AB023, NOVEL POLYENE ANTIBIOTICS

I. TAXONOMY OF THE PRODUCING ORGANISM, FERMENTATION AND ANTIFUNGAL ACTIVITY

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AB023 is a complex of polyene antibiotics produced by an actinomycete, SD581, which was isolated from a Kenyan soil sample. The two main components, pentaene antibiotics AB023a and AB023b, have antifungal activity against some phytopathogenic fungi, particularly against *Botrytis cinerea* (MIC of 5 μg/ml).

In the course of a screening program aimed at the discovery of new compounds active against phytopathogenic fungi, we isolated a new complex of polyene antibiotics. This paper describes the discovery and fermentation of antibiotics AB023a and AB023b, the taxonomy of the producing organism and the antifungal properties of AB023a. The isolation and structure elucidation of antibiotic AB023 are reported in a companion paper[1].

Materials and Methods

Microorganism

Strain SD581 was isolated from a soil sample collected in Nairobi, Kenya. The screening procedure was designed for the isolation of actinomycetes. The soil was suspended in water, diluted and plated on a medium consisting of starch 2%, glucose 1%, $CaCO_3$ 0.3%, casein hydrolysate 0.2%, NZ-Amine (Sheffield) 0.2%, yeast extract 0.2%, meat extract 0.2%, agar 2%. The medium was supplemented with cycloheximide (50 μg/ml). The plates were incubated for 20 days at 28°C, in the dark.

Discovery Screen

Antibiotics AB023 were found in a screen for substances with activity against a panel of phytopathogenic fungi consisting of *Botrytis cinerea*, *Fusarium moniliforme* and *Pythium ultimum*. *Candida albicans* was also included. All the test strains were from the collection of Institute of Plant Pathology—University of Milan (Italy).

Strain SD581 was selected because its fermentation broth had activity against *B. cinerea*, *F. moniliforme* and *C. albicans* in agar diffusion tests.

Taxonomic Studies

Methods described by the International Streptomyces Project (ISP)[2], WAKSMAN[3], and WILLIAMS *et al.*[4] were used to determine the morphological and physiological characteristics of the strain SD581.

Fermentation

The seed medium consisted of glucose 1.0%, soluble starch 2.0%, cotton seed meal 0.2%, casein hydrolysate 0.2%, yeast extract 0.2%, meat extract 0.2% and $CaCO_3$ 0.3%. The pH was adjusted to 7.0 before sterilization. Vegetative growth was obtained in two stages: Stage 1) a 500-ml Erlenmeyer flask containing 50 ml of the seed medium was inoculated with a spore suspension from a mature slant culture

and incubated for 72 hours at 28°C; stage 2) the resulting culture was transferred to a 2-liter baffled flask containing 1 liter of the seed medium and incubated further for 72 hours at 28°C. For production of the antibiotics, a 40-liter CHEMAP fermenter was charged with 25 liters of a production medium consisting of glycerol 1.5%, cotton seed meal 1.0% and $CaCO_3$ 0.3%, in tap water. The pH was adjusted to 6.5~7.0 before sterilization at 121°C for 1 hour. The fermenter was inoculated with the entire second stage seed culture and the fermentation was carried out at 28°C. Agitation was 300 rpm, aeration was 0.26 v/v/m and the head pressure was 0.15 kg/cm^2.

Fermentation Analyses

Cell growth was determined as percent packed pellet volume after centrifugation at 5,000 rpm for 20 minutes. Accumulation of antibiotics during fermentation was monitored by HPLC[1].

In Vitro Activity

The MICs for AB023a were determined by an agar dilution method in Potato Dextrose Agar. MIC for *Micrococcus luteus* was determined in nutrient agar (OXOID).

Results

Discovery

The AB023-producing culture was selected from other isolates for its strong activity against *B. cinerea*. The fermentation broth was sufficiently active to confirm interest in the antibiotic and the isolation of the active components was accomplished using *B. cinerea* as test organism.

Taxonomy of the Producing Strain

The cultural and physiological characteristics of strain SD581 are reported in Tables 1, 2 and 3.

A scanning electron micrography of strain SD581 is shown in Fig. 1: Morphological characters are those of *Streptomyces* with *Rectiflexibiles* smooth spore chains. Following the indications of WILLIAMS *et al*[5], SD581 characteristics were analyzed using the MATIDEN computer program[6]. Its close affinity was to *Streptomyces exofoliatus* cluster; the identification scores were: Willcox probability = 0.999, taxonomic distance = 0.413 with a standard error = 0.664.

Table 1. Cultural characteristics of strain SD581.

Medium		Cultural characteristic	Medium		Cultural characteristic
Yeast extract - malt extract agar (ISP 2)	G:	Abundant	Glycerol - asparagine agar (ISP 5)	G:	Abundant
	AM:	Grey		AM:	Grey
	R:	Light yellow		R:	Light yellow
	SP:	Absent		SP:	Absent
Oatmeal agar (ISP 3)	G:	Poor	Peptone - yeast extract - iron agar (ISP 6)	G:	Poor
	AM:	Grey		AM:	Grey
	R:	Light yellow		R:	Light yellow
	SP:	Absent		SP:	Absent
CZAPEK's agar	G:	Poor	Tyrosine agar (ISP 7)	G:	Poor
	AM:	Grey		AM:	White
	R:	Light yellow		R:	Light yellow
	SP:	Absent		SP:	Absent
Starch agar (ISP 4)	G:	Abundant	Nutrient agar	G:	Abundant
	AM:	Grey		AM:	White
	R:	Light yellow		R:	Light yellow
	SP:	Absent		SP:	Light yellow

Observations after incubation for 20 days at 28°C. G; Growth, AM; aerial mycelium, R; reverse, SP; soluble pigment.

Table 2. Physiological characteristics of strain SD581.

Test	Reaction
Lipolysis	+
Pectin hydrolysis	−
Arbutin degradation	+
Growth	
at 4°C	−
at 28°C	+
at 37°C	+
at 45°C	−
with NaCl (7% w/v)	−
with Na-azide (0.01% w/v)	−
with phenol (0.1% w/v)	−

Fig. 1. Scanning electron micrography of strain SD581 grown on Potato Dextrose Agar for 15 days.

Bar represents 10 μm.

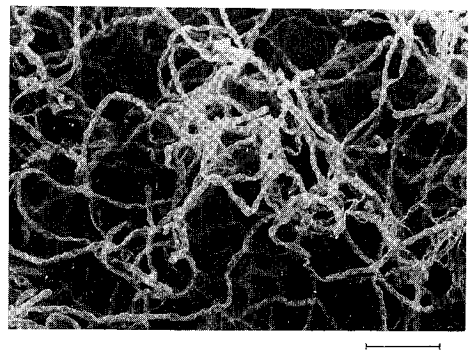


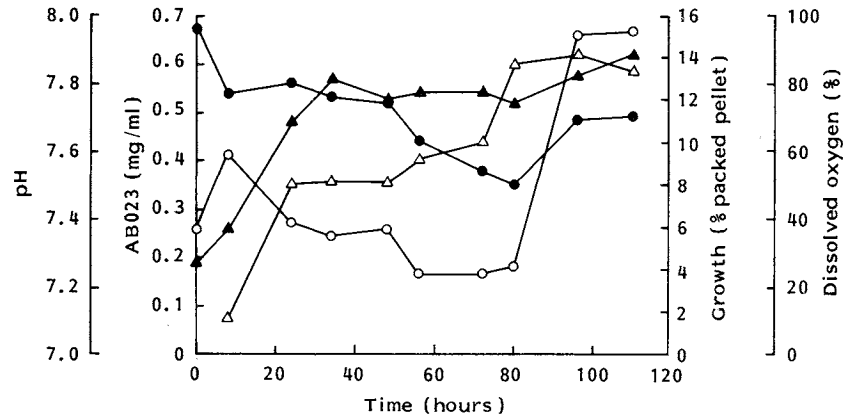
Table 3. Utilization of various compounds as the sole source of carbon by strain SD581.

Carbon source	Growth	Carbon source	Growth
Adonitol	−	α-Methyl-D-glucoside	−
Arabinose	−	*N*-Acetyl-D-glucosamine	−
Cellobiose	−	Raffinose	−
L-Cysteine	+	Sorbitol	−
Galactose	−	Sucrose	−
Glycerol	+ +	Trehalose	−
L-Histidine	+	Valine	+
L-Hydroxyproline	+	Xylitol	−
meso-Inositol	−	Xylose	−
Lactose	−	2-*keto*-Gluconate	−
Maltose	+ +	D-Melezitose	−

The API 20C Auxanogram (Api System SA) was used. Incubation was at 28°C for 20 days.
+ +; Good utilization, +; poor utilization, −; no utilization.

Fig. 2. Time course of the AB023 fermentation.

△ AB023 (mg/ml), ▲ growth (% packed pellet), ○ pH, ● % dissolved oxygen.



Fermentation

Growth, pH, dissolved oxygen and accumulation of antibiotics AB023 are plotted in a time course study of fermentation with strain SD581 (Fig. 2). A maximum potency of 0.62 mg/ml was achieved in

96 hours.

Biological Activity

The MICs of AB023a for some phytopathogenic fungi are given in Table 4. The highest activity was observed against *B. cinerea* (MIC of 5 μg/ml). Antibiotic AB023b has a substantially similar pattern of activity (data not shown).

Table 4. Biological activity of AB023a.

Organism	MIC (μg/ml)
Botrytis cinerea	5
Helminthosporium teres	10
Fusarium roseum	10
F. moniliforme	25
Rhizoctonia solani	10
Colletotricum coffeanum	10
Piricularia orizae	10
Pythium ultimum	100
Candida albicans	10
Micrococcus luteus	100

Discussion

Strain SD581 was assigned to the *Streptomyces* genus on the basis of morphological observation. A good fitting of strain SD581 with *S. exofoliatus* cluster was indicated by the high value of Willcox probability, the low value of taxonomic distance and the small standard error[7].

Strain SD581 was deposited, according to the Budapest Treaty, in the National Collection of Industrial and Marine Bacteria, 23 St. Machar Drive Aberdeen—U.K., and was assigned the accession number NCIMB 40212.

Acknowledgments

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