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Avermectins, New Family of Potent Anthelmintic Agents: Producing Organism and Fermentation

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The avermectins are a complex of chemically related agents which exhibit extraordinarily potent anthelmintic activity. They are produced by a novel species of actinomycete, NRRL 8165, which we have named *Streptomyces avermitilis*. The morphological and cultural characteristics which differentiate the producing organism from other species are described. The avermectins have been identified as a series of macrocyclic lactone derivatives which, in contrast to the macrolide or polyene antibiotics, lack significant antibacterial or antifungal activity. The avermectin complex is fully active against the gastrointestinal nematode *Nematospiroides dubius* when fed to infected mice for 6 days at 0.0002% of the diet. Fermentation development, including medium modification and strain selection, resulted in increasing the broth yields from 9 to 500 μg/ml.

Of the several thousand microbial fermentation products which have been described, only a few have been reported to have anthelmintic activity. Of those which do, many belong to the aminoglycoside family of antibiotics. Hygromycin B (6, 10) and antibiotic G-418 (11) are active against both nematodes and cestodes. The destomycins (12, 16) are active only against nematodes, whereas paromomycin (22) and antibiotic complex S15-1 (1) are cestocidal. Antibiotics of other structural classes which are active against nematodes include the netropsin relative anthelvencin (15) and two cytosine-containing antibiotics, aspiculamycin (9) and anthelmycin (8). Three other fermentation products, myxin (7), the thaimycins (3), and the axenomycins (4), are cestocidal.

We have found a group of anthelmintic agents unrelated to any of the above compounds. They are produced by an actinomycete which was isolated at Kitasato Institute from a soil sample collected at Kawana, Ito City, Shizuoka Prefecture, Japan. It had been sent to Merck Sharp & Dohme Research Laboratories for testing in the various screening programs there. Early tests indicated that whole broth was active against *Nematospiroides dubius* in mice over at least an eightfold range without notable toxicity. Subsequently, the anthelmintic activity was isolated and identified as a family of closely related compounds whose structures are shown in Fig. 1.

The complex, which has been named avermectin, is highly active against a variety of nematodes. The complex is fully active against *N. dubius* when fed to infected mice for 6 days at 0.0002% of the diet. The individual components, although differing in their potencies, are active against other nematode species when administered orally or parenterally at 0.1 mg/kg or less; e.g., avermectin B_{1a} is greater than 95% effective against gastrointestinal nematodes and lungworms of cattle at a single oral dose of 0.1 mg/kg or against hookworms of dogs at 0.005 mg/kg (5).

In this paper, we describe the characteristics of the producing culture *Streptomyces avermitilis* MA-4680 (NRRL 8165) and the production of avermectin by fermentation. Subsequent papers will describe the isolation and chromatographic properties (13) and the anthelmintic activity (5). Details of the elucidation of the structures are reported elsewhere (G. Albers-Schönberg, B. H. Arison, J. C. Chabala, A. W. Douglas, P. Eskola, M. H. Fisher, A. Lusi, H. Mrozik, J. L. Smith, and R. L. Tolman, J. Am. Chem. Soc., in press).

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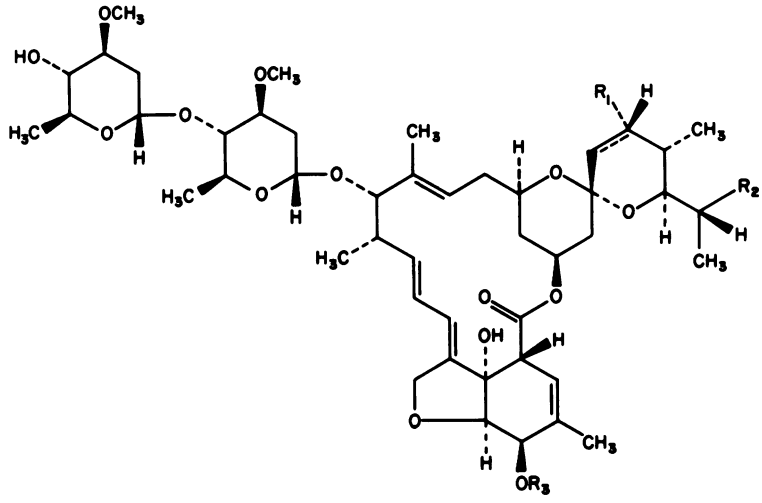

Avermectin	R_1	R_2	R_3
A1a		C_2H_5	CH_3
A1b		CH_3	CH_3
A2a	OH	C_2H_5	CH_3
A2b	OH	CH_3	CH_3
B1a		C_2H_5	H
B1b		CH_3	H
B2a	OH	C_2H_5	H
B2b	OH	CH_3	H

Where R_1 is absent, the double bond (====) is present.
Both sugars are α-L-oleandrose.

FIG. 1. *Structure of the avermectins.*

MATERIALS AND METHODS

Taxonomic studies. The morphological and cultural characteristics of the microorganism which produces avermectin were determined by use of the media and methods described by Shirling and Gottlieb (17). Previously described taxonomic keys and cultural descriptions (2, 18–21, 23) were used to compare the culture with recognized genera and species.

Fermentation studies. Fermentations were performed in 250-ml Erlenmeyer flasks incubated at 28°C on a rotary shaker (2-inch [ca. 5.08-cm] throw, 220 rpm). Vegetative growth stored in a liquid nitrogen refrigerator in seed medium containing 20% glycerol was used as the culture source for the experiments described here. Inocula were grown for 1 or 2 days in baffled flasks containing 50 ml of seed medium. Seed medium I contained (in grams per 1,000 ml of water) the following: cerelose, 1.0; soluble starch, 10.0; beef extract, 3.0; Ardamine pH (Yeast Products, Inc.), 5.0; *N*-Z-Amine E (Sheffield Chemical), 5.0; $MgSO_4 \cdot 7H_2O$, 0.05; KH_2PO_4, 0.182; Na_2PHO_4, 0.190; and $CaCO_3$, 0.5. The pH was adjusted to 7.0 before the addition of the $CaCO_3$. In later experiments, seed medium II, consisting of the following (in grams per 1,000 ml of water), was used: lactose, 20.0; distillers' solubles (Brown-Forman Distillers' Corp.), 15.0; and Ardamine pH, 5.0. The pH was adjusted to 7.0 before autoclaving. All media were sterilized by autoclaving at 120°C for 20 min. Production flasks, containing 40 ml of medium, were inoculated with 2 ml of the seed culture.

Reducing sugar was determined with a Technicon Autoanalyzer by a modification of the ferricyanide method of Park and Johnson (14). Growth was estimated by measuring the packed mycelial volume from 10 ml of broth centrifuged in a 15-ml graduated centrifuge tube.

Mouse assay. Anthelmintic assays were performed in mice infected with *N. dubius*. Male Charles River CD-1 white mice (15 to 20 g) were infected by gavage with larvae of *N. dubius*. After inoculation, they were fed for 6 days with milled Purina Lab Chow in which the test material had been mixed. The mice were returned to the normal diet and, at 14 days postinfection, examined for the production of eggs and the presence or absence of worms. The results of the assay were scored subjectively as fully active (no eggs, no worms), moderately active, slightly active, and inactive.

TLC assay. For the thin-layer chromatography (TLC) densitometric assay, mycelia from 10 ml of broth were removed by centrifugation. The mycelial cake was mixed with 10 ml of acetone–1 NHCl (100:1, vol/vol) and shaken for about 2 min. A 5-ml amount of $CHCl_3$ was added, and the mixture was again shaken. The mixture was centrifuged, and the upper (mycelial) layer was discarded. The lower phase (~8

ml) was removed and evaporated to dryness under vacuum. The residue was taken up in 0.1 ml of $CHCl_3$. A 2-μl sample was applied to the origin of a silica gel 60-F254 plate (E. M. Laboratories, Inc.). The plate was developed for 30 to 40 min with chloroform–ethyl acetate–methanol–methylene chloride (9:9:1:2). The avermectins can be detected under short-wave ultraviolet light or by spraying with 1% ceric sulfate in 10% sulfuric acid followed by heating at 100°C for about 10 min.

For a quantitative assay, the plate was scanned at 245 nm with a double-beam densitometer (Schoeffel Instrument Corp.). The peak heights were compared with those of a standard extract which had been calibrated by high-pressure liquid chromatography (HPLC) assay (13). A scan of this standard is shown in Fig. 2. Only the four major components, avermectins A_{1a}, A_{2a}, B_{1a}, and B_{2a}, were detected since the b components were present in only trace amounts. Total avermectin was determined as the sum of the four individual components.

Isolation of *S. avermitilis* MA-4848. A suspension of spores of *S. avermitilis* MA-4680 was irradiated with ultraviolet light and plated on a dextrose-mineral salts medium supplemented with 1 g of tryptophan per liter. The colonies which grew were replicated onto plates of the same medium lacking tryptophan. "Auxotrophs" were picked and tested for production of avermectin. MA-4848 was the highest producing isolate. However, subsequent tests demonstrated that this culture does not require tryptophan, and it has no known difference from MA-4680 other than much greater ability to produce avermectin. It has been deposited with the American Type Culture Collection as ATCC 31271.

RESULTS AND DISCUSSION

Taxonomy. Taxonomic studies of culture MA-4680 revealed it to be a *Streptomyces* species with sporophores forming spirals as side branches on aerial mycelia. The spirals are compact but become more open as the culture ages (Fig. 3A). Spores were in chains of more than 15 and were usually spherical to oval. The spore surface was smooth as revealed by electron microscopy (Fig. 3B). Sporulation occurred on oatmeal agar, glycerol–asparagine agar, inorganic salts–starch agar, and egg albumin agar. The culture grew well at 28 and 37°C but not at 50°C. These characteristics, plus those summarized in Tables 1 and 2, were compared with those of known species in the classical references (2, 18–22). No *Streptomyces* species is described as having the brownish-gray spore mass color, smooth spore surface, spiral sporophore structure, production of melanoid pigments, and cultural and carbon utilization patterns that together comprise the distinctive species *S. avermitilis*.

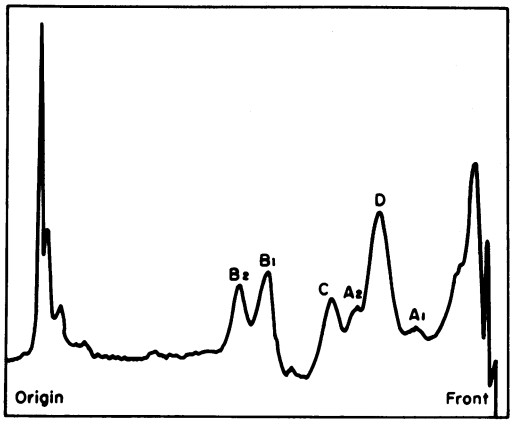

FIG. 2. *Densitometer trace of a mycelial extract of S. avermitilis MA-4680 (NRRL 8165) used as a standard in the TLC assay. The peaks of the four major avermectin components are labeled A_1, A_2, B_1, and B_2. Peak C is an artifact of the medium, and Peak D is a fermentation product unrelated to the avermectins.*

Assays. In early studies, avermectin was determined by its anthelmintic activity since it lacked detectable antimicrobial activity. The anthelmintic activity was associated with the mycelia and absent from the filtrate. Chromatographic assays were developed once the identity of the anthelmintic product was known. The TLC assay was used for much of the work presented here. It offered the advantages of sensitivity and rapidity. The four major components were resolved adequately although there was some interference by extraneous ultraviolet-absorbing materials (peaks C and D in Fig. 2). Peak C is an artifact derived from the medium, and peak D is a fermentation product unrelated to the avermectins.

The HPLC assay (13) is more precise and capable of resolving all of the anthelmintic components. At times it was used to confirm the results of the TLC assays. In one experiment, a comparison of the anthelmintic assay with the HPLC assay gave the following correlation of total avermectin complex to anthelmintic activity: full activity, 54 μg/25 g of feed; moderate activity, 34 μg/25 g of feed; slight activity, 26 μg/25 g of feed.

Fermentation. Numerous complex media were screened for their ability to support the production of avermectin. Two media which gave high yields are described in Table 3. Whole broth from *S. avermitilis* MA-4680 grown on medium A was fully active in the mouse assay at 6 ml/25 g of feed and slightly to moderately active at 3 ml/25 g of feed, indicating a yield of about 9 μg/ml.

Medium A served as the basis for initial medium development. Suitable substitutes for Amidex were dextrin 600, soluble starch, corn starch, mannose, and dextrose. Glycerol, blackstrap molasses, lactose, *N*-acetylglucosamine, sucrose, glucosamine, inositol, xylose, raffinose, and dextran 100C gave reduced yields of avermectin.

Suitable substitutes for distillers' solubles in medium A were cornmeal, cottonseed meal, peanut meal, whole egg powder, wheat gluten, and soybean meal. Primary yeast, corn steep, and whole milk powder were less effective substitutes. Elimination of distillers' solubles from medium A without substitution only slightly reduced avermectin production.

Autolyzed yeast (Ardamine pH) was required for avermectin production in medium A. A vitamin mix, yeast nucleic acids, monosodium glutamate, or a trace

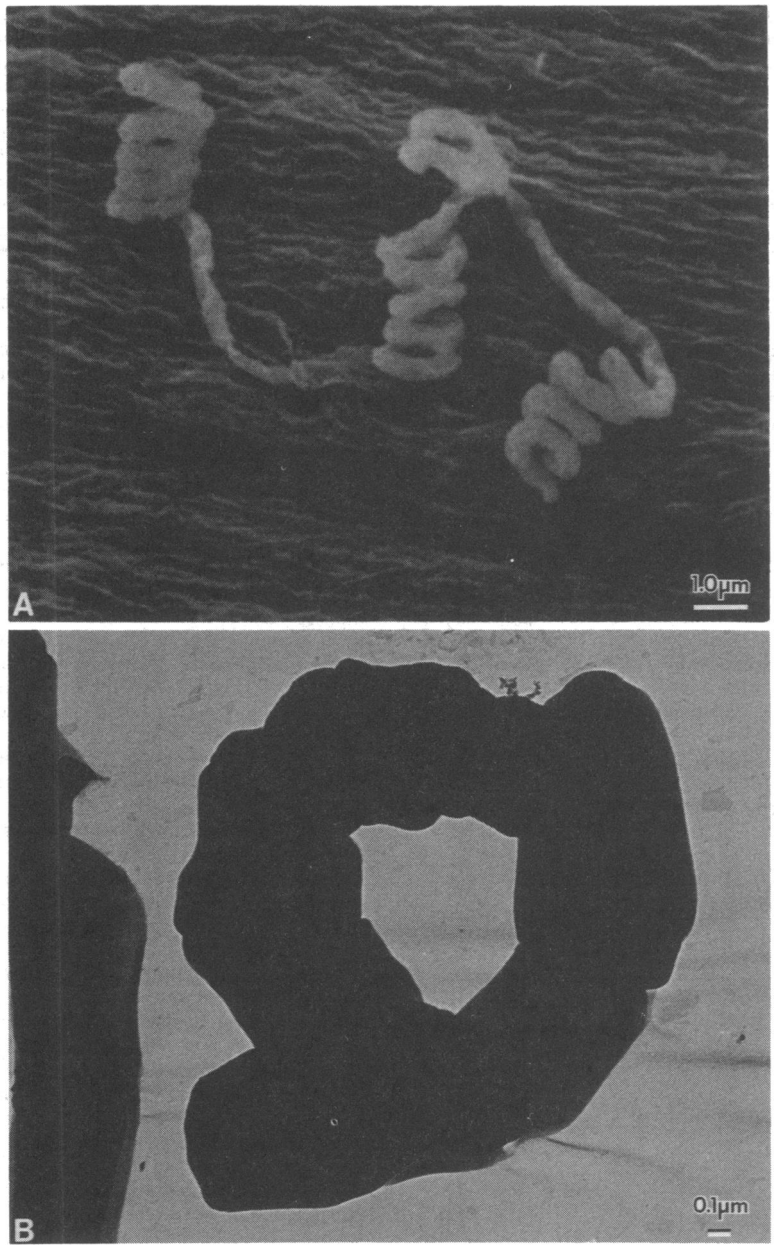

FIG. 3. *(A) Scanning electron micrograph of S. avermitilis MA-4680 (NRRL 8165) showing sporophores forming compact to slightly open spirals; (B) transmission electron micrograph showing the smooth surface of spherical to oval spores.*

element mix could not replace Ardamine. Elimination of $CoCl_2 \cdot 6H_2O$ from medium A slightly stimulated avermectin production.

The effects of various additions to medium A were determined by the anthelmintic assay. Of 12 vitamins tested at 2.5 μg/ml, only biotin stimulated the production of avermectin. The vitamins without effect were folic acid, thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, niacin, inositol, *p*-aminobenzoic acid, ascorbic acid, calcium pantothemate, alpha-tocopherol, and lipoic acid. Vitamin K_5 at 10 or 50 μg/ml was also without effect. Eighteen amino acids were tested at 0.5 and 2.5 mg/ml. Of these, only DL-tryptophan (0.5 mg/ml) and possibly DL-methionine (2.5 mg/ml) stimulated production. The amino acids without effect were glycine, L-cystine, L-cysteine hydro-

chloride, L- and DL-aspartic acid, L-asparagine·H_2O, L-arginine, L-alanine, L-hydroxyproline, DL-isoleucine, DL-leucine, L-glutamine, DL-serine, L-tyrosine, DL-phenylalanine, DL-histidine, and DL-homoserine. The addition of 0.5 mg of KH_2PO_4 per ml or 0.25 mg of $MgSO_4 \cdot 7H_2O$ per ml to medium A inhibited production, whereas sodium chloride (1 mg/ml) was without effect. A mixture of trace elements stimulated production although this effect was not always reproducible. The element or elements responsible have not been identified.

The components of medium B were determined by

TABLE 1. *Cultural characteristics of S. avermitilis MA-4680 (NRRL 8165)*[a]

Medium	Vegetative growth	Aerial mycelia	Soluble pigment
Oatmeal agar (ISP medium 3)[b]	Reverse—very dark brown	Powdery, brownish gray (41i)[c] mixed with white	Brown
Cazpek-Dox agar	Poor, colorless	Scant, grayish	Light grayish tan
Egg albumin agar	Tan	Moderate, light grayish yellowish brown (3ge)[c] mixed with white	Light yellowish brown
Glycerol-asparagine agar (ISP medium 5)[b]	Reverse—yellowish brown	Powdery, brownish gray (41i)[c] mixed with white	Light yellowish brown
Inorganic salts-starch agar (ISP medium 4)[b]	Reverse—grayish-yellowish brown	Powdery, light brownish gray (4ig)[c] edged with darker brownish gray (41i)[c]	Light yellowish brown
Yeast extract-dextrose-salts agar	Reverse—dark brown	Moderate, brownish white	Brown
Yeast extract-malt extract agar (ISP medium 2)[b]	Reverse—dark brown	Moderate, brownish white	Brown
Peptone-iron-yeast extract agar	Dark brown	None	Dark brown to black
Nutrient agar	Tan	Sparse, grayish	Light brown
Nutrient starch agar	Tan	Sparse, grayish white	Light brown
Nutrient gelatin agar	Tan	Sparse, grayish white	Light brown
Gelatin stab	Brown ring	None	Greenish brown
Skim milk agar	Dark brown	None	Dark brown
Litmus milk	Dark brown growth ring	None	Dark brown
Skim milk	Dark brown growth ring	None	Dark brown
Potato plug	Tan	Brown mixed with grayish white	Grayish brown
Loeffler blood serum	Grayish tan	None	Some browning of medium
Nutrient tyrosine agar	Reverse—dark brown to black	Sparse, grayish	Dark brown

[a] All readings were taken after 3 weeks at 28°C.
[b] See reference 17.
[c] Color number designations were taken from *Color Harmony Manual*, 4th ed., Container Corp. of America, Chicago, 1958.

TABLE 2. *Physiological properties of S. avermitilis MA-4680 (NRRL 8165)*

Characteristic	Observation[a]
Carbohydrate utilization[b]	Good growth: glucose, arabinose, fructose, inositol, lactose, maltose, mannitol, mannose, raffinose, rhamnose, sucrose, xylose No growth: cellulose
Melanin production[c]	Positive
H_2S production[c]	Positive
Hydrolysis of starch[d]	Good
Liquefaction of gelatin[e]	Good
Hydrolysis of casein[f]	Good
Peptonization of milk[g]	Complete, becoming alkaline (pH 8)
Liquefaction of Loeffler blood serum	None
Decomposition of tyrosine[h]	None

[a] All readings were taken after 3 weeks at 28°C.
[b] Pridham-Gottlieb basal medium plus 1% carbon source (17).
[c] Peptone-iron-yeast extract agar (17).
[d] Nutrient starch agar (23).
[e] Nutrient gelatin agar; gelatin stab (23).
[f] Skim milk agar (23).
[g] Litmus milk; skim milk (23).
[h] Nutrient tyrosine agar (23).

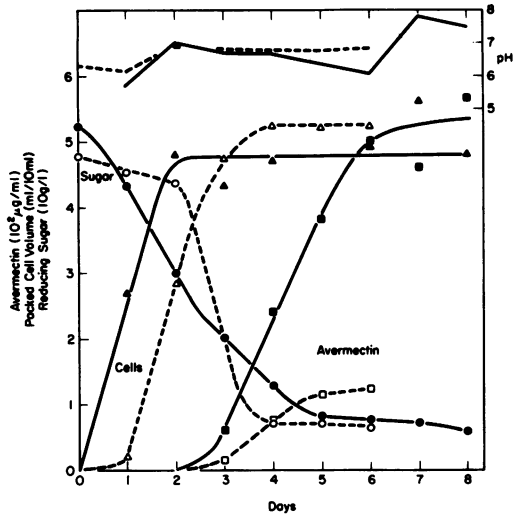

FIG. 4. *Comparison of the fermentation of S. avermitilis MA-4680 (NRRL 8165) (open symbols, dashed lines) and MA-4848 (ATCC 31271) (closed symbols, solid lines) in 250-ml shake flask cultures. Reducing sugar (○, ●) and packed cell volume (△, ▲) were determined as described in the text. Total avermectins (□, ■) were determined by HPLC assay (13). Each point is the average of duplicate flasks removed at 24-h intervals.*

substitution in a basic medium consisting of 3% carbon and energy source, 1.5% nitrogen source, and 0.15% vitamin source. It was determined that dextrose was superior to dextrin, sucrose, or lactose as a carbon and energy source for the production of avermectin. Peptonized milk nutrient was superior to Ferm-Amine I, Sheftene WP 100, and prime meat peptone as the nitrogen source. Autolyzed yeast (Ardamine pH) was the best vitamin source. The addition of polyglycol P-2000 stimulated the production of avermectin threefold from 17 to 53 μg/ml, as determined by the TLC assay. This is probably the single most effective modification of the medium which was made.

TABLE 3. *Complex media for the production of avermectin*

Medium	Component and amt
A	Amidex[a], 40 g; distillers' solubles[b], 7 g; Ardamine pH[c], 5 g; $CoCl_2 \cdot 6H_2O$, 0.05 g; distilled water, pH 7.3, 1 liter
B	Cerelose[d], 45 g; peptonized milk nutrient[e], 24 g; Ardamine pH[c], 2.5 g; polyglycol P-2000[f], 2.5 ml; distilled water, pH 7.0, 1 liter

[a] Corn Products Refining Co.
[b] Brown-Forman Distillers' Corp.
[c] Yeast Products, Inc.
[d] Corn Products Refining Co.
[e] Humko Sheffield Chemical Co.
[f] The Dow Chemical Co.

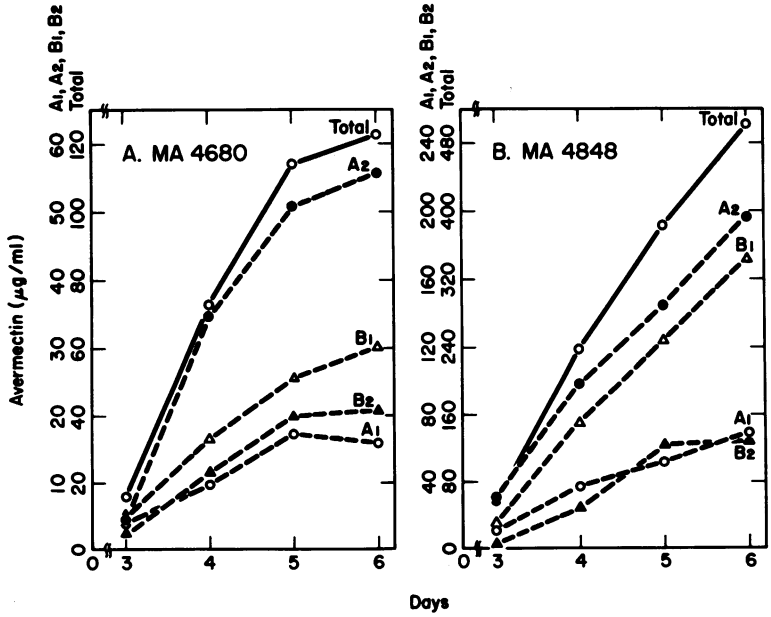

FIG. 5. *Comparison of the production of avermectins A_1 (○), A_2 (●), B_1 (△), and B_2 (▲) by S. avermitilis MA-4680 (NRRL 8165) and MA-4848 (ATCC 31271). The individual components were determined by HPLC assay, and the total avermectin (○, solid line) is the sum of the four components. Note the difference in scales of the ordinates in (A) (MA-4680) and (B) (MA-4848). The data are from the experiments in Fig. 4, and each point represents the average of duplicate flasks removed at 24-h intervals.*

The levels of each of the three components (Cerelose, peptonized milk nutrient, and Ardamine pH) were varied in a three-factor medium optimization study. Two successive studies, each followed by computer analysis of the data, led to the final optimum composition of medium B given in Table 3.

Figure 4 compares the time course of the avermectin fermentation on medium B by *S. avermitilis* MA-4680 and a high-producing isolate, MA-4848 (ATCC 31271). Typical tropophase-idiophase kinetics were exhibited. Both cultures began producing avermectin sometime between 2 and 3 days when growth had ceased. The production by MA-4680 proceeded at a slower rate and was essentially complete by 5 days when the concentration reached 120 μg/ml. The production by MA-4848 proceeded much more rapidly and at a nearly linear rate until the concentration reached 500 μg/ml at 6 days.

Figure 5 compares the production of the four major components of the avermectin complex by *S. avermitilis* MA-4680 (Fig. 5A) and MA-4848 (Fig. 5B) as determined by HPLC assay. Although there was some variability in the relative proportions of the four components, A_1 was usually produced in the least amount (about 10 to 15% of the total), whereas A_2 and B_1 were produced in larger amounts (each about 30 to 40% of the total).

From the structures (Fig. 1), one could infer a precursor-product relationship among the various components. For example, the A components could be produced by methylation of the B components, and A_1 and B_1 could be produced by dehydration of A_2 and B_2. However, the data shown in Fig. 5 do not indicate any such relationship. All four of the major components appeared to be produced concomitantly although at different rates.

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LITERATURE CITED

1. **Brown, W. E., J. Szanto, E. Meyers, T. Kawamura, and K. Arima.** 1977. Taeniacidal activity of streptothricin antibiotic complex S15-1 (SQ-21, 704). J. Antibiot. **30**:886–889.
2. **Buchanan, R. E., and N. E. Gibbons (ed.).** 1974. Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
3. **Cassinelli, G., E. Cotta, G. D'Amico, C. Della Bruna, A. Grein, R. Mazzoleni, M. L. Ricciardi, and R. Tintinelli.** 1970. Thaimycins, new anthelmintic and antiprotozoal antibiotics produced by *Streptomyces michiganensis* var. *amylolyticus* var. *nova*. Arch. Mikrobiol. **70**:197–210.
4. **Della Bruna, C., M. L. Ricciardi, and A. Sanfilippo.** 1973. Axenomycins, new cestocidal antibiotics. Antimicrob. Agents Chemother. **3**:708–710.
5. **Egerton, J. R., D. A. Ostlind, L. S. Blair, C. H. Eary, D. Suhayda, S. Cifelli, R. F. Riek, and W. C. Campbell.** 1979. Avermectins, new family of potent anthelmintic agents: efficacy of the B_{1a} component. Antimicrob. Agents Chemother. **15**:372–378.
6. **Goldsby, A. I., and A. C. Todd.** 1957. A new swine anthelmintic. North Am. Vet. **38**:140–144.
7. **Greenberg, E., J. Berger, G. Beskind, R. Cleeland, H. N. Prince, and E. Titsworth.** 1967. Studies on the *in vitro* and *in vivo* chemotherapeutic properties of the antibiotic myxin. Chemotherapy (Basel) **12**:272–281.
8. **Hamill, R. L., and M. M. Hoehn.** 1964. Anthelmycin, a new antibiotic with anthelmintic properties. J. Antibiot. Ser. A **17**:100–103.
9. **Haneishi, T., M. Arai, N. Kitano, and S. Yamamoto.** 1974. Aspiculamycin, a new cytosine nucleoside antibiotic. III. Biological activities, *in vitro* and *in vivo*. J. Antibiot. **27**:339–342.
10. **Kelley, S. W., L. Harris, M. A. Alexander, and L. S. Olsen.** 1960. Hygromycin B for removing *Thysanosoma actinioides*, fringed tapeworms from feedlot lambs. J. Am. Vet. Med. Assoc. **136**:505–507.
11. **Loebenberg, D., M. Counelis, and J. A. Waitz.** 1975. Antibiotic G-418, a new *Micromonospora*-produced aminoglycoside with activity against protozoa and helminths: antiparasitic activity. Antimicrob. Agents Chemother. **7**:811–815.
12. **Malakhova, E. I., and M. N. Fedotova.** 1974. Effectiveness of destonate-20 against nematodes in pigs and birds. Byull. Vses. Inst. Gelmintol. **12**:43–45.
13. **Miller, T. W., L. Chaiet, D. J. Cole, L. J. Cole, J. E. Flor, R. T. Goegelman, V. P. Gullo, H. Joshua, A. J. Kempf, W. R. Krellwitz, R. L. Monaghan, R. E. Ormond, K. E. Wilson, G. Albers-Schönberg, and I. Putter.** 1979. Avermectins, new family of potent anthelmintic agents: isolation and chromatographic properties. Antimicrob. Agents Chemother. **15**:368–371.
14. **Park, J. T., and M. J. Johnson.** 1949. A submicrodetermination of glucose. J. Biol. Chem. **181**:149–151.
15. **Probst, G. W., M. M. Hoehn, and B. L. Woods.** 1966. Anthelvencins, new antibiotics with anthelmintic properties, p. 789–795. Antimicrob. Agents Chemother. 1965.
16. **Shimura, M., Y. Sekizawa, K. Iinuma, H. Naganawa, and S. Kondo.** 1975. Destomycin C, a new member of destomycin family antibiotics. J. Antibiot. **28**:83–84.
17. **Shirling, E. B., and D. Gottlieb.** 1966. Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. **16**:313–340.
18. **Shirling, E. B., and D. Gottlieb.** 1968. Cooperative description of type cultures of *Streptomyces*. II. Species descriptions from first study. Int. J. Syst. Bacteriol. **18**: 69–189.
19. **Shirling, E. B., and D. Gottlieb.** 1968. Cooperative description of type cultures of *Streptomyces*. III. Additional species descriptions from first and second studies. Int. J. Syst. Bacteriol. **18**:279–392.
20. **Shirling, E. B., and D. Gottlieb.** 1969. Cooperative description of type cultures of *Streptomyces*. IV. Species descriptions from second, third and fourth studies. Int. J. Syst. Bacteriol. **19**:391–512.
21. **Shirling, E. B., and D. Gottlieb.** 1972. Cooperative description of type cultures of *Streptomyces*. V. Additional descriptions. Int. J. Syst. Bacteriol. **22**:265–394.
22. **Tanowitz, H. B., and M. Wittner.** 1973. Paromomycin in the treatment of *Diphyllobothrium latum* infection. J. Trop. Med. Hyg. **76**:151–152.
23. **Waksman, S. A.** 1961. The actinomycetes, vol. 2. The Williams & Wilkins Co., Baltimore.