A NEW ANTIBIOTIC, XK-46

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(Received for publication January 31, 1974)

A new antibiotic XK-46 is produced optimally at 46°C by a thermophilic *Streptomyces* sp. MK-46. This antibiotic is a color indicator, red in acidic and blue in alkaline solutions, soluble in organic solvents and slightly soluble in water. XK-46 is active against Gram-positive bacteria and *Proteus vulgaris*, and strongly inhibits tyrosine hydroxylase activity.

A new antibiotic XK-46 is produced by a thermophilic *Streptomyces* sp. MK-46 which can grow between 30~55°C. This antibiotic is a color indicator, red in acidic and blue in alkaline solutions, is active against Gram-positive bacteria and *Proteus vulgaris* but not against other Gram-negative bacteria, yeast and fungi. The compound is also found to inhibit strongly tyrosine hydroxylase activity. XK-46 seems similar to litmocidin from the data on elemental analysis, molecular weight and visible spectrum but differs from it in paper chromatographic behavior.

Characteristics of the strain and the isolation and properties of the antibiotic are reported in this paper.

Characteristics of the Strain MK-46

The strain (the laboratory number MK-46 and American Type Culture Collection number ATCC 21729) belongs to the genus *Streptomyces* and its cultural characteristics on various media after culturing for 5 days at 45°C are described in Table 1. The aerial mycelia were white or gray and the soluble pigment red at acid pH and blue at alkaline pH. The strain utilized D-glucose, D-mannitol and L-rhamnose readily, utilized D-fructose and D-raffinose only slightly and did not utilize D-arabinose, L-inositol, D-xylose and saccharose as a carbon source. As seen in Table 2, the strain could grow between 30~55°C with optimal growths at 40~52°C. The temperature for maximal antibiotic production was 46°C. Some other physiological properties were as follows: pH range for growth was 5.0~9.0, the liquefaction of gelatin and hydrolysis of starch were positive, chromogenic action was positive, milk was peptonized, nitrate was not reduced, and cellulose was not decomposed.

Production and Isolation of XK-46

A medium containing 2 % glucose, 0.5 % dried yeast, 1 % distillers solubles, 0.05 % $MgSO_4 \cdot 7H_2O$, 0.03 % KCl and 0.1 % $CaCO_3$ (pH 7.0 before sterilization) was used for antibiotic production. The strain was cultured at 45°C for 30 hours with 15 liters/minute aeration and 300 rpm stirring in a 30-liter jar fermentor. The culture broth was adjusted to pH 4.0 with HCl, the antibiotic was extracted with the half volume of chloroform and the chloro-

Table 1. Cultural characteristics of *Streptomyces* sp. MK-46

Medium	Growth	Substrate mycelium	Aerial mycelium	Formation of aerial mycelium	Soluble pigment
Glucose-asparagine agar	Moderate	Brown (5 le)	Greenish gray (24 1/2dc)	Moderate	Reddish brown (5 ie)
Glycerol-asparagine agar	Moderate	Cocoa brown (5 ni)	Gray (3 dc)	Moderate	brown (4 li)
Starch-agar	Moderate	Violet (7 ie)	Greenish gray (1 fc)	Moderate	Violet (9 ig)
Tyrosine agar	Good	Chocolate brown (5 po)	Gray (2 dc)	Good	Cocoa brown (5 ni)
Nutrient agar	Good	Chocolate brown (5 po)	Gray (3 dc)	Good	Ebony brown (2 po)
Yeast ext.-malt ext. agar	Good	Ebony brown (8 pn)	Gray (3 fe)	Good	Ebony brown (8 pn)
Oat meal agar	Moderate	Violet (6 1/2 pi)	Gray (2 ih)	Moderate	Violet (6 1/2 ig)

Streptomyces sp. MK-46 was cultured at 45°C for 5 days. The indications in the parentheses are in accordance with the color classification of Color Harmony Manual (Container Corporation of America).

form layer was concentrated. The concentrate was put on a 500-ml silica gel column which was subsequently washed with 1 liter of chloroform, and XK-46 was eluted with a solvent mixture of chloroform - methanol (30:1). The active fraction was concentrated to obtain a red powder. This product was dissolved in 50 ml of ethylether. After removing the residue, the filtrate was concentrated to 5 ml and stored overnight in a refrigerator. The compound was crystallized from ethylether to yield about 100 mg of a red crystalline substance.

Table 2. Optimal temperature of growth and production of XK-46

Temp. (°C)	Cell growth	Production
20	−	−
25	−	−
30	+	−
35	++	±
40	+++	+
46	+++	++
52	+++	+
55	+	±
57	−	−

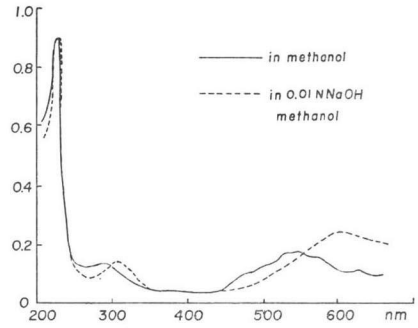
Streptomyces sp. MK-46 was cultured for 24 hours in the medium described in "Production and Isolation of XK-46". The extent of cell growth or production of XK-46 is recorded as no growth or production −, poor ±, good to very good +, ++, +++

Properties of XK-46

XK-46 is a color indicator, red at acid pH, violet at neutral pH and blue at alkaline pH. As seen in Fig. 1, XK-46 has maximal absorbancy at 224 nm, 286 nm, 495 nm (shoulder), 530 nm, 573 nm and 617 nm in methanolic solution, and 226 nm, 310 nm and 600~610 nm in 0.01 N NaOH-methanol. The antibiotic is soluble in alcohols, acetone, chloroform, ethylacetate, ethylether and benzene, slightly soluble in water, and insoluble in *n*-hexane and petroleum ether. Its melting point was not sharp but it began to melt at

74~76°C. The elemental analysis was : C 59.24, H 6.70. Nigrogen was not present. The molecular weight was 400~420 (determined by vaporpressure osmometor), and $[\alpha]_D^{23}$ was +212° (*c* 0.0024, acetone). In paper chromatography, Rf values of XK-46 were 0.38 with 20 % aqueous ammonium chloride, 0.04 with water-saturated *n*-butanol, 0.75 with *n*-butanol - acetic acid - water (3:1:1), 0.29 with water-saturated ethylacetate and 0.00 with water-saturated *n*-butanol containing 2 % piperidine and 2 % *p*-toluenesulfonic acid. In silica gel thin-layer chromatography, the compound had the following Rf values : 0.70 with benzene - ethylacetate - methanol - acetic acid (10 : 5 : 5 : 1), 0.77 with benzene - ethylacetate - acetic acid (5 : 5 : 1), 0.68 with benzene - chloroform - ethylacetate - acetic acid (10 : 5 : 5 : 1) and 0.48 with ethylether - chloroform - ethylacetate - acetic acid (10 : 5 : 5 : 1). The Rf value in cellulose thin-layer chromatography using an Eastman Kodak chromagram sheet was 0.57 with water-saturated *n*-butanol. The IR spectrum in KBr tablet is shown in Fig. 2. When XK-46 was kept at pH 2 or pH 6 in methanolic solution for 5 hours at 50°C, the activity did not decrease, but at pH 10, only 4 % activity remained. It is obvious that XK-46 is stable in acidic but not in alkaline solutions.

Fig. 1. Ultraviolet and visible spectra of XK-46



As seen in Table 3, XK-46 is active against Gram-positive bacteria, *Proteus vulgaris*, *Mycobacterium phlei*, *Mycobacterium koda* and some strains resistant to known antibiotics, but is not active against other Gram-negative bacteria, yeast and fungi. As seen in Fig. 3, XK-46 has a potent inhibitory action on tyrosine hydroxylase which was partially purified from beef adrenal medulla homogenates by a method described by NAGATSU *et al.*[1] and assayed accord-

Fig. 2. IR spectrum of XK-46

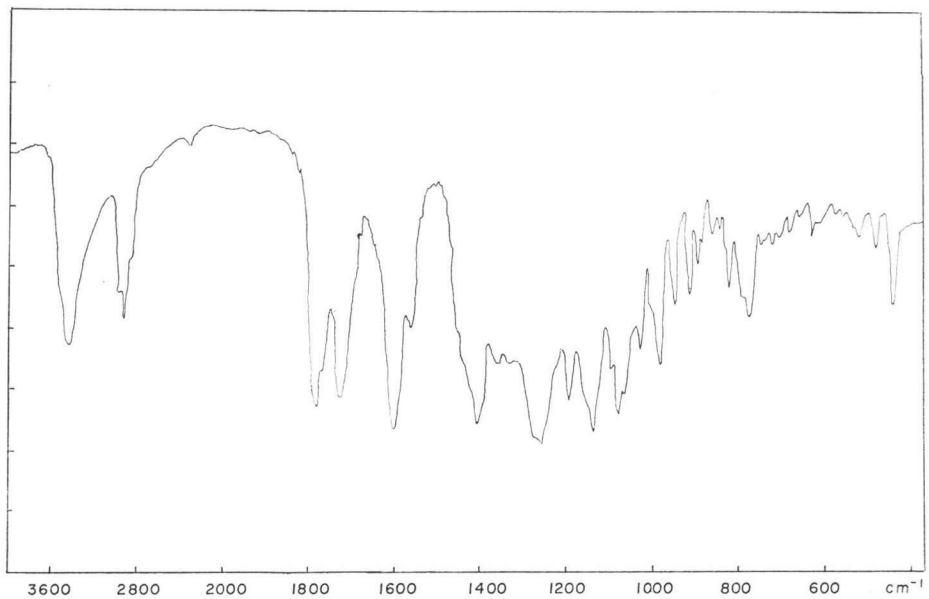
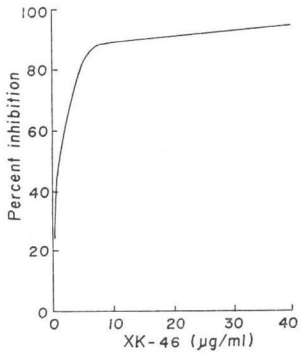


Fig. 3. Inhibiton of tyrosine hydroxylase by XK-46.

The reaction mixture contained 200 μ moles of phosphate buffer (pH 6.0), 0.1 μ mole of L-tyrosine containing 1×10^5 c. p. m of L-tyrosine ^{14}C, 100 μ moles of mercaptoethanol, 1 μ mole of 2-amino-4-hydroxy-6,7-dimethyltetrahydropteridine and methanolic solution of XK-46 in the indicated concentration. Incubation was carried on at 30°C for 15 minutes. The effect of XK-46 on the enzyme reaction was studied by determining 3,4-dihydroxyphenylalanine produced.



ing to AYUKAWA *et al.*[2] XK-46 had also a slight anti-tumor activity against EHRLICH ascites carcinoma and Sarcoma 180 solid tumors. The acute toxicity of XK-46 was 12.5 mg/kg of LD_{50} in mice after intraperitoneal injection.

Discussion

XK-46, a pH indicator, soluble in organic solvents and slightly soluble in water, does not contain nitrogen. These considerations and its anti-bacterial spectrum would make

Table 3. Minimal inhibitory concentration of XK-46

Organism	MIC (μg/ml)
Streptococcus faecalis ATCC 10541	2.6
Staphylococcus aureus ATCC 6538p	0.08
Staph. aureus KY 8942 (R-KM, PM, SM)	0.16
Staph. aureus KY 8946 (R-Mac)	0.16
Bacillus subtilis No. 10707	0.04
Sarcina lutea ATCC 9341	1.3
Neisseria catarrhalis ATCC 7900	1.3
Mycobacterium avium F (KB 44)	>83
Mycobacterium phlei IFO 3158	0.65
Mycobacterium koda (KB 47)	2.6
Mycobacterium smegmatis ATCC 10143	83
Candida albicans ATCC 10231	>83
Aspergillus niger KY 179	>83
Escherichia coli ATCC 4352	21
Escherichia coli ATCC 26	>83
Escherichia coli ATCC 11775	>83
Proteus vulgaris ATCC 6897	2.6
Proteus vulgaris KY 4296 (R-NA)	1.3
Proteus mirabilis KY 4293	>83
Proteus morgani KY 4298	>83
Proteus rettgeri KY 4288	>83
Shigella sonnei ATCC 9290	>83
Salmonella typhosa ATCC 9992	>83
Serratia marcescens ATCC 4003	>83
Klebsiella pneumoniae ATCC 10031	>83
Pseudomonas aeruginosa BMH 1	>83
Aerobacter aerogenes ATCC 13048	>83

Assayed with agar dilution method at pH 7.0 for 17 hours. Resistances to antibiotics. KM: Kanamycin, PM: Paromomycin, SM: Streptomycin, Mac: Macrolide antibiotics, NA: Nalidixic acid.

Table 4. Comparison of chemical and physical properties of XK-46 with some known antibiotics

	XK-46	Wr-141[3]	Thermorubin[4]	Litmocidin[5,6]
Elemental analysis	C 59.24 H 6.70	59.7 5.0	64.15 4.24	59.11 5.46
$[\alpha]_D$	+212	−310	−14	
M.P. (°C)	74	196~197	190	144~146
UV_{max}(nm)	224 286 495 (sh) 530 573 617	244 286 497 527 568 636	300 328	460~480 510~530 560~570
Mol. weight	400~420	400±40	432	418

XK-46 similar to Wr-141[3], thermorubin[4] and litmocidin[5,6]. However, as seen from the comparative data given in Table 4, XK-46 is different from Wr-141 and thermorubin, since Wr-141 has maximal ultraviolet absorbance at 244 nm and 286 nm and an $[\alpha]_D$ −310°. Similarly, thermorubin has maximal ultraviolet absorbance at 300 nm and 328 nm and an $[\alpha]_D$ −14°, while XK-46 has peaks at 224 nm and 286 nm and $[\alpha]_D$+212. Although XK-46 is very similar to litmocidin from the data obtained on elemental analysis, visible spectrum and molecular weight, its Rf values 0.04 on paper and 0.57 on cellulose thin-layer chromatography using water-saturated *n*-butanol as solvent, are different from the corresponding values for litmocidin, which were 0.06 and 0.69, respectively. It can therefore be concluded that XK-46 is a new antibiotic.

The authors express their sincere thanks to Dr. A.C. SINCLAIR and his associates of the Abbott Laboratories for their instructions.

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