X-14547A, A NEW IONOPHOROUS ANTIBIOTIC PRODUCED BY *STREPTOMYCES ANTIBIOTICUS* NRRL 8167

DISCOVERY, FERMENTATION, BIOLOGICAL PROPERTIES AND TAXONOMY OF THE PRODUCING CULTURE

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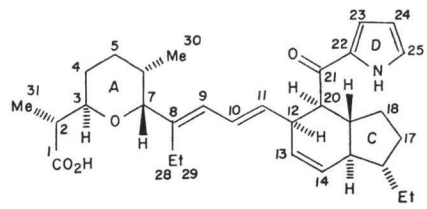
X-14547A is a novel antibiotic produced by a new strain of *Streptomyces antibioticus* (NRRL 8167). The antibiotic is active *in vitro* against Gram-positive bacteria and is capable of complexing and transporting divalent as well as monovalent metal cations.

In the course of our search for new antibiotics from soil cultures, a strain of *Streptomyces antibioticus* (NRRL 8167) was found which produced an antibiotic with activity mainly against Gram-positive bacteria. The antibiotic activity, similar to that of the polyether antibiotics, was shown to be solvent extractable and quite stable. Isolation and chemical characterization of the antibiotic X-14547A (the structure shown in Fig. 1) are described in the following report[1]. In this report we present the taxonomy of the producing organism, fermentation conditions for production of X-14547A, and biological properties of the antibiotic.

Fig. 1. Structure of antibiotic X-14547A



Taxonomy of the Producing Organism

The organism producing the antibiotic X-14547A was isolated from a soil sample collected at Martinsville, Virginia, U.S.A., in 1974. The culture was identified as a strain of *Streptomyces antibioticus*[2,3], and deposited at NRRL (Northern Regional Research Laboratory, Peoria, Ill.) where it has been assigned the number 8167. This strain is very similar in phenotypic properties to the type strain of the species (ATCC 8663). The organism produces a substrate mycelium which does not fragment and an aerial mycelium forming chains of spores of the *rectus-flexibilis* type. The spores measure 1.26~1.42 μm by 0.69~0.91 μm, and have a smooth surface (Plate 1). The cell walls of the organism contain the LL-isomer of diaminopimelic acid[4]. Growth characteristics of strain NRRL 8167 are summarized in Table 1, and morphological and physiological properties of the strain in comparison with those of the type strain of the species, ATCC 8663, are given in Table 2. Differences between the two strains include nitrate reduction, melanin production, utilization of L-arabinose and antibiotic production.

Table 1. Cultural characteristics of *Streptomyces antibioticus* NRRL 8167

Agar medium	Amount of growth Degree of sporulation	Spore mass color	Color of reverse substrate mycelium
Yeast malt extract (ISP-2)[a]	moderate to abundant growth; well sporulated; slightly hygroscopic	3*fe* (silver gray) mostly; some tufts of *a* (white)	2*nl* (covert brown) at center; 2*ca* (light ivory) at edge
Oatmeal (ISP-3)[a]	abundant growth; well sporulated	3*fe* (silver gray)	3*fe* (silver gray) at center and 2*dc* (natural) at edge
Inorganic salts-starch (ISP-4)[a]	abundant growth; well sporulated	3*fe* (silver gray); with edges and flecks of *b* (oyster white)	2*dc* (natural)
Glycerol-asparagine (ISP-5)[a]	moderate growth; moderately to well sporulated; slightly hygroscopic	2*fe* (covert gray) with patches and edges of *b* (oyster white)	2*ec* (bisquit) mostly; 2*ge* (covert tan) at edge
Peptone-yeast extract-iron (ISP-6)[a]	moderate growth; no sporulation; dark brown soluble pigment	*i* (gray) where not sporulated	*i* (gray) where not sporulated
Tyrosine (ISP-7)[a]	poor growth; some sporulation; slight amount of brown soluble pigment	2*dc* (natural)	3*li* (beaver)
CZAPEK-DOX	poor growth; sparsely sporulated	*b* (oyster white)	*b* (oyster white)
BENNETT'S[b]	moderate growth; well sporulated; hygroscopic	2*fe* (covert gray)	3*lg* (adobe brown)
SABOURAUD dextrose (Difco)	moderate growth; no sporulation	3*ie* (camel) where not sporulated	3*ie* (camel) where not sporulated
Thermoactinomyces fermentation (Difco)	abundant growth; well sporulated; hygroscopic	3*fe* (silver gray) mostly; with tufts of *b* (oyster white)	3*pn* (dark brown) at center; and 3*ng* (yellow maple) at edge
ATCC 5[c]	moderate growth; well sporulated; hygroscopic	3*fe* (silver gray); also areas of 3*dc* (natural)	3*pl* (mustard brown)
Amidex[d]	abundant growth; well sporulated; slightly hygroscopic	3*fe* (silver gray) mostly; edges of *b* (oyster white)	3*pl* (mustard brown) at center; 2*cb* (ivory tint) around edge
Starch casein[e]	abundant growth; well sporulated; hygroscopic	3*fe* (silver gray); *b* (oyster white) in one area	2*dc* (natural)

Agar plates were read after 14 days of incubation at 28°C. The color scheme used was the Color Harmony Manual (Container Corporation of America, 4th edition, 1958).

a) See reference (2) for ISP media.

b) Yeast extract, 0.1%; beef extract, 0.1%; N-Z Amine A (Humko-Sheffield Chem. Co., Lyndhurst, N.J.), 0.2%; dextrose, 1%; agar, 1.8%, pH 7.3.

c) American Type Culture Collection, Catalog of Strains, 11th Edition, 1974.

d) Amidex (Corn Products Co., Decatur, Ill.), 1%; N-Z Amine A, 0.2%; beef extract, 0.1%; yeast extract, 0.1%; $CoCl_2 \cdot 6H_2O$, 0.002%, agar, 2%, pH adj. to 7.3[5].

e) Soluble starch, 1%; casein, 0.1%; K_2HPO_4, 0.05%; $MgSO_4$, 0.05%; agar, 1.5%; pH adj. to 7.4.

Fermentation

The vegetative inoculum was developed by transferring spores of *S. antibioticus* NRRL 8167 to a 6-liter Erlenmeyer flask containing 2 liters of a medium with the following composition (in g/liter): Tomato pomace 5.0, distiller's solubles 5.0, O.M. peptone (Oscar Mayer & Co., Madison, Wisconsin) 5.0, debittered dried yeast 5.0, corn starch 20.0, $CaCO_3$ 1.0, and K_2HPO_4 1.0. The pH of this medium was adjusted to 7.0 before autoclaving. The inoculated flask was incubated for 72 hours at 28°C on a rotary shaker operating at 250 rpm. Four liters of the resulting culture was used to inoculate a 100-gallon fermentor containing 60 gallons of the following medium (in g/liter): glucose 10.0, black-

Plate 1a. *Streptomyces antibioticus* NRRL 8167. Typical *rectus-flexibilis* spore chains with some atypical loose spiral chains. 14 days on ISP-3 agar. ×2670.

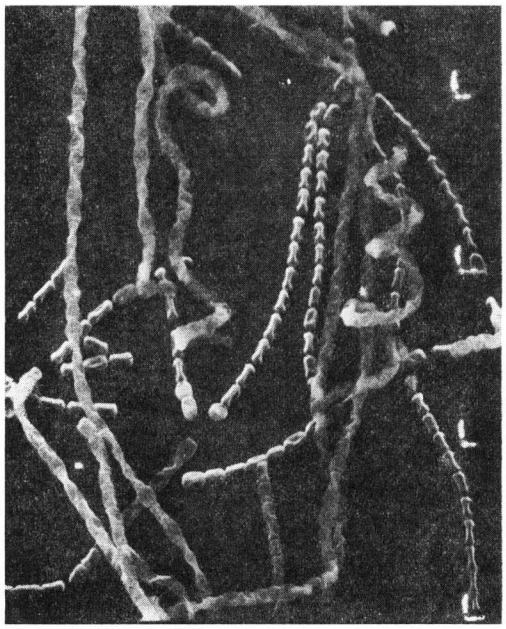


Plate 1b. *Streptomyces antibioticus* NRRL 8167. Smooth spores. 14 days on ISP-3 agar. ×9070.

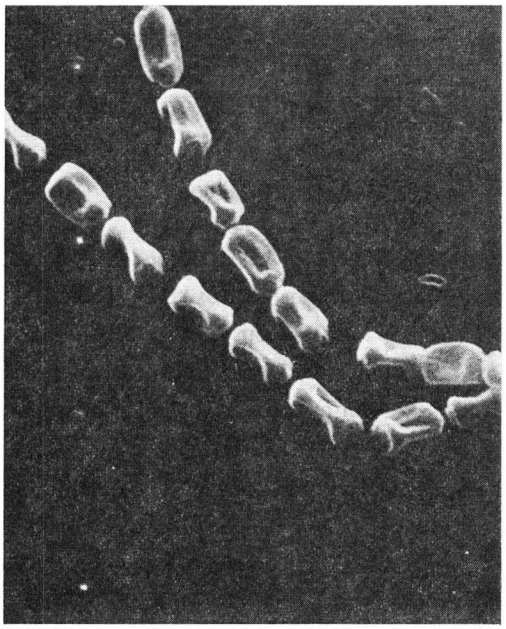


Table 2. Morphological and physiological characteristics of *Streptomyces antibioticus* NRRL 8167 and *S. antibioticus* ATCC 8663

Test	NRRL 8167	ATCC 8663	Test	NRRL 8167	ATCC 8663
H_2S, ISP-6	+	+	Sucrose utilization*	−	−
Melanin, ISP-7	+ weak	−	Cellulose utilization*	−	−
Spore surface	smooth	smooth	Reverse side pigment	−	−
Color of spore mass	gray	gray	Soluble pigment	−	−
Spore chain form	*rectus-flexibilis*	*rectus-flexibilis*	Streptomycin sensitivity, 10 μg disc	+	not tested
D-Glucose utilization*	++	++	Nitrate reduction	−	+
D-Xylose utilization*	++~+	±	Casein hydrolysis	+	+
L-Arabinose utilization*	++	+~±	Gelatin hydrolysis	+	+
L-Rhamnose utilization*	++	++	Starch hydrolysis	+	+
D-Fructose utilization*	++	++	ISP-1 darkening	+	+
D-Galactose utilization*	++	++	NaCl (%) tolerance	5	not tested
Raffinose utilization*	−	−	Temperature growth range °C	10~37	not tested
D-Mannitol utilization*	++	++	DAP isomer	LL	LL
i-Inositol utilization*	++	++	Antibiotic production	X-14547A	−
Salicin utilization*	−	−			

++=strong positive response; −=negative response.

* These tests were done in carbon-utilization agar (Bacto ISP-9 medium) containing 1.0% of the indicated carbon source.

strap molasses 20.0, HySoy T (Sheffield Chem. Co., Union, N.J.) 5.0, $CaCO_3$ 2.0. The pH of the medium was adjusted to 7.2 with NaOH before sterilization. The tank was aerated at 3 cfm and stirred at a rate of 280 rpm. Results from a typical X-14547A fermentation in a 100-gallon fermentor is shown in Table 3.

Table 3. Time course of X-14547A production

Time (Day)	Potency* Diameter of inhibition zone in mm
2	0
3	0
4	11.0
5	12.8
6	12.0

* Whole broth was assayed against *Bacillus megaterium* ATCC 8011 using agar diffusion cup-plate assay.

Biological Properties

Antibiotic X-14547A is active *in vitro* primarily against Gram-positive bacteria, having a spectrum of activity similar to other ionophore antibiotics (Table 4).

Antibiotic X-14547A is effective as a growth promotant for ruminants, increasing the efficiency of feed utilization by these animals[6]. The acute toxicity of the antibiotic in mice is 129 mg/kg p.o.

X-14547A facilitates extraction of monovalent and divalent cations from an aqueous solution into a non-miscible organic solvent[7]. The antibiotic also transports Rb^+ and Ca^{2+} across a solvent barrier ($CHCl_3$) from one aqueous phase into another (Fig. 2)[8]. The ability to transport Ca^{2+} is shared with only a few of the known carboxylic acid ionophorous antibiotics, lasalocid, A23187, ionomycin and lysocellin[9,10,11].

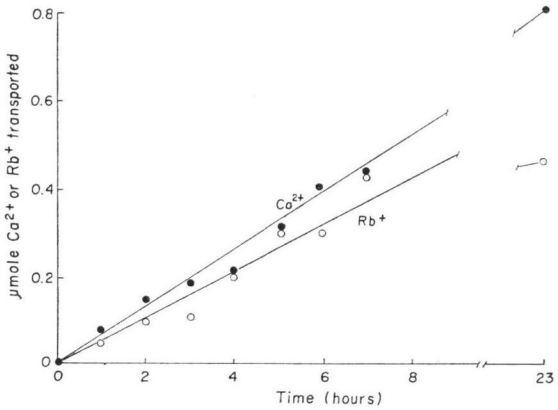
Table 4. Antimicrobial spectrum of antibiotic X-14547A, lasalocid A and antibiotic A23187

Test organism	MIC (μg/ml)*		
	Antibiotic X-14547A	Lasalocid A	Antibiotic A23187
Pseudomonas aeruginosa ATCC 8709	>100	>100	>100
Proteus vulgaris ATCC 6380	>100	>100	>100
Escherichia coli ATCC 27856	>100	>100	>100
Klebsiella pneumoniae ATCC 27858	>100	>100	>100
Serratia marcescens ATCC 27857	>100	>100	>100
Serratia sp. ATCC 93	>100	>100	>100
Acinetobacter calcoaceticus ATCC 10153	>100	>100	>100
Staphylococcus aureus ATCC 6538 P	0.2	1.6	0.19
Sarcina lutea ATCC 9341	0.1	3.1	0.045
Bacillus megaterium ATCC 8011	0.1	0.3	0.79
Bacillus sp. E ATCC 27859	0.2	0.2	0.19
Bacillus subtilis NRRL 558	0.1	1.6	0.09
Bacillus sp. TA ATCC 27860	0.2	1.6	0.045
Mycobacterium phlei ATCC 355	3.1	12.5	1.57
Streptomyces cellulosae ATCC 3313	0.8	6.3	6.25
Paecilomyces varioti ATCC 26820	>100	0.6	>100
Penicillium digitatum ATCC 26821	>100	>100	1.57
Candida albicans NRRL 477	>100	>100	0.39
Saccharomyces cerevisiae ATCC 4226	>100	>100	>100

* Lowest two-fold dilution giving a zone of inhibition in an agar diffusion assay.

Fig. 2. Time course of Ca^{2+} and Rb^{+} transport by antibiotic X-14547A in a U-tube

The U-tube system described by ASHTON and STEINRAUF[8] was employed for the assay. A glass U-tube was filled with 5 ml of a chloroform solution of antibiotic X-14547A (2×10^{-4} M). Two ml of an aqueous buffer (Tris-HCl, 20 mM, pH 9.5) containing 1 mM [^{45}Ca]calcium chloride or [^{86}Rb] rubidium chloride was placed in one arm and an equal volume of the same buffer solution with unlabelled calcium or rubidium chloride in the other arm. The reaction was started by the addition of the respective labelled metal chloride and the chloroform phase separating the two aqueous phases was then gently stirred with a magnetic stirrer. The rate of appearance of radioactive calcium or rubidium in the label-free side was determined by counting samples (50 μl) taken from both aqueous phases with 10 ml Aquasol (New England Nuclear, Boston, Mass.) in an Intertechnique liquid scintillation spectrometer.



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