SCREENING OF ANTIVIRAL ANTIBIOTICS FROM ACTINOMYCETES : CORRELATION BETWEEN ANTIVIRAL ACTIVITY, CYTOTOXICITY AND ANTIBACTERIAL ACTIVITY

(Studies on Antiviral and Antitumor Antibiotics. XI)

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We have used the agar-diffusion method[1] to look for antibiotics, with an inhibitory effect on Newcastle disease virus (NDV) multiplication in primary cultures of chick embryo fibroblasts (CEF), produced by bacteria, fungi, yeasts and actinomycetes from the type culture collection of our laboratory. Actinomycetes and fungi showed more antiviral activity than bacteria and yeasts[2]. Recently, we have screened soil actinomycetes for antiviral antibiotics with the result reported here.

Many antibiotics from actinomycetes have been reported, some of which are active against viruses. To avoid finding known antibiotics we looked for antiviral materials without antibacterial activity and also selected actinomycetes with specific medium requirements for antibiotic production. Three media were employed :

A Medium : glucose 20 g, Pharmamedia (Traders Oil Mill Co., U. S. A.) 20 g, gluten meal (Nihon Shokuhin Kagaku Kogyo Co., Japan) 20 g, Ebios (Ebios Yakuhin Kogyo Co., Japan) 5 g, $CaCO_3$ 3 g and tap water 1,000 ml.

K Medium : glucose 20 g, Pharmamedia 20 g, gluten meal 20 g, Ebios 5 g, KH_2PO_4 15 g, K_2HPO_4 30 g and tap water 1,000 ml.

M Medium : glycerine 20 g, Pharmamedia 20 g, gluten meal 20 g, Ebios 5 g, methionine 10 g, $CaCO_3$ 3 g and tap water 1,000 ml.

Table 1 shows correlation between antiviral and antibacterial activities. Out of 850 strains tested, 106 strains inhibited NDV growth in cultured cells and 28.2 % of them were active on *Escherichia coli*, *Staphylococcus aureus* and/or *Sarcina lutea*. About 73 % of the antivirals with antibacterial activity were potent inhibitors of growth of *S. lutea*. The correlation between antibacterial activity and cytotoxicity is shown in Table 2. In total, 65 strains out of 230 which showed antiviral activity or cytotoxicity inhibited the growth of *S. lutea*, a higher ratio than found with anti-*E. coli* and anti-*S. aureus* activities. But most antibiotics which had both anti-*S. aureus* activity and antiviral activity or cytotoxicity were also active on *S. lutea* (Tables 1 and 2). Many antitumor antibiotics have anti-*Sarcina* activity[4]. Antitumor activity in general indicates a cytotoxic effect on cell metabolism, and

Table 1. Correlation between antiviral and antibacterial activities

Activities	Frequency					
	K Medium only (number)	M Medium only (number)	K & M Media (number)	Total (number)	% of 102 strains	% of 850 strains tested
NDV −E, St and Sa	21	17	38	76 } 102	74.5	9.0 } 12.1
NDV +E, St and/or Sa	0	13	13	26 }	25.5	3.1 }
+E	0	6	7	13	12.7	1.5
+St	0	5	11	16	15.7	1.9
+Sa	0	11	11	22	21.6	2.6
+Sa and St	0	5	8	13	12.7	1.5

Actinomycetes isolated from soil samples were inoculated in each medium (10 ml/20×200 mm test tube) and cultivated for 3 days at 30°C with shaking. An equal volume of acetone was added to the whole broth, and antiviral activity was examined by paper-disc agar-diffusion method[1] employing a virus-cell system of NDV Miyadera strain and primary cultures of CEF[3]. Antibacterial activity on *Escherichia coli* I. A. M. 1182 (E), *Staphylococcus aureus* I. A. M. 1058 (St) and *Sarcina lutea* I. A. M. 1099 (Sa) was tested with the same samples.

Table 2. Correlation between cytotoxicity and antibacterial activity

Activities	Frequency					
	K Medium only (number)	M Medium only (number)	K & M Media (number)	Total (number)	% of 124 strains	% of 850 strains tested
CT(−NDV) −E, St and Sa	5	16	34	55 } 124	44.3	6.5 } 14.6
CT(−NDV) +E, St and/or Sa	19	40	10	69 }	55.7	8.1 }
+E	2	7	6	15	12.1	1.8
+St	16	3	18	37	29.9	4.4
+Sa	2	18	23	43	34.7	5.1
+Sa and St	2	17	17	36	29.0	4.2

The methods were the same in Table 1. Strains which exerted only cytotoxicity (CT) are listed in this table.

Table 3. Dose response of anti-NDV activity of antibiotic A528 (quinomycin B)

Concentration (mcg/ml)	CTZ (mm)	AVZ (mm)	Concentration (mcg/ml)	CTZ (mm)	AVZ (mm)	Concentration (mcg/ml)	CTZ (mm)	AVZ (mm)
1,000	53.4	—	31.3	43.3	—	0.98	30.7	—
500	50.6	—	15.6	41.5	—	0.49	18.5	25.7
250	49.2	—	7.81	40.9	—	0.25	—	17.8
125	47.1	—	3.91	36.6	—	0.12	—	14.9
62.5	45.0	—	1.95	34.9	—	0.06	—	13.0

The assay method of antiviral activity was the same as in Table 1. Paper discs impregnated with the antibiotic solution of concentrations indicated were placed on hardened soft agar overlayers, and antiviral activity and cytotoxicity were measured and expressed as zone diameter. CTZ; cytotoxic zone caused by the antibiotic. AVZ; antiviral zone free of plaque-formation.

antiviral activity of many antibiotics may also result from a disturbance of host cell metabolism. Thus a relationship between anti-*Sarcina* activity and effect on animal cell metabolism may be expected.

One hundred and twenty-four strains exerted only a cytotoxic effect without showing an antiviral zone free of plaques in plate assay method, and 42 strains displayed a cytotoxic zone larger than 30 mm in diameter. Some antiviral antibiotics give similar effects at high concentrations, for example antibiotic A528 which was identified as quinomycin B[5] (Table 3). Some of the potent cytotoxic principles may inhibit multiplication of NDV in plate assay method at lower concentrations.

Culture medium specificity was examined with 91 strains which showed antiviral activity when cultivated in Medium K and/or M, with the results shown in Table 4. More than 60 % of the strains which produced antiviral principles in unusual media such as Medium K and/or M also produced activity in Medium A, but it is not known if the active principle was the same in different media.

Table 4. Culture medium specificity in production of antiviral antibiotics

	Media					
	K only	M only	K & M	K & A	M & A	K, M & A
Number	14	13	8	11	19	26
Frequency (%)	15.4	14.3	8.8	12.1	20.9	28.5

Ninety-one strains which showed antiviral activity were cultivated in the three media and antiviral activity was examined according to the methods described in the legend to Table 1.

Out of 106 strains, 7 strains were selected for isolation of antiviral antibiotics based on biological activity and medium specificity. All antibiotics isolated were found to be new by chemical and spectrophotometric analysis with one exception which was identified as quinomycin B. One of the newly isolated antibiotics had antibacterial activity but the others had only antiviral activity as far as examined. Their chemical and physical properties and biological aspects of the anti-

viral effect on multiplication of RNA and DNA viruses are now under study and will be reported separately.

References

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