NOTES

A NEW ANTIBIOTIC, OS-1804

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In the course of our screening program for new antibiotics from actinomycetes, a new antibiotic, OS-1804, active against gram-positive and gram-negative bacteria and some fungi, was obtained from cultured broth and mycelia of *Streptomyces* strain OS-1804 which had been isolated from a soil sample collected in Sanriku-cho, Iwate prefecture, Japan.

This strain showed satisfactory growth on various media and its aerial mycelia developed plentifully from the substrate mycelia. The spores are cylindrical and their size is 1.2~1.5×0.4~0.6 μ. The spores showed a smooth surface without spiny or hairy structures. Taxonomical studies indicated a close resemblance to *Streptomyces michiganensis* CORBAZ, ETTLINGER, KELLER-SCHIERLEIN and ZÄHNER 1957[1], except for utilization of arabinose and production of antibiotic OS-1804. We therefore, concluded that this strain was *S. michiganensis* OS-1804.

The fermentation was carried out for 4~6 days at 28°C in a 30-liter fermenter containing 20 liters of the following medium: soybean meal 2.0%, starch 1.0%, glycerin 1.0%, dried yeast 0.3%, NaCl 0.5%, $(NH_4)_2SO_4$ 0.2%, K_2HPO_4 0.1%, $CaCO_3$ 0.3% (pH 7.0 before sterilization). The potency of OS-1804 in the fermentation, assayed by the paper disc method using *Sarcina lutea* PCI 1001, reached a maximum value at approximately 120 hours. The cultured broth was centrifuged and the mycelia were extracted with methanol (8 liters) at pH 2.0. After the extract was adjusted to pH 7.0 with 6 N sodium hydroxide, it was evaporated to give a dark brown powder. The powder was dissolved in water and the residue was filtered off. An active substance in the broth supernatant was separately recovered by the following procedure. Activated carbon (150 g) was added to the broth supernatant (15 liters). The mixture was stirred for 30 minutes and filtered. After the carbon cake had been washed with 60% aqueous acetone, the antibiotic was eluted twice with 6 liters of 60% aqueous acetone acidified with 6 N hydrochloric acid (pH 2.0). The eluent was evaporated to a nearly aqueous solution (4 liters) under reduced pressure and adjusted to pH 7.0 with 6 N sodium hydroxide. This concentrate and the active extract from the mycelia were combined. The mixture was passed through an Amberlite IRC-50 (NH_4^+) column and eluted with 0.5 N hydrochloric acid after washing with 0.5 N ammonium hydroxide. The eluent was adsorbed on Amberlite XAD-2 and eluted with deionized water. Further purification was carried out by a Sephadex G-10 column eluted with deionized water and an Avicel column eluted with PrOH - EtOH - H_2O (10: 1: 1). The active fractions from the Avicel column were evaporated to dryness under reduced pressure. The active residue was dissolved in a small amount of methanol and precipitated with acetone. This precipitate was washed with ethyl ether to give a white hygroscopic powder of OS-1804 hydrochloride (120 mg). Physicochemical properties of the antibiotic OS-1804 hydrochloride are as follows: mp. 117~122°C, $[\alpha]_D^{20}$ +3.0 (*c* 1.0, MeOH). Anal. Found: C, 47.06; H, 8.79; N, 20.99; Cl, 15.70. Calcd. for $C_{18}H_{37}N_7O_2 \cdot 2HCl$: C, 47.06; H, 8.61; N, 21.48; Cl, 15.53. The UV spectrum in methanol showed only end absorption. The IR spectrum (KBr) is depicted in Fig. 1. OS-1804 hydrochloride is soluble in water, dimethyl sulfoxide and methanol but insoluble in acetone, ethyl acetate, chloroform and ethyl ether. The antibiotic shows positive tests with the SAKAGUCHI, DRAGENDORFF and RYDON-SMITH reagents but is completely inert to anthrone, potassium permanganate and ninhydrin reagents.

OS-1804 forms its reinecke salt as red crystals, mp. 116~118°C, Anal. Found: C, 30.16; H, 5.05; N, 25.73; Cr, 10.96. Calcd. for $C_{18}H_{37}N_7O_2 \cdot 2[HCr(NH_3)_2(SCN)_4]$: C, 30.60; H, 5.03; N, 26.03; Cr, 10.17. The CMR spectrum of the hydrochloride measured in D_2O indicated the presence of 18 carbons. The molecular formula of OS-1804 was suggested to be $C_{18}H_{37}N_7O_2$.

The antimicrobial spectrum of OS-1804 by agar dilution method is shown in Table 1. The

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antibiotic was effective against gram-positive bacteria, some gram-negative bacteria, and filamentous fungi but inactive for yeasts. It is the characteristic feature of the antibiotic that it is effective against plant pathogens such as *Piricularia oryzae, Sclerotinia cinerea* and *Alternaria kikuchiana*. The acute toxicity (LD_{50}) of OS-1804 in mice was 100 mg/kg intraperitoneally.

Among the known antibiotics, laterosporamine[2] most resembles OS-1804 with respect to physical and chemical properties. However, the melting point of laterosporamine reinecke salt is 152~156°C, whereas OS-1804 reinecke salt melts at 116~118°C. Furthermore thaymycins[3] produced by a variant of *S. michiganensis*, *S. michiganensis* var. *amylolyticus* are obviously different from OS-1804 because the former is an acidic substance and the basic nature of the latter is indicated by paper electrophoresis.

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References

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Table 1. Antimicrobial activity of OS-1804

Test organisms	MIC (μg/ml)
Bacillus subtilis PCI 219	50
Bacillus cereus IFO 3001	100
Sarcina lutea PCI 1001	0.1
Staphylococcus aureus FDA 209P	25
Staphylococcus aureus JC-1	25
Mycobacterium smegmatis ATCC 607	12.5
Aerobacter aerogenes ATCC 9621	6.25
Escherichia coli NIHJ	1.56
Shigella sonnei E33	6.25
Salmonella typhimurium	>100
Pseudomonas aeruginosa P-3	>100
Nocardia asteroides	0.39
Xanthomonas oryzae	100
Candida albicans	>100
Saccharomyces sake	>100
Cryptococcus neoformans	100
Microsporum gypseum	50
Aspergillus niger	0.39
Aspergillus fumigatus	>100
Trichophyton rubrum	6.25
Trichophyton interdigitale	6.25
Trichophyton mentagrophytes	3.13
Piricularia oryzae	0.78
Sclerotinia cinerea	<0.09
Alternaria kikuchiana	<0.09
Botrytis cinerea	6.25
Cochliobolus miyabeanus	0.78

Method: agar dilution method.
Media: bacteria, nutrient agar; fungi and yeast, potato dextrose agar.
Solvent: H_2O
Incubation time: bacteria, 48 hours, fungi and yeast, 96 hours.

Fig. 1. IR spectrum of OS-1804 hydrochloride