NOTE

A NEW ANTIBIOTIC, XK-19-2

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A new antibiotic XK-19-2 is produced by *Streptomyces* sp. MK-19 and it is a basic peptide antibiotic containing lysine, threonine or serine, arginine, glycine, proline, aspartic acid, histidine, alanine, isoleucine and phenylalanine. This antibiotic has antibacterial activity against gram-positive and gram-negative bacteria, but has no antitumor activity. In this paper, some characteristics of the strain, the isolation and some properties of this antibiotic are reported.

Characteristic of the Strain MK-19

This strain (the laboratory number: MK-19) belongs to the genus *Streptomyces*. The spores were not observed on almost all media shown in Table 1, but were confirmed on starch-agar medium. The surface of the spores was smooth. This strain formed whorls and ball-like bodies and the sporophore was straight or a little flexuous. The cultural characteristics of this strain on various media after culturing for 3 weeks at 30°C are shown in Table 1. Soluble pigments were not observed in almost all media tested, except the gelatin and LOEFFLER's serum medium. This strain utilized glucose, inositol, glycerin and mannose, utilized slightly fructose and raffinose, but did not utilize mannitol, arabinose, salicin, lactose, rhamnose, saccharose and xylose, as carbon source. Other physiological properties were as follows: the growth temperature was 25~35°C, growth pH was 5.5~8.5, the liquefaction of gelatin was positive, hydrolysis of starch was positive, the formation of tyrosinase was negative, milk was peptonized and coagulated, nitrate was not reduced, cellulose was not decomposed and chromogenic action was generally negative but occasionally positive.

These properties were compared with known species of *Streptomyces* described in "The Actinomyces" vol. II by S. A. WAKSMAN and "Identification Keys for Antibiotics-producing *Streptomyces*" vol. I by T. ARAI *et al.* It was then recognized that this strain is close to *Streptomyces verticillus*.

Production and Isolation of XK-19-2

A medium containing 2% glucose, 2% defatted soy bean powder and 0.1% $CaCO_3$ at pH 7.2 before sterilization was used for the production of the antibiotic. The strain was cultured at 30°C for 65 hours under 15 liters/min. aeration and 300 rpm stirring, by use of 30-liter jar fermentor. The cultured broth was adjusted to pH 4.0 with HCl and filtered *in vacuo*. The filtrate was adjusted to pH 8.0 with ammonia water and passed through a column of 600 ml Amberlite IRC-50 (NH_4^+). After washing with 3 liters of water, two antibiotics (XK-19-1 and XK-19-1-2) were eluted with 0.3 N aqueous ammonia. The column was then washed with 3 liters of water and the antibiotic XK-19-2 was eluted with 0.5 N HCl. The active fraction of XK-19-2 from IRC-50 was neutralized with ammonia and passed through the column of active carbon. After washing with water, XK-19-2 was eluted with 80% aqueous methanol (pH 2 with HCl), neutralized with Dowex 44 (OH^-) and freeze-dried to obtain the crude powder. The crude powder of XK-19-2 was dissolved in a mixture of *n*-butanol - pyridine - acetic acid - water (3 : 2 : 1 : 2), passed through 500 ml of a cellulose column which has been prepared with the mixed solvent described above and eluted with the same solvent. The active fraction was concentrated for removal of solvent and the aqueous solution of crude XK-19-2 was passed through a 50-ml CM-sephadex C-25 column. After washing with 150 ml of 0.1 M ammonium formate, XK-19-2 was eluted with 0.18~0.25 M ammonium formate by gradient elution. XK-19-2 was checked with silica gel thin - layer chromatography using the mixture of *n*-butanol - pyridine - acetic acid - water (3:2:1:2). The Rf value of XK-19-2 was 0.15 on a bioautogram using *Bacillus subtilis* as the test organism. After concentration, the aqueous solution of XK-19-2 was passed through a column of CG-50 (H^+) and the antibiotic was

Table 1. Cultural characteristics of *Streptomyces* sp. MK-19

Medium	Growth	Substrate mycelium	Aerial mycelium	Formation of aerial mycelium	Soluble pigment
Yeast extract malt extract agar	Good	Yellowish brown S: (2 ni) R: (2 ng)	Grayish white (b)	Moderate	None
Starch agar	Good	Yellowish brown S: (2 gc) R: (1 1/2 gc)	White (a)	Moderate	None
Glycerol-asparagine agar	Good	Yellowish brown S: (2 lg) R: (2 lg)	White (a)	Moderate	None
Glucose-asparagine agar	Good	Yellowish brown S: (2 ec) R: (2 gc)	Cream-color (3 ba)	Poor	None
CZAPEK's agar	Poor	Colorless	White (a)	Poor	None
Nutrient agar	Good	Yellowish brown S: (2 lg) R: (2 lg)	Grayish white (b)	Moderate	None
Glycerol-calcium malate agr	Good	Yellowish brown S: (2 ie) R: (2 ie)	Greenish cream color (2 ba)	Poor	None
Plain agar	Poor	Colorless	Colorless	Poor	None
Egg albumin agar	Poor	Colorless	Colorless	Poor	None
Tyrosine agar	Moderate	Yellowish brown S: (2 ie) R: (2 ie)	Greenish cream color (2 ba)	Moderate	None
Peptone-glucose agar	Good	Yellowish brown S: (2 ie) R: (2 ie)	Grayish white (b)	Moderate	None
Gelatin	Moderate	Grayish green S: (1 1/2 gc) R: (1 1/2 gc)		None	Brown (3 pl)
Litmus milk	Poor[a)]			None	None
Glucose-CZAPEK liquor	Moderate	Grayish brown (2 ec)			
Filter paper cellulose	Poor	Colorless		None	None
Potato	Moderate	Yellowish brown S: (2 lg) R: (2 lg)	Reddish grayish white (b) (5 cb)	Moderate	None
Cellulose-meat peptone agar	Moderate	Yellowish brown S: (2 ie) R: (2 ie)		None	None
LOEFFLER's serum	Good	Yellowish brown S: (2 gc) R: (2 gc)		None	Blackish brown (4 nl)

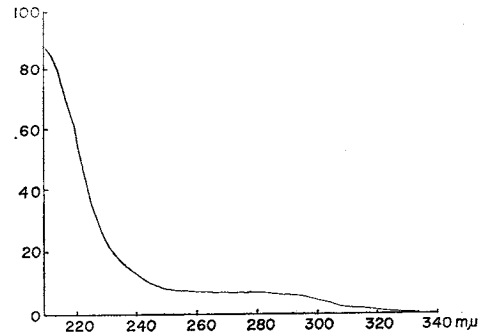
Streptomyces sp. MK-19 was cultured at 30°C for 3 weeks.

The indications within the parentheses are in accordance with the color classification of Color Harmony Manual (Container Corporation of America).

S: Color of surface of substrate mycelium. R: Color of the reverse side of substrate mycelium a) Growth was invisible to naked eyes, but coagulation and peptonization were observed.

eluted with 0.5 N HCl and neutralized with Dowex 44 (OH^-). These procedures were repeated. Twenty mg of white powder of XK-19-2 was eventually obtained with addition of the 10 volumes of acetone.

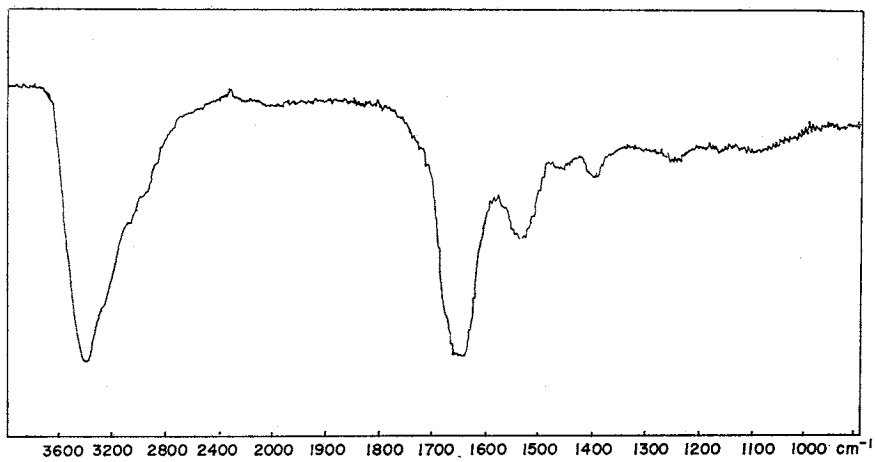
Fig. 1. UV spectrum of XK-19-2.



Properties of XK-19-2

As seen in Fig. 1, XK-19-2 had only end absorption in the UV spectrum. From the IR spectrum (Fig. 2), it was thought that this compound might be peptide, because it had typical peptide peaks at 3400 cm^{-1}, 1650 cm^{-1} and 1540 cm^{-1}. Its melting point was >200°C(dec.)

Fig. 2. IR spectrum of XK-19-2. (KBr)



and the elemental analysis was: C 35.82, H 8.15 and N 15.22. XK-19-2 is soluble in water and methanol, slightly soluble in ethanol and insoluble in acetone, butanol and other organic solvents. Rf values of XK-19-2 by paper chromatography are 0.97 with 20% aqueous ammonium chloride, 0.00 with water-saturated *n*-butanol, 0.01 with *n*-butanol-acetic acid-water (3:1:1), 0.00 with water-saturated ethyl acetate and 0.00 with water saturated *n*-butanol containing 2% *p*-toluene sulfonic acid and 2% piperidine. By silica gel thin-layer chromatography, it had the following Rf values: 0.34 with methanol —10% aqueous ammonium acetate —10% ammonia water (10:9:1) and 0.15 with *n*-butanol-pyridine-acetic acid-water (3:2:1:2). Lysine, threonine or serine, arginine, glycine, proline, aspartic acid, histidine, alanine, isoleucine and phenylalanine were detected by amino acid analysis of the acid hydrolyzate of XK-19-2 (hydrolyzed in 6 N HCl and 110°C for 24 hours), and the molar ratio were 5 : 4 : 4~3 : 4~3 : 1~2 : 1 : 1 : 1 : 1 : 1, respectively.

As seen in Table 2, XK-19-2 had a broad antibacterial spectrum against gram-positive and negative bacteria and also active against many bacteria which were resistant against some antibiotics, but had no antitumor activity against EHRLICH ascites carcinoma and Sarcoma 180 solid tumors. Acute toxicity of XK-19-2 was 5.1 mg/kg of LD_{50} on mice with the intravenous injection.

Discussion

XK-19-2 is a peptide antibiotic consisting of lysine, threonine or serine, arginine, glycine, proline, aspartic acid, histidine, alanine, isoleucine and phenylalanine, and it has only end absorption in the UV spectrum and its melting point is >200°C (dec.). From these results, XK-19-2 is similar to solemycin.[1] However, XK-19-2 is different from solemycin, because

Table 2. Minimal inhibitory concentration of XK-19-2 by agar dilution method

Micoorganisms	MIC (μg/ml)*
Streptococcus faecalis ATCC 10541	>83
Staphylococcus aureus ATCC 6538P	0.16
S. aureus KY 8942 (R-KM, PM, SM)	0.65
S. aureus KY 8953 (R-KM, PM, NM, SM, TC, EM, Pen. G, SA)	>21
Bacillus subtilis No. 10707	0.082
B. cereus ATCC 9634	0.16
B. mycoides ATCC 9463	0.082
Serratia marcescens ATCC 4003	>83
Sarcina lutea ATCC 9341	>83
Klebsiella pneumoniae ATCC 10031	0.082
Neisseria catarrhalis ATCC 7900	0.16
Aerobacter aerogenes ATCC 13048	>83
Escherichia coli ATCC 26	0.0051
E. coli K-12 ML 1629 (R-NM, KM, PM, SM, CM, TC, SA)	0.65
E. coli ML 1878 (R-SM)	0.16
E. coli ML 3306 (R-KM, PM, NM)	0.04
E. coli EcR_3 (R-KM, NM, PM, SM, SPM, TC, CM)	0.16
Pseudomonas aeruginosa BMH No. 1	83
Proteus vulgaris ATCC 6897	5.1
Shigella sonnei ATCC 9290	0.32
Salmonella typhosa ATCC 9992	0.32
Mycobacterium phlei IFO 3158	0.16
M. avium FKB 44	0.65
M. smegmatis ATCC 607	>21
Candida albicans ATCC 10231	>21
Aspergillus niger	>21

NM: Neomycin, KM: Kanamycin, PM: Paromomycin, SM: Streptomycin, TC: Tetracycline, CM: Chloramphenicol, Pen. G: Penicillin G, EM: Erythromycin, SA: Sulfonamide, SPM: Spectinomycin
* Assayed on pH 8.0 nutrient agar at 30°C for 17 hours

the m.p. of solemycin is 188°C and its acute toxicity is low in contrast with a high toxicity of XK-19-2, LD_{50} 5.1 mg/kg iv in mice.

It is already known that *Streptomyces verticillus* produces phleomycins[2] and bleomycins[3]. But XK-19-2 is different from phleomycins and bleomycins from the following reasons: (1) these antibiotics have UV absorbancy at 244 and 285~295 mμ[2,3], while XK-19-2 has only end absorption, (2) amino acid components of XK-19-2 different from these of phleomycins and bleomycins[4], and (3) XK-19-2 does not show any antitumor activity[3,5]. Accordingly it can be concluded that XK-19-2 is a new antibiotic.

XK-19-1-1 and XK-19-1-2, by-products of XK-19-2, are considered to be antibiotics belonging to the streptothricin antibiotics, since both antibiotics have the delayed toxicity. Especially, XK-19-1-2 is similar to the LL-AC 541 group antibiotics[6~8] because it has streptoline, glycine and N-methylgulosamine, but not β-lysine.

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