Phosphate
Glucose (g l^{-1})	16·0, 1·0*	16·0	16·0
$(NH_4)_2SO_4$ (g l^{-1})	6·0	2·0†, 1·0‡	6·0
K_2HPO_4 (g l^{-1})	1·0	1·0	0·5§
NaCl (g l^{-1})	1·0	1·0	1·0
$MgSO_4$. $7H_2O$ (g l^{-1})	1·0	1·0	1·0
$CaCl_2$. $2H_2O$ (g l^{-1})	0·1	0·1	0·1
$ZnSO_4$. $7H_2O$ (mg l^{-1})	3·575	3·575	3·575
$CuSO_4$. $5H_2O$ (mg l^{-1})	6·25	6·25	6·25
$NiCl_2$. $6H_2O$ (mg l^{-1})	1·25	1·25	1·25
$FeSO_4$. $7H_2O$ (mg l^{-1})	3·45	3·45	3·45

* Morphological studies.
† Ammonia limiting culture when 75% of glucose was used.
‡ Ammonia limiting culture when 50% of glucose was used.
§ Phosphate limiting just before glucose exhaustion.

Shake flask experiments

Cultures of JH801 were stored as spore suspensions in plastic tubes at −20 °C. An aliquot (20 ml) of spore suspension was pre-germinated in a 10 ml tube containing 5 ml GAY medium. Pre-germination was carried out at 30 °C for 2 d on a rotary shaker (150 rev min^{-1}). Seed cultures (50 ml for optimal pellet formation) were transferred into 250 ml Erlenmeyer flasks containing 100 ml GAY medium. The cultivations were carried out for 7 d at 30 °C and 150 rev min^{-1} on a rotary shaker.

Analytical methods

Samples containing 70–100 g of the cultivation medium were withdrawn from the bioreactor at 1–6 h intervals. The biomass concentration was measured by filtering the sample on a dry and pre-weighed filter, washing with 40 ml 0·9% (w/v) NaCl solution, followed by drying at 105 °C for 24–48 h and measuring the weight gain. The extracellular glucose concentration was measured by flow injection analysis. The analysis was based on enzymatic oxidation of glucose by glucose oxidase and determination of the formed hydrogen peroxide by a chemiluminescence reaction with luminol (Benthin *et al.* 1991).

Exhaust gas

The exhaust gas was analysed for oxygen and carbon dioxide by paramagnetic and infrared analysis (both Servomex Analyser Series 1400; Servomex Int. Ltd, East Sussex, UK). The airflow was measured by a mass flow controller (Brooks 5850 Tr, Brooks Instrument Division, The Netherlands).

Quantification of the morphology

Methods described by the International *Streptomyces* Project (ISP; Williams *et al.* 1983a, 1983b) were used to determine the morphological and physiological characteristics of the strain JH801. The morphology was quantified using an automatic image analysis system consisting of a microscope (Nikon Optiphot-2; Nikon, Japan), a CCD camera (Sony, Japan) and an image analyser (Quantimet 600; Leica, Germany). Spores were counted using the image analysis system combined with a counting chamber.

Genotoxin activity

The genotoxicity of extracts was screened by a bacterial repair assay using a DNA repair-proficient *Escherichia coli* WP2

trpE56 (Green and Muriel 1976) and its repair-deficient derivative CM871 *trpE65*, *urvA155*, *recA56*, *lexA* (Tweats *et al.* 1981), donated by Professor B.A. Bridges (University of Sussex, Brighton, UK). The bacteria were spread as a soft agar overlay on M9 minimal plates (Sambrook *et al.* 1989) containing 20 mg L-tryptophan ml^{-1}. Dimethyl sulphoxide (DMSO) solutions of the fractions were pipetted in 100 μl aliquots into wells (diameter 9 mm, depth 4 mm) made in the test plates. After overnight incubation at 37 °C the inhibition zones around the wells were measured. A difference of more than 5 mm in the diameter of inhibition zones between the repair-proficient and -deficient strains was considered a positive response. Green and Muriel (1976) have reported that the logarithm of genotoxic activity is proportional to the radius of the inhibition zone. This hypothesis was tested experimentally by using two dilution series of fermentation samples ($n = 2$). According to the results, the range from 2 to 8 mm of the radius of the inhibition zone is linear to the logarithm of the concentration. The correlation coefficient of the regression line was calculated to be 0·998. In the fermentation experiments the radius of the measured inhibition zone was transformed, using this correlation, to arbitrary units (abt. unit).

Extraction and purification of active compound

The cultivation medium from shake flask cultures (2500 ml) was filtered to remove the biomass. The supernatant fluid was alkalized by adding 1/5 volume (500 ml) 1 mol l^{-1} NH_4OH and extracted three times with 1/10 volume (250 ml each) chloroform. The extracts were combined and concentrated to dryness *in vacuo* to yield a crude solid.The crude solid was dissolved in water, filtered and re-extracted with chloroform. This solid was applied to a thin-layer chromatography (TLC) silica gel plate (Silica 60f254; Merck) which was sprayed with 50 ml 0·1 mol l^{-1} KOH in methanol. The plate was developed with a mixture of cyclohexane : toluene : diethylamine (85 : 10 : 15, v/v/v). The active fraction was visualized under a u.v. lamp (254 nm), homogenized and transferred to a plastic syringe. The active fraction migrated to R_F 0·15–0·20, and was subsequently eluted with methanol (5 × silica volume) and evaporated to dryness. The residue was fractionated by semi-preparative high-performance liquid chromatography (HPLC). The stationary phase consisted of LiChrospher RP-8 (5 mm; Merck) in a 125 × 10 mm column. Elution was isocratic at a flow rate of 2 ml min^{-1} using methanol : 0·05 mol l^{-1} ammonium acetate (70 : 30) as the mobile phase. The eluent was monitored at 264 nm and a fraction with 5 min retention time was collected. After fractionation methanol was evaporated *in vacuo* and the residue was alkalized by adding 1/5 volume 1 mol l^{-1} NH_4OH and extracted three times with 1/10 volumes of chloroform. The chloroform phase was washed twice with water and evaporated to dryness. The pure compound (25 mg) was lyophilized and stored at −20 °C.

Identification

The u.v. spectrum was measured with a Hitachi 220 double-beam spectrophotometer (Tokyo, Japan). The i.r. spectrum was measured in KBr disks on a Nicolet 510P FT-IR spectrometer (Madison, WI, USA). Mass spectra were obtained using a VG TRIO 2 quadrupole mass spectrometer (VG Analytical, Manchester, UK). ^1H- and ^{13}C-nuclear magnetic resonance (NMR) spectra were measured with a Bruker AM 400WB spectrometer (Karlsruhe, Germany) in $CDCl_3$ at 299 K trimethyl silane (TMS) as internal standard.

RESULTS

Taxonomy and morphology of JH801

The strain grew well on both chemically defined media and natural organic media. The colour of the aerial mycelium was yellow (yellow-colour series). The spore chains were straight rectus flexibilis (RF) type with a smooth spore surface (Table 2). Based on diamino acid diagnosis of the peptidoglycan (LL-diaminopimelic acid), absence of mycolic acids and the fatty acid pattern strain, JH801 was considered to belong to the genus *Streptomyces*. The chemotaxonomic characteristics of strain JH801 were compared with those of *Streptomyces anulatus* DSM40236 type strain and no significant morphological difference was observed between the two cultures (Table 2).

To obtain a definite species identification, partial sequence analysis of the 16S rDNA was carried out. It was found that the sequence of JH801 was only remotely related to that of *S. anulatus* DSM40236 type strain (97·6%). However, chemotaxonomical, morphological and sequence data clearly indicated JH801 to be a member of the *S. anulatus* cluster.

Streptomyces anulatus JH801 belongs to the group of filamentous actinomycetes which are characterized by polarized growth in the hyphal tips. Tip extension and branching therefore become important variables in the study of growth and product formation of *S. anulatus* JH801. Based on the model proposed by Nielsen and Krabben (1995), it is possible to describe the morphology by five parameters, i.e. (1) the specific growth rate estimated from the total hyphal length; (2) the maximum tip extension rate, K_{tip}; (3) a saturation constant related to tip extension, K_t; (4) the specific branching frequency, K_{bran}; and (5) the hyphal diameter, D_h. The estimated parameters for *S. anulatus* JH801 are listed in Table 3 together with data from two filamentous fungi.

Comparison of the different strains clearly shows that filamentous actinomycetes are much smaller in size than filamentous fungi, i.e. the diameter is approximately six times smaller. The maximum tip extension for *S. anulatus* JH801

Table 2 Taxonomical comparison of strian JH801 with *Streptomyces anulatus*

	Strain JH801	*S. anulatus* DSM40236
Spore chain morphology	RF	RF
Spore surface	Smooth	Smooth
Colour of aerial mycelium	Yellow	Yellow
Diffusible pigment	Yellow/brown	Yellow/brown
Melanin	Positive	Positive
Diamino acid of the peptidoglucan	LL-diaminopimelic acid	LL-diaminopimelic acid
Carbon utilization		
L-arabinose	Positive	Positive
D-xylose	Positive	Positive
L-rhamnose	Positive	Positive
D-raffinose	Negative	Negative
D-mannitol	Positive	Positive
i-inositol	Negative	Negative
D-fructose	Positive	Positive
D-saccharose	Positive	Negative

RF, Rectus flexibilis.

Table 3 Morphological parameters for different filamentous organisms

Strain	μ_{max} (h^{-1})	K_{tip} ($\mu m\ tip^{-1}$ h^{-1})	K_t (μm)	K_{bran} ($tip\ \mu m^{-1}$ h^{-1})	D_h (μm)
Streptomyces anulatus JH801	0·20	3·2	11	0·0164	0·6
Penicillium chrysogenum P8	0·16	10	60	0·0044	4
Aspergillus oryzae	0·27	35	148	0·0023	3·2

Data for *P. chrysogenum* are taken from Nielsen and Kraben (1995). Data for *A. oryzae* are taken from Spohr *et al*. (1997).

is three times smaller than that for *Penicillium chrysogenum* and 10 times smaller than that for *Aspergillus oryzae* but all the strains have specific growth rates lying in the same range. According to Bergter (1978), *S. anulatus* JH801 must therefore be more densely branched, which also can be seen in Table 3, i.e. K_{bran} for *S. anulatus* JH801 is significantly higher than for the two filamentous fungi.

Submerged cultivation

A series of batch cultivations was carried out in order to study growth and genotoxin production. Two batch cultivations, where glucose was the limiting substrate, were carried out in order to study the general metabolism of *S. anulatus* JH801. An additional number of batch cultivations, where nitrogen or phosphate were the limiting substrates, were carried out in order to study the mechanisms for production of genotoxins.

General physiology of *Streptomyces anulatus* JH801

Figure 1 shows the results of a batch cultivation with glucose as the limiting substrate and Table 4 summarizes the general physiology of *S. anulatus* JH801. The maximum specific growth rate was calculated from a linear regression on a semilogarithmic plot of the biomass concentrations. The 'true' yield coefficient (γ_{sx}; see eqn 1) (Nielsen and Villadsen 1994) was estimated from the glucose consumption using a maintenance coefficient of 0·05 C-mol glucose (C-mol biomass h)$^{-1}$ (taken from Nielsen 1993). The yield coefficients for *S. anulatus* JH801 were calculated setting up

Table 4 Physiological data obtained for *Streptomyces anulatus*

	Batch	
	BS8.14	BS8.15
μ (h^{-1})	0·20	0·21
Y_{sx} C-mol biomass C-mol glucose^{-1}	0·55	0·51
γ_{sx} C-mol biomass C-mol glucose^{-1}	0·62	0·63
Y_{sco2} C-mol CO_2 C-mol glucose^{-1}	0·42	0·45
Y_{xN} N-mol C-mol biomass^{-1}	0·25	0·25
Total C (%)	97·3	96·3

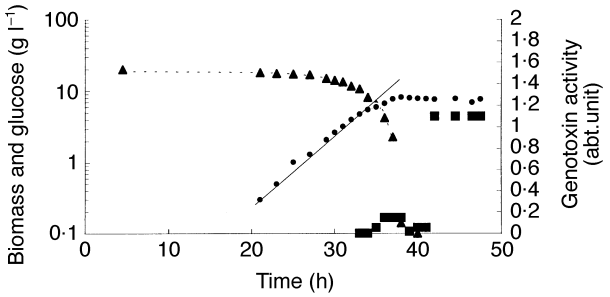

Fig. 1 Profiles for a batch cultivation with *Streptomyces anulatus* JH801. ●, Biomass concentrations; ▲, glucose concentration; ■, genotoxin activity, – – –, glucose uptake calculated from eqn 1, with a true yield coefficient of 0·62 C-mol glucose C-mol biomass^{-1} and with a maintenance coefficient of 0·05 C-mol glucose C-mol biomass^{-1} h^{-1} (taken from Nielsen (1993)). ——, Constant specific growth rate of $\mu = 0{\cdot}20$ h^{-1}

elemental balances for carbon and nitrogen during the exponential growth phase of the batch cultivations.

Only 3% of the carbon was missing in BS8·14 and 4% in BS8·15 indicating that almost all substrates and products are accounted for in the carbon balance. This was also confirmed by measurements of very low levels of acetate, lactate, glycerol, ethanol and gluconic acid by HPLC. The results therefore clearly show that *S. anulatus* JH801 utilizes glucose to produce mainly biomass (50–55%) and CO_2 (40–45%).

$$ds/dt = -((1/\gamma_{sx})\mu + m_s)x \qquad (1)$$

Mass balances for batch cultivations where nitrogen became the first limited substrate were also set up. The yield was found to be 0·62 C-mol biomass C-mol glucose^{-1} if calculated just until the point of nitrogen exhaustion; after this it decreased to 0·26 C-mol biomass C-mol glucose^{-1}. Comparison of the biomass, glucose and CO_2 data indicated that glycogen stores were built up during nitrogen starvation.

Production kinetics of genotoxins

The batch cultivation experiments showed that genotoxin production occurred at the end of cultivation near glucose depletion (see Fig. 1). Quantitatively, the main production of genotoxins in these experiments took place approximately 4 h after the stationary phase was reached. A possible explanation could be that genotoxin production is inhibited by the presence of glucose and that expression of relevant genes first takes place after its depletion. Some time was required to build up the necessary enzymes and this led to a lag phase before high genotoxic activities could be measured. Four fed-batch cultivations with a constant feed rate of 1 g l^{-1} h^{-1} were made to see if glucose repression played a role in genotoxin production. Figure 2 shows the results from one of the fed-batch cultivations.

During the fed-batch cultivation, an overshoot in glucose occurred because glucose consumption was lower than estimated. At 70 h, the glucose concentration was very low (it is observed that genotoxin activity cannot be detected before 90 h of cultivation). During this time, a very low genotoxin production starts, but the activity disappears again towards

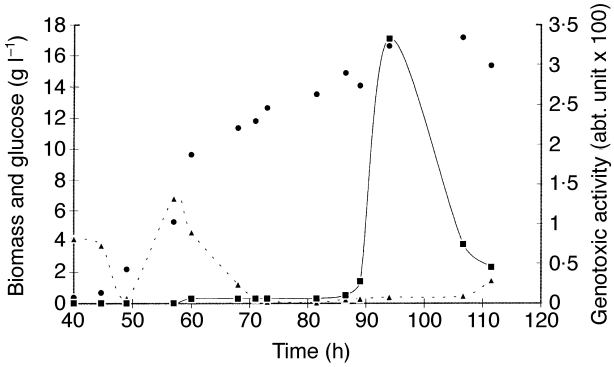

Fig. 2 Fed-batch profiles for biomass (●) and glucose concentrations (▲) together with genotoxin activity (■) for *Streptomyces anulatus* JH801

the end. The maximum genotoxin activity during the cultivations was 0·036 abt. units, which is 50 times lower than the maximum genotoxin activity measured during the batch cultivations. From these results it can be concluded that genotoxin production is not a growth-related process because the biomass concentration increases without an additional increase in genotoxin production.

Morphological changes during genotoxin production

As it was not possible to establish a correlation between the physiology of *S. anulatus* JH801 and genotoxin production, a possible correlation to morphology was studied.

By comparing genotoxin production and morphological data, there appeared to be no change in the total average hyphal length observed, and only a small increase in the total number of tips, at the time when genotoxin production takes place. As none of the quantitative measurements seemed to be related to genotoxin production, the samples were qualitatively analysed microscopically to see whether any differences could be detected. In the qualitative analysis, it was investigated whether characteristics such as spores, swollen hyphae, swollen tips, lysed structures or fragmented tips could be detected. It was found (Fig. 3) that, at the time when genotoxin was produced, some spore formation and disintegration of hyphal structures could be detected, and approximately 30 h later the spore concentration started to increase dramatically. Figure 3 and Table 5 clearly show that

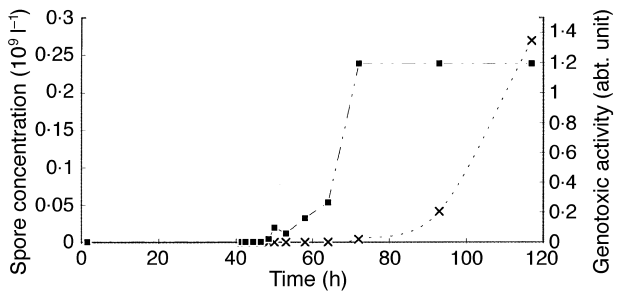

Fig. 3 Spore concentrations (×) and genotoxin activity (■) for a batch cultivation with *Streptomyces anulatus* JH801

Table 5 The final spore concentration and genotoxin activity for batch cultivations where different substrates became the first limiting substrate

	Spore concentration (10^9 spores l^{-1})	Genotoxin activity (arbitrary unit)
Glucose	0·27	1·2
Phosphate	0·13	0·42
Ammonia	0·25	0·006

S. anulatus JH801 is among the few *Streptomyces* strains that sporulate in submerged cultures.

Batch cultivations where glucose, ammonia or phosphate were the limiting substrate were set up to test if a relation could be established between spore formation and genotoxin production. In all cultivations, the growth was exponential until the levels of glucose, nitrogen or phosphate became limiting and spore formation appeared in the stationary phase. Table 5 summarizes the final spore concentration and the final genotoxin activity. It can be concluded that phosphate limitation seems to reduce both spore and genotoxin production whereas ammonia limitation only reduces genotoxin activity and not the final spore concentration. This clearly shows that sporulation is not glucose-repressed but is more likely to be related to a nutritional downshift. Furthermore, genotoxin production seems to be ammonia-dependent, which is consistent with the presence of nitrogen in this secondary metabolite (see later).

Identification of one of the active genotoxins

Bioassay-directed fractionation of the $CHCl_3$ extract by silica gel semipreparative TLC gave an active fraction which, on further purification by reversed-phase (C8) HPLC, yielded a slightly brownish compound identified as dihydroabikoviromycin on the basis of NMR techniques combined with mass, i.r. and u.v. spectral analysis (Table 6, Fig. 4). The mass spectrum gave a 100% signal at m/e = 163·1 corresponding to $C_{10}H_{13}NO$, but the ^{13}C-NMR spectrum revealed an additional carbon signal from an impurity, as was shown later. It was uncertain if two or three quaternary carbon atoms were actually present, although five CH, two CH_2 groups and one olefinic or nitrogen-bond methyl were

Table 6 Physicochemical properties of dihydroabikoviromycin

Appearance	Yellow powder (25 mg)
Molecular formula	$C_{10}H_{13}NO$
EI-m.s. $(M)^+$	162·97 (calculated for $C_{10}H_{13}NO$, 163·1)
m/z (%)	163 (17) 147 (9) 134 (34) 120 (83) 106 (87) 91 (100) 79 (53) 77 (46) 65 (13) 63 (4)
u.v. λ_{max} nm (MeOH)	244 (850)
i.r. ν_{max} cm^{-1} (KBr)	3428, 3189, 2928, 2853, 1630, 1439, 1024, 955, 897, 791
^{1}H NMR ($CDCl_3$)	See Fig. 4
^{13}C NMR ($CDCl_3$) d	39·90 (t, C-2), 26·43 (t, C-3), 59·30 (d, C-4), 64·50 (s, C-4a), 140·39 (s, C-5), 134·79 (d, C-6), 131·41 (d, C-7), 63·22 (d, C-7a), 114·68 (d, C-8), 14·00 (q, C-9)

NMR, Nuclear magnetic resonance.

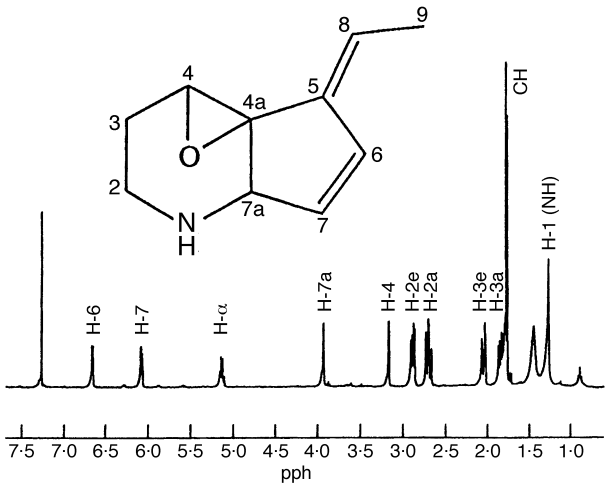

Fig. 4 Chemical structure and ^{13}C-nuclear magnetic resonance spectra of dihydroabikoviromycin

confirmed unequivocally by their C,H correlation. Using these structural elements, a sub-structure search with Anti-Base (Laatsch 1994) was performed. The result was further reduced by excluding aromatics and carbonyl groups whose absence was obvious from chemical shifts. Only dihydroabikoviromycin (Fig. 4) and its N-hydroxy derivative were found in this search, and the data of the former proved to be identical in every respect with those of the genotoxic activity from *S. anulatus*.

DISCUSSION

It can be concluded that *S. anulatus* JH801 is among the few *Streptomyces* strains that sporulate in submerged cultures (the first *Streptomyces* strain to show submerged sporulation, *S. griseus*, was found by Kendrick and Ensign (1990)). In general, *Streptomyces* strains sporulate relatively easily on solid media, with no special medium requirements reported (Glazebrook *et al.* 1990). Submerged sporulation is a far more infrequent event. Daza *et al.* (1989) found that glucose, ammonium and phosphate concentrations influence sporulation. Furthermore, it was observed that glucose did not inhibit sporulation in cases of ammonia exhaustion. Our results agree with these findings. There is evidence that sporulation and genotoxic production are related. No morphological changes were detectable during the stationary phase when genotoxin production took place. The only changes that could be seen were formation of a few spores and lysed mycelium structures later in the batch cultivation. When sporulation was not induced, no genotoxin production took place. The lag phase between the increase in genotoxic activity and spore formation was approximately 30 h.

Production of genotoxin is shown to take place when the strain is grown in a defined medium with glucose and ammonia as the only carbon and nitrogen sources, respectively. Genotoxin production in batch cultures occurs very late after glucose depletion. The production sharply increases, remaining constant thereafter. The fed-batch experiments also showed that high stress conditions with glucose limitation alone cannot trigger production.

Dihydroabikoviromycin has previously been isolated from the culture medium of *S. olivaceus* SF-973 (Trinci 1971). Tsuruoka and co-workers (1973) used it as an example of enzymatic conversion of an inactive antibiotic (dihydroabikoviromycin) to an antiviral and antifungal antibiotic (abikoviromycin) (Ogawa *et al.* 1973). Later, a related antibiotic, N-hydroxydihydroabikoviromycin (together with abikoviromycin and dihydroabimoviromycin), with antimicrobial activity against *Klebsiella pneumoniae*, was isolated from another streptomycete (Takahashi *et al.* 1986). In the previous studies, dihydroabikoviromycin has been reported as biologically inactive (Ogawa *et al.* 1973; Tsuruoka *et al.* 1973; Takahashi *et al.* 1986). The results reported demonstrate that, by using specific screening methods, new bioactivities can be detected even among compounds that have previously been regarded to be of secondary interest.

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