Chapter 2

Isolation and Characterization of the Producing Organism

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I. Source of Streptomyces avermitilis and discovery of the avermectins

II. Taxonomy of Streptomyces avermitilis

III. Isolation of the avermectins

IV. Antibacterial and antifungal activity

Of the several thousand fermentation products that had been described through 1978, only a few were reported to have anthelmintic activity. Among these were anhehlycin, anthelvencin, the antibiotic complex S-15-1, aspiculomycin, the axenomycins, G-418, hygromycin B, the destomycins, myxin, paromomycin, and the thaimycins. Only hygromycin B has been used commercially; and, until the last decade, synthetic compounds dominated the anthelmintic market. The discovery of the avermectins changed that dramatically.

I. Source of Streptomyces avermitilis and Discovery of the Avermectins

The microorganism that produces the avermectins was isolated from a soil sample collected near a golf course at Kawana, Ito City, Shizuoka Prefecture, Japan. Research scientists at the Kitasato Institute in Tokyo, under the direction of Dr. Satoshi Omura, isolated this culture. This group is noted for its skill in isolating exceptional microorganisms from soil; and, as part of a joint research project between the Kitasato Institute and Merck Sharp & Dohme Research Laboratories (MSDRL), the cultures isolated at the Kitasato Institute are tested in MSDRL’s screening programs. These cultures quickly gained a reputation for being a rich source of new activities, and they are used to test many new screening assays. One of the new assays being developed at MSDRL was based on the administration of test substances to mice infected with *Nematodirus dubius*, and it was decided to test all of the Kitasato cultures in that assay.
The avermectin-producing strain was among one group of 50 Kitasato cultures grown at MSDRL and screened by the *N. dubius* assay. This strain, originally designated OS-3153, was active but quite toxic in the first assay and in a confirmatory assay. In subsequent tests, however, this culture exhibited good activity with little or no toxicity. The unknown activity received the code designation C-076, and the culture was entered into the MSDRL culture collection as MA-4680. Several accounts of the discovery of the avermectins have been published (Burg *et al.* 1979; Campbell *et al.* 1984; Stapley and Woodruff 1982; and Woodruff and Burg 1986).

II. Taxonomy of *Streptomyces avermitilis*

The taxonomy of the producing organism, MA-4680, was established using the classical method of Shirling and Gottlieb (1966) and a number of published taxonomic keys (Buchanan and Gibbons 1974; Shirling and Gottlieb 1968a, 1968b, 1969, 1972; Waksman 1961). The taxonomic description has been published (Burg *et al.* 1979) as follows:

Taxonomic studies of culture MA-4680 revealed it to be a *Streptomyces* species with sporophore forming spirals as side branches on aerial mycelia. The spirals are compact but become open as the culture ages (Figure 2.1). Spores were in chains of more than 15 and were usually spherical to oval. The spore surface was smooth as revealed by electron microscopy (Figure 2.2). 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 2.1 and 2.2, were compared with those of known species in the classical references. . . . No *Streptomyces* species

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![Figure 2.1](image_url)  
**Figure 2.1.** Scanning electron micrograph of *S. avermitilis* MA-4680 showing sporophores forming compact to open spirals.