ALBORIXIN, A NEW ANTIBIOTIC IONOPHORE: TAXONOMY, ISOLATION AND BIOLOGICAL PROPERTIES

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Alborixin is an ionophorous antibiotic of the nigericin group isolated from cultures of a strain of *Streptomyces albus*. It is active against Gram-positive bacteria and is coccidiostatic, but it is very toxic. The antibacterial principle was extracted with organic solvent from the mycelium, isolated in crystalline form and named alborixin.

A new ionophorous antibiotic is produced by a *Streptomyces* sp 3840. Alborixin is active against Gram-positive bacteria and *Eimeria tenella* (chicken coccidiosis).

This report presents the taxonomy of this new *Streptomyces* species, the fermentative production, the isolation and biological properties of alborixin.

Taxonomy

Strain 3840 was isolated from a soil sample collected in Versailles (France).

Most of the taxonomic studies were carried out in accordance with the methods adopted by the International Streptomyces Project.[1]

Media recommended by WAKSMAN[2] were also used.

The various media were inoculated with a washed mycelial suspension from a broth culture shaken at 28°C for 48 hours.

Morphological Characteristics

Strain 3840 showed good growth on various media. It was first grown on STARON potato-glucose-corn steep medium.

(1) Microscopic characteristics: branched substrate mycelium develops aerial mycelia with spore-bearing aerial hyphae, straight or flexuous.

Under an electron microscope the spores show a smooth or warty surface.

The spores are oval-shaped or ellipsoidal, 0.8~1 μ in diameter (Fig. 1).

(2) Appearance on various media: The cultural characteristics of strain 3840 shown in Table 1 were observed after two weeks of incubation at 27°C on the designated media.

The stock culture of strain 3840 was maintained as agar slants (STARON potato-corn steep agar) freeze-dried spores, freeze-dried mycelia, or frozen mycelia.

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Fig. 1. Electron micrograph of the spores of strain 3840 (×7,000).

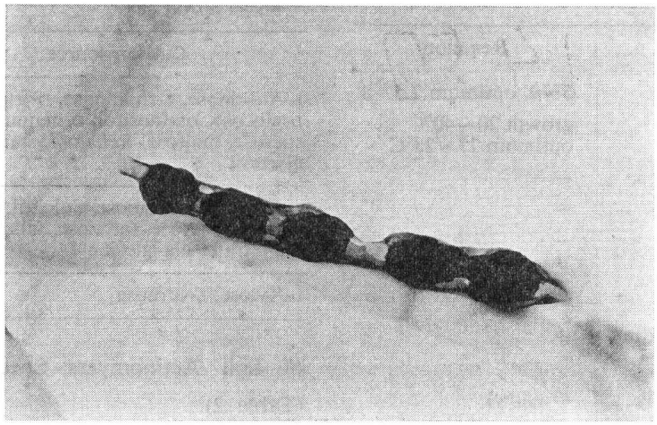


Table 1. Characteristics of strain 3840

Medium	Growth	Aerial mycelium	Diffusible pigment
CZAPEK's agar (WAKSMAN 1)	good, colorless	abundant, white	none
Glycerol nitrate agar (WAKSMAN 1)	moderate,colorless,	white, forming rings around the colonies	none
Glucose-asparagine agar (WAKSMAN 3)	poor, colorless	poor, white	none
Glucose tyrosine agar (WAKSMAN 11)	poor, colorless	poor, white	none
Glycerol citrate agar (WAKSMAN 4)	good, colorless	white, forming rings around the colonies	none
Nutrient agar	moderate, colorless	moderate	none
BENNETT's agar	good growth, colorless to brownish	good, white with water drops	none
EMERSON agar	good growth, colorless to brownish	moderate, whitish to yellowish	none
Yeast malt agar (WAKSMAN 31)	good growth, colorless to brownish	moderate, white	none
Carrot plug	none	none	none
Potato plug	none	none	none
Potato dextrose agar (Difco)	none	none	none
Potato peptone glycerine agar (P medium of SHINOBU)	moderate, colorless to brownish	moderate, white	none
Potato glucose corn steep agar (STARON's medium)	good growth, colorless to brownish	abundant, white	none
SABOURAUD medium (Difco)	none	none	none
Oatmeal agar	good growth	abundant, white	none

(3) Physiological characteristics: Physiological characteristics were investigated according to the International Streptomyces Project[1] and the Physiological and Cultural Study for the Identification

Table 2. Physiological properties of streptomycete strain 3840

Parameters	Reaction
pH Range	5~9, optimum 7.5~8
Temperature	growth 20~40°C optimum 27~28°C
Melanin formation	−
H_2S Production	−
Tyrosinase reaction	−
Nitrate reduction	+
Starch hydrolysis	+
Liquefaction of gelatin	+
Coagulation of milk	+
Peptonization of milk	+ (slow)
Cellulose decomposition	−

Table 3. Utilization of carbon sources by *Streptomyces* strain 3840

Carbon source	Growth
L-Arabinose, L-rhamnose, D-fructose, D-glucose, DL-inositol, D-mannitol, sucrose, maltose, trehalose, starch, glycerol	good
D-Galactose, D-ribose, dulcitol, sorbitol, lactose, raffinose, salicin, Na-citrate, Na-succinate	moderate
L-Xylose, L-sorbose	none

of Soil Actinomyces Species by SHINOBU[3] (Table 2).

Utilization of carbon sources could not be examined on the PRIDHAM and GOTTLIEB medium[4] because there was no growth on it; it was investigated with CZAPEK's medium (WAKSMAN's medium 1) (Table 3).

In summary, the strain 3840 belongs to genus *Streptomyces* and forms branched aerial mycelium, straight or flexuous. Its outward appearance is powdery and white. The spores are smooth.

Soluble pigment is never observed and melanin reaction is negative, so it presents all the characteristics of a *Streptomyces albus,* though sporophores have no spirals, only hooks.

Fermentation of Alborixin

Strain 3840 was grown in several media at various pH and temperature values.

Several sources of carbon and nitrogen were found suitable, the best ones being dextrose as a source of carbon and corn steep or casamino acids as sources of nitrogen.

Ferrous sulfate or zinc sulfate were found to improve remarkably alborixin production.

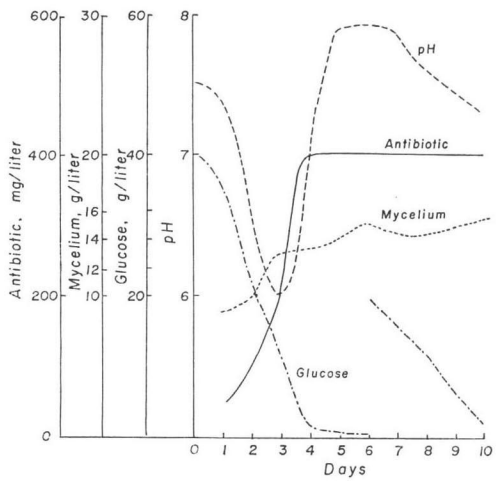
Strain 3840 was maintained as frozen mycelium for inoculation in a 500-ml Erlenmeyer flask filled with 100 ml of an inoculum medium consisting of: dextrose 40 g, soyabean flour 10 g, corn steep solid 3 g, $CaCO_3$ 5 g, tap water 1,000 ml.

The flask was agitated on a rotary shaker at 200 rev/min for 2 days at 27°C and transferred to a 2-liter fermentor filled with 1 liter of the same medium and cultured at 27°C for 24 hours.

The resulting seed culture was then transferred to a 20-liter glass fermentor containing 12 liters of the following fermentation medium: dextrose 40 g, corn steep solid 10 g, $FeSO_4 \cdot 7H_2O$ 0.5 g, $CaCO_3$ 5 g, tap water to 1,000 ml; pH after sterilization, 8.

The fermentation was carried out with stir-

Fig. 2. Changes occurring during fermentation in dextrose-corn steep medium.



ring at 400 rpm and 6 liters per minute aeration for 4~7 days at 27°C.

Results obtained during a typical fermentation are shown in Fig. 2.

During the fermentation and isolation process the antimicrobial activity was determined by the broth dilution method using *Bacillus cereus*.

Isolation and Purification

Alborixin was extracted from the mycelial cake, after filtration with a Celite aid.

Isolation and purification are given in the preceding paper.[5]

Biological Properties

The antimicrobial spectrum against bacteria and fungi was determined by the conventional serial dilution method. It is given in Table 4.

Table 4. Inhibitory concentration of alborixin*

Organism	MIC (mcg/ml)
Bacillus cereus	0.4
Bacillus megaterium	<0.1
Streptococcus lactis	0.8
Staphylococcus aureus	0.2
Corynebacterium xerosis	0.8
Escherichia coli	>100
Serratia marcescens	>100
Proteus morganii	>100
Pseudomonas aeruginosa	100
Aspergillus niger	100
Candida parapsilosis	>100
Aerobacter aerogenes	100

* Tests in nutrient broth for bacteria, SABOURAUD medium for fungi 24 hours at 30°C.

Alborixin is active against Gram-positive bacteria. It is as active as monensine against *Eimeria tenella*.

The LD_{50} of alborixin determined in mice subcutaneously was 150 mg/kg.

References

1) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313~340, 1966
2) WAKSMAN S. A.: The actinomycetes. Vol. II. The Williams and Wilkins Co., Baltimore, 1961
3) SHINOBU R. B.: Physiological and cultural study for the identification of soil actinomyces species. Mem. Osaka Univ. B. Nat. Sci. 7: 1~76, 1958
4) PRIDHAM, T. G. & D. GOTTLIEB: The utilization of carbon compounds by some Actinomycetales as an aid for species determination. J. Bact. 56: 107~114, 1948
5) GACHON, P.; C. FARGES & A. KERGOMARD: Alborixin, a new antibiotic ionophore: Isolation, structure, physical and chemical properties. J. Antibiotics 29: 603~610, 1976