Journal of Natural Products
Vol. 58, No. 7, pp. 1081-1086, July 1995

ANTIMICROBIAL METABOLITES FROM A BACTERIAL SYMBIONT

JIANXIONG LI,* GENHUI CHEN, JOHN M. WEBSTER,

Department of Biological Sciences, Simon Fraser University, Burnaby, Vancouver, B.C., Canada V5A 1S6

and EVA CZYZEWSKA[1]

Department of Chemistry, Simon Fraser University, Burnaby, Vancouver, B.C., Canada V5A 1S6

ABSTRACT.—Two types of antibiotics, namely, indoles and dithiolopyrrolones, have been isolated and identified from *Xenorhabdus bovienii* A2. Compounds **1** and **2** showed strong activity against *Cryptococcus neoformans*, compounds **3** and **4** showed strong activity against *Botrytis cinerea*, and compounds **1**, **3**, and **4** showed significant activity against *Phytophthora infestans* (**2** was not tested). In addition, two lower homologues of xenorhabdins **5** and **6**, namely, 6-(*N*-3′-methylbutanamido)-4,5-dihydro-1,2-dithiolo[4,3-b]pyrrol-5-one [**7**] and 6-(*N*-butanamido)[4,5-dihydro-1,2-dithiolo[4,3-b]pyrrol-5-one [**8**], have been isolated and characterized for the first time.

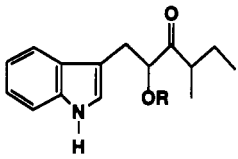
Bacteria of the genera *Xenorhabdus* and *Photorhabdus* (Enterobacteriaceae) are symbiotically associated with insect pathogenic nematodes of the genera *Steinernema* and *Heterorhabditis*, respectively (1,2), and exhibit antibacterial and antifungal activity (3–5). After the nematodes penetrate the hemocoel of an insect, they release these bacteria which proliferate and kill the host insect. These bacteria produce antibiotics which inhibit the growth of other microbial flora in the insect cadavers (3–6).

Most species of *Xenorhabdus* and *Photorhabdus* exist in two forms, designated primary and secondary, when cultured in vitro (7,8). The primary form, but less so the secondary form of *Xenorhabdus* and *Photorhabdus*, exhibits a wide spectrum of antibiotic activity (3–5), and four types of antibiotics produced by these bacteria have been described: (a) indole derivatives from a *Xenorhabdus* sp. (9); (b) hydroxystilbene derivatives from *P. luminescens* (9,10); (c) organically soluble dithiolopyrrolones, the xenorhabdins, from *Xenorhabdus* sp. Q1 strain, *X. bovienii*, and strains of *X. nematophilus* (11), and (d) H_2O-soluble benzopyran-1-one derivatives, the xenocoumacins from *Xenorhabdus* sp. Q1 strain, and *X. nematophilus* All strain (12). Each strain of these bacteria has been reported to produce only one type of antibiotic, with the exception of *Xenorhabdus* sp. Q1 strain which produces xenorhabdins and xenocoumacins (8). Among these compounds, xenocoumacin 1 has proved to be active against animal and human pathogenic fungi (12).

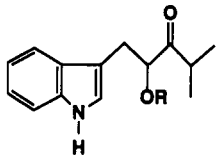
Recently, strong activity has been reported against fungal pathogens of plants by *Xenorhabdus* spp. (4). This observation has led to the isolation and identification of antifungal metabolites from *X. bovienii*.

The liquid medium from cultures of the primary form of *X. bovienii* A2, grown over a period of 4 days, was subjected to solvent extraction with EtOAc either directly or, after removing the cells, by centrifugation. The bioactivity of the primary form was tested at this and each subsequent step in the isolation process using *Bacillus subtilis* and *Botrytis cinerea* for antibacterial and antifungal activity, respectively (13). The bioactive EtOAc extract was subsequently separated by Si gel cc to give bioactive compounds **1–4** and other bioactive fractions. The other bioactive fractions were then separated by reversed-phase hplc to yield bioactive

[1]Present address: School of Engineering Science, Simon Fraser University.



1 R=Ac
3 R=H

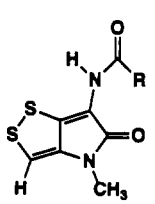


2 R=Ac
4 R=H

compounds **5**, **6**, and **8**, along with a bioactive fraction from which component **7** was obtained by repeated hplc.

As the ^{1}H-nmr, ir, and ms data of compounds **1–4** were very similar or identical to those of the four indole derivatives previously isolated and identified from a *Xenorhabdus* sp. (9), they were assigned as 3-(2′-acetoxy-3′-oxo-4′-methylhexyl)-indole, 3-(2′-acetoxy-3′-oxo-4′-methylpentyl)-indole, 3-(2′-hydroxy-3′-oxo-4′-methylhexyl)-indole, and 3-(2′-hydroxy-3′-oxo-4′-methylpentyl)-indole, respectively. The position of the hydroxyl group in compound **3** was confirmed to be at C-2′ rather than C-1′ by observation of the long-distance coupling of the proton attached to C-1′ (3.04 ppm, ^{1}H nmr) to the carbon in the indole ring (110.89 ppm, ^{13}C nmr). The optical rotations of **1**, **3**, and **4** (the amount of **2** available for testing was insufficient for this measurement, but it probably has the same configuration as **4**) are similar to those reported in the literature (9). Therefore compounds **1–4** isolated from *X. bovienii* A2 are most likely the same as those reported from a different strain of *Xenorhabdus* (9), rather than being their stereoisomers.

A similar study on the ^{1}H- and ^{13}C-nmr, ir, uv, and ms data of compounds **5** and **6** showed them to be identical to 6-(*N*-hexanamido)-4,5-dihydro-1,2-dithiolo[4,3-b]pyrrol-5-one and 6-(*N*-3′-methylhexanamido)-4,5-dihydro-1,2-dithiolo[4,3-b]pyrrol-5-one (xenorhabdins), respectively, which have been isolated previously from *Xenorhabdus* spp. (11). Our data showed **8** and **7** are lower homologues of **5** and **6**, respectively, with two fewer carbons in each case.



5 R=$CH_2CH_2CH_2CH_2CH_3$
6 R=$CH_2CH_2CH_2CH(CH_3)_2$
7 R=$CH_2CH(CH_3)_2$
8 R=$CH_2CH_2CH_3$

The ms of **8** showed a molecular ion at *m/z* 256. Loss of the fragment of C_4H_6O led to the same principal daughter ion at *m/z* 186 as those in the ms of **5** and **6** (composition $C_6H_6N_2OS_2$). The base peak (*m/z* 186) of each ms showed only a limited tendency for further fragmentation, in agreement with the assignment of this ion as a heterocyclic aromatic. The uv spectra of **5–8** were all very similar, suggesting that the same chromophore is present in all four compounds. This supports the conclusion that they are members of a homologous series with a common nucleus of $C_6H_6N_2OS_2$. Signals at δ 7.46 (br), 6.63, and 3.35 in the ^{1}H-nmr spectrum of **8** were identical to those in the ^{1}H-nmr spectra of **5–7**, further suggesting the presence of the same heterocyclic ring system, while signals at higher magnetic field in the ^{1}H-nmr spectrum, together with ms data, indicated that a butanoyl group was present. Based on this analysis, the structure of **8** was assigned and is consistent with the ^{13}C-nmr data obtained.

The ms of **7** showed a molecular ion at *m/z* 270, indicating an additional methylene to the C_4H_6O portion of **8**, while signals at higher magnetic field in the nmr spectrum indicated that a 3-methyl-

butanoyl group was present. Accordingly, the structure of **7** was assigned. These two lower homologues, **7** and **8**, were isolated as natural products for the first time in this study, although other lower homologues, thiolutin and aureothricin, have been characterized previously (11).

The preliminary bioassays showed that all these purified compounds are active against *B. cinerea* and *B. subtilis* and additional tests on the four indole derivatives showed them to be active against fungi of medical and agricultural importance (Table 1). Compounds **1** and **2** exhibited strong activity against *C. neoformans*, with **3** and **4** active against *B. cinerea,* and **1**, **3**, and **4** active against *Phytophthora infestans* (**2** was not tested). Only moderate activity was detected against the other organisms tested. These antibiotics have been demonstrated as strong antibacterial agents (9,14). The antifungal activity of the dithiolopyrrolones **5–8** was detected for the first time during this isolation process, although some related dithiolopyrrolones have known antifungal activity (15). Further detailed investigation of the effect of these natural dithiolopyrrolones against different fungi is needed. The present results reinforce the hypothesis that antimicrobial substances produced by *Xenorhabdus* play an essential role in minimizing microbial putrefaction of the insects infected by the *Steinernema/Xenorhabdus* and *Heterorhabditis/Photorhabdus* complexes (8).

In conclusion, two types of antibiotic substances (indole derivatives, **1–4**, and dithiolopyrrolones, **5–8**) have been isolated and identified from *X. bovienii* A2 and their antifungal and antibacterial activity demonstrated. Two lower homologues of dithiolopyrrolones or xenorhabdins (**7** and **8**) were isolated from this bacterial genus for the first time. More studies on the efficient production, stereochemistry (for **1–4**), antimicrobial spectra of activity, and mechanism of bioactivity of these substances, and on the possible synergistic effect among these antibiotics are needed before their full potential can be realized.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded on a Bruker WM400 spectrometer in $CDCl_3$, using residual $CHCl_3$ as internal standard. The 2D nmr spectrum for the long-range ^{1}H-^{13}C correlation of compound **3** was obtained with the inv4lplrnd program in $CDCl_3$ (16). Low-resolution ms were obtained on a Hewlett-Packard 5985B gc/ms system operating at 70 eV using a direct probe. Cims were obtained with isobutane on the same instrument as described above. High-resolution ms were recorded on a Kratos MS80 instrument. Ir spectra were recorded on a Perkin-Elmer S99B spectrometer.

TABLE 1. Antifungal Minimum Inhibitory Concentrations (MIC) (μg/ml) of Compounds **1–4**.

Test organism	Compounds			
	1	**2**	**3**	**4**
Aspergillus fumigatus ATCC 13073	>100	>100	>100	>100
A. flavus ATCC24133	>100	>100	>100	>100
Botrytis cinerea[a]	>100	>100	12	12
Candida tropicales CBS94	>100	>100	>100	>100
Cryptococcus neoformans ATCC14117	50	25	>100	>100
Phytophthora infestans[b]	100	NA	100	50
Pythium ultimum[c]	>100	NA	>100	>100

[a]Originally isolated from an Anjou pear and provided by Dr. P. Sholberg, Agriculture Canada Research Station, Summerland, British Columbia, Canada.

[b]Originally from Ciba Geigy, Switerland (isolate CB215), and provided by Dr. Z. Punja, Department of Biological Sciences, Simon Fraser University.

[c]Originally isolated from bean roots planted in the Fraser Valley, British Columbia, Canada and provided by Dr. J. Rahe, Department of Biological Sciences, Simon Fraser University.

Mps (uncorrected) were determined on a Fisher-Johns melting point apparatus. Optical rotations were measured on an Autopol II, automatic polarimeter. Hplc analysis was performed on a Waters 510 instrument with a Waters 484 uv detector.

CULTURE CONDITIONS.—*Xenorhabdus bovienii* and its nematode symbiont *S. feltiae* used in this study were collected from soil in British Columbia, Canada and maintained in culture in this laboratory as described previously (4). Last-instar larvae of the greater wax moth, *Galleria mellonella*, were infected with infective juvenile (IJ) nematodes, carrying *X. bovienii*, at a rate of 25 IJs/larvae. After 24 to 48 h the dead insect larvae were surface disinfected by dipping them into 95% EtOH and igniting them. The cadavers were aseptically dissected, hemolymph was streaked onto an NBTA medium (nutrient agar supplemented with 0.025 grams of bromothymol blue and 0.04 gram of 2,3,5-triphenyltetrazolium chloride per liter) and incubated in the dark at room temperature. The resulting primary form of *X. bovienii* A2 was maintained and subcultured at 14-day intervals. Inocula of the primary form were prepared by adding one loopful of the culture to 50 ml of tryptic soy broth (TSB) in a 100-ml Erlenmeyer flask. Cultures were shaken at 120 rpm on an Eberbach gyrorotary shaker for 24 h at 25°. Bacterial fermentation was initiated by adding 100 ml of this bacerial culture to 900 ml of TSB in a 2,000-ml flask. The flask was incubated in the dark at 25° on an Eberbach gyrorotary shaker. After 96 h, the culture was immediately centrifuged (12,000×g, 20 min, 4°) to separate the bacterial cells.

EXTRACTION AND ISOLATION.—The cell-free material was extracted with EtOAc four times, and the combined extracts were dried with anhydrous Na_2SO_4 and filtered through glass wool. The filrate was concentrated on a rotary evaporator below 30° under vacuum to yield a brown oil. The above isolation process was repeated 10 times to yield approximately 2.8 g of the oil from 10 liters of culture broth.

The crude extracts were processed through a Si gel (200 g Si gel 60, 40×5 cm, EM Science, Darmstadt, Germany) chromatographic column with 50% Et_2O in hexane as the eluent. After compounds **1** (45 mg/liter), **2** (18 mg/liter), **3** (100 mg/liter), and **4** (40 mg/liter) were eluted both in pure form and in a mixture, the bioactive fractions A and B were obtained by eluting with 70% Et_2O in hexane.

Fraction A was separated by hplc on a C_{18} prep. column (Spherisorb 10 [ODS(1)], 250×10 mm, 10 micro, Phenomenex, Torrance, CA) programmed (10% MeOH in H_2O for 5 min and gradually increasing to 90% MeOH in H_2O in 25 min, isocratic for 5 min) at 2.5 ml/min. The eluate was monitored at 254 nm. Active peak 1 (*R*, 31.5 min), peak 2 (*R*, 32.7 min), peak 3 (*R*, 33.5 min) (compound **5**, 2 mg/liter), peak 4 (*R*, 34.5 min) (compound **6**, 1 mg/ml) were collected. Peak 1 was further separated by hplc on the same column with 50% CH_3CN in H_2O as the eluent to give compound **7** (*R*, 12.9 min, 0.2 mg/liter).

Fraction B was purified by hplc using the same conditions and process as those for fraction A. Compound **8** (*R*, 30.3 min, 0.5 mg/liter) was obtained.

Compound **1**.—Obtained as an oil; $[\alpha]^{25}D$ +57° (*c*=1.1, $CHCl_3$); ^1H nmr ($CDCl_3$) δ 8.06 (1H, brs, NH), 7.64 (1H, d, *J*=7.5 Hz), 7.37 (1H, d, *J*=7.5 Hz), 7.21 (1H, t, *J*=7.5 Hz), 7.15 (1H, t, *J*=7.5 Hz), 7.05 (1H, d, *J*=2.2 Hz), 5.46 (1H, dd, *J*=4.5 and 8 Hz), 3.30 (1H, dd, *J*=4.5 and 15 Hz), 3.18 (1H, dd, *J*=7 and 15 Hz), 2.63 (1H, sext., *J*=7 Hz), 2.07 (3H, s), 1.73 (1H, m), 1.39 (1H, m), 0.98 (3H, d, *J*=6.5 Hz), 0.86 (3H, t, *J*=7 Hz); eims *m/z* 287 (M^+, 10), 227 (M^+-60, 10), 170 (47), 130 (100), 103 (10), 84 (25), 77 (20), 57 (35); cims *m/z* 288 (M+1, 44), 228 (100).

Compound **2**.—Obtained as an oil; ^1H nmr ($CDCl_3$) δ 8.06 (1H, brs, NH), 7.64 (1H, d, *J*=7.5 Hz), 7.37 (1H, d, *J*=7.5 Hz), 7.21 (1H, t, *J*=7.5 Hz), 7.15 (1H, t, *J*=7.5 Hz), 7.05 (1H, d, *J*=2.2 Hz), 5.46 (1H, dd, *J*=4.5 and 8 Hz), 3.27 (1H, dd, *J*=4.5 and 15 Hz), 3.19 (1H, dd, *J*=7 and 15 Hz), 2.72 (1H, hept., *J*=7 Hz), 2.07 (3H, s, $COCH_3$), 1.09 (3H, d, *J*=6 Hz), 0.99 (3H, d, *J*=6 Hz); eims *m/z* 273 (M^+, 25), 213 (M^+-60, 15), 170 (60), 130 (100), 103 (6), 84 (12), 77 (8); cims *m/z* 274 (M+1, 48), 214 (100).

Compound **3**.—Obtained as an oil; $[\alpha]^{25}D$ +87° (*c*=1.0, $CHCl_3$); ir (film) ν max 3410, 2967, 2932, 1705, 1457, 742 cm^{-1}; ^1H nmr ($CDCl_3$) δ 8.06 (1H, brs, NH), 7.63 (1H, d, *J*=7.5 Hz), 7.36 (1H, d, *J*=7.5 Hz), 7.20 (1H, t, *J*=7.5 Hz), 7.15 (1H, t, *J*=7.5 Hz), 7.12 (1H, d, *J*=2.3 Hz), 3.52 (1H, d, *J*=5.5 Hz, OH), 4.60 (1H, m), 3.32 (1H, dd, J=4.5 and 15 Hz), 3.04 (1H, dd, *J*=7 and 15 Hz), 2.77 (1H, sext., *J*=6.5 Hz), 1.65 (1H, m), 1.42 (1H, m), 0.99 (3H, d, *J*=6.5 Hz), 0.88 (3H, t, *J*=7 Hz); ^{13}C nmr δ 11.4, 14.8, 27.1, 29.8, 43.1, 76.2, 110.9, 111.2, 118.6, 119.5, 122.2, 122.8, 127.4, 136.1, 215.8; eims *m/z* 245 (M^+, 10), 130 (100), 92 (5), 77 (3), 57 (2); cims *m/z* 246 (M^++1, 100), 130 (55); hrms *m/z* calculated for $C_{15}H_{19}NO_2$ 245.1416, found 245.1416.

Compound **4**.—Obtained as an oil; $[\alpha]^{25}D$ +86° (*c*=1.1, $CHCl_3$); ^1H nmr ($CDCl_3$) δ 8.05 (1H, brs, NH), 7.63 (1H, d, *J*=7.5 Hz), 7.36 (1H, d, *J*=7.5 Hz), 7.20 (1H, t, *J*=7.5 Hz), 7.14 (1H, t, *J*=7.5 Hz), 7.11 (1H, d, *J*=2.3 Hz), 4.67 (1H, m), 3.45 (1H, d, *J*=5.5 Hz, OH), 3.31 (1H, dd, *J*=4.5 and 15 Hz), 3.06 (1H, dd, *J*=7 and 15 Hz), 2.88 (1H, hept., *J*=7 Hz), 1.13 (3H, d, *J*=7 Hz),

1.02 (3H, d, J=6.5 Hz); eims m/z 231 (M^+, 15), 130 (100), 103 (5), 77 (10).

Compound **5**.—Obtained as a solid; mp 172–174°; eims, uv, ir, and ^{1}H-nmr (Me_2CO-d_6) data identical or very similar to those reported (11); ^{1}H nmr ($CDCl_3$) δ 7.43 (1H, br s, CO-NH), 6.63 (1H, s, H-3), 3.35 (3H, s, N-Me), 2.35 (2H, t, CO-CH_2, J=7.4 Hz), 1.70 (2H, m, CH_2), 1.35 (4H, m, CH_2-CH_2), 0.90 (3H, t, J=6.9 Hz); ^{13}C nmr ($CDCl_3$) δ 171.4, 167.0, 136.9, 132.6, 114.1, 108.6, 36.5, 31.3, 27.8, 25.1, 22.3, 13.8.

Compound **6**.—Obtained as a solid; mp 154–156°; eims, uv and ir identical or very similar to those reported (11); ^{1}H nmr ($CDCl_3$) δ 7.43 (1H, br s, CO-NH), 6.63 (1H, s, H-3), 3.35 (3H, s, N-Me), 2.32 (2H, t, CO-CH_2, J=7.6 Hz), 1.69 (2H, m, CH_2), 1.34 (1H, m, CH), 1.24 (2H, m, CH_2), 0.88 (6H, d, J=7.0 Hz); ^{13}C nmr ($CDCl_3$) δ 171.4, 167.0, 136.9, 132.7, 114.4, 108.8, 38.4, 36.7, 27.8, 27.8, 23.3, 22.5.

Compound **7**.—Obtained as a solid; uv (MeOH) λ max 389, 315 nm; ^{1}H nmr ($CDCl_3$) δ 7.42 (1H, br s, CO-NH), 6.63 (1H, s, H-3), 3.35 (3H, s, N-Me), 2.20 (2H, d, CO-CH_2, J=7.6 Hz), 1.2–1.6 (1H, m, CH), 1.00 (6H, d, J=6.4 Hz); eims m/z 272 (2), 271 (3), 270 (M^+, 22), 188 (10), 187 (11), 186 (100), 185 (9), 105 (9), 69 (9), 57 (14); hrms m/z calcd for $C_{11}H_{14}N_2O_2S_2$, 270.0497, found 270.0498.

Compound **8**.—Obtained as a solid; uv (MeOH) λ max 390, 310 nm; ir (KBr) ν max 3448, 1654, 1560, 1458 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 7.63 (1H, br s, CO-NH), 6.63 (1H, s, H-3), 3.35 (3H, s, N-Me), 2.32 (2H, t, CO-CH_2, J=7.3 Hz), 1.72 (2H, sext., CH_2, J=7.4 Hz), 0.99 (3H, t, J=7.4 Hz); ^{13}C nmr ($CDCl_3$) δ 171.3, 167.0, 136.7, 132.4, 114.2 (these first 5 signals were extremely weak due to the limited amount of the sample available and were therefore estimated from the spectrum), 108.5, 38.4, 27.7, 18.8, 13.6; eims m/z 258 (3), 257 (4), 256 (27), 188 (10), 187 (11), 186 (100), 185 (9), 130 (8); hrms m/z calcd for $C_{11}H_{14}N_2O_2S_2$ 256.0340, found 256.0338.

ANTIMICROBIAL BIOASSAYS (13).—Determination of minimum inhibitory concentration (MIC) values (13): Active compounds **1–4** were dissolved in DMSO, filter sterilized with a 0.2-μm filter and diluted into 2 ml of potato dextrose broth in a 5-ml vial, resulting in a final DMSO concentration of <0.4% (v/v). The active compounds were serially diluted twofold to produce culture media containing dilutions from 200 μg/ml of active ingredient in order to determine the MIC value. Each dilution was replicated three times with both liquid media and agar plates. The two yeast test candidates (*C. tropicales* and *C. neoformans*) were grown on potato dextrose agar (PDA) for 24 h at 25°, then scraped from the plate by flooding the plate with 0.8% saline and diluted with the saline to make inocula containing 2.5 to 2.8×10^7 cells per ml. *Aspergillus* spp. and *B. cinerea* were grown on PDA for 7 days at 25° before the conidia were harvested by flooding the plate with sterile, distilled water and diluting to make the final inocula of 2.5 to 3.0×10^6 conidia per ml. The inocula were then added to the culture media with the test chemicals. The tests on *P. ultimum* and *P. infestans* were performed on water agar (1.5%) and rye agar (17), respectively. The chemicals were diluted with distilled H_2O, mixed with equal amounts of agar. A mycelium plug (0.5 cm in diameter) was placed in the center of each plate containing agar and the test chemical. Replicates were incubated at 35° (*B. cinerea, P. ultimum,* and *P. infestans* were inoculated at 24°) and the MIC value visually determined after 24 h (48 h for *B. cinerea*, 96 h for *P. infestans*). The MIC value is defined as the lowest concentration of active compound which prevents the growth of the test organism under the above conditions.

ACKNOWLEDGMENTS

We thank Dr. K.N. Slessor for helpful discussions and the critical review of the manuscript, Dr. A. Tracey for helpful discussions about nmr spectral data, Mrs. M. Tracey for nmr spectra, Mr. G. Owen for ms spectra, and Mr. K. Ng for experimental assistance. The research was supported by funding from Agro-BioTech Research Corporation, Canada and the Natural Sciences and Engineering Research Council of Canada.

LITERATURE CITED

1. G.M. Thomas and G.O. Poinar, *Int. J. System. Bacteriol.*, **29**, 352 (1979).
2. R.J. Akhurst, *Int. J. System. Bacteriol.*, **33**, 38 (1983).
3. R.J. Akhurst, *J. Gen. Microbiol.*, **128**, 3061 (1982).
4. G. Chen, G.B. Dunphy, and J.M. Webster, *Biol. Control.*, **4**, 157 (1994).
5. M. Barbercheck and H.K. Kaya, *J. Invertebr. Pathol.*, **55**, 225 (1990).
6. G.O. Poinar and G.M. Thomas, *Parasitology*, **56**, 385 (1966).
7. R.J. Akhurst and N.E. Boemare, *J. Gen. Microbiol.*, **134**, 1835 (1988).
8. R.J. Akhurst and G.B. Dunphy, in: "Parasites and Pathogens of Insects." Ed. by N.E. Beckage, S.N. Thompson, and B. Federici, Academic Press, New York, 1993, Vol. 2, pp. 1–23.
9. V.J. Paul, S. Frautschy, W. Fenical, and K.H. Nealson, *J. Chem. Ecol.*, **7**, 589 (1981).
10. W.H. Richardson, T.M. Schmidt, and K.H. Nealson, *Appl. Environ. Microbiol.*, **54**, 1602 (1988).
11. B.V. McInerney, R.P. Gregson, M.J. Lacey,

R.J. Akhurst, G.R. Lyons, S.H. Rhodes, D.R.J. Smith, L.M. Engelhardt, and A.H. White, *J. Nat. Prod.*, **54**, 774 (1991).
12. B.V. McInerney, W.C. Taylor, M.J. Lacey, R.J. Akhurst, and R.P. Gregson, *J. Nat. Prod.*, **54**, 785 (1991).
13. K.D. Hickey, "Methods for Evaluating Pesticides for Control of Plant Pathogens," The American Phytopathological Society, St. Paul, MN, 1986.
14. L. Sundar and F.N. Chang, *J. Gen. Microbiol.*, **139**, 3139 (1993).
15. P.R. Deb and B.K. Dutta, *Curr. Sci.*, **53**, 659 (1984).
16. A. Bax and M.F. Summers, *J. Am. Chem. Soc.*, **108**, 2093 (1986).
17. C.E. Caten and J.L. Jinks, *Can. J. Bot.*, **46**, 329 (1967).

Received 27 November 1994