0.3% tetraethylammonium hydroxide. The maximum absorbancy of the resultant red color was measured at 504 mμ against the reagnet blank between 10 and 25 minutes.

The calibration curve was linear in the range of 25—130 μg/ml of glucosamine HCl solution. Glucosamine and the other 2-amino-2-deoxy sugars showed similar colors under these conditions (Method A and Colorimetric method), while various N-substituted derivatives, glucosaminides, mucopolysaccharides, most of the other sugars and amino acids have no color by Method A as indicated in Table I. After hydrolysis with HCl, various derivatives of 2-amino-2-deoxy sugars and mucopolysaccharides gave a blue color as shown in Table I.

Details of the experiment and the reaction mechanism will be reported in the near furture.

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A Contact Antitumor Activity of *Bacillus natto* on Solid Type Ehrlich Carcinoma Cells

We have investigated extensively the specificity of metabolic and hydrolytic activities of soil bacteria and *E. coli* on benzoic acid derivatives, amino acids and acyl-amino acids and also on the acylase activity of ascites tumor cells.[1,2] Based on these reuslts, we have been trying to search for some familiar bacteria, which have high selective toxicity on human tumor cells. The first choice was the bacillus of 'Natto' (fermented Japanese beans) which is a popular and cheap daily food for Japanese. Here we wish to report the contact antiutmor effect of a strain (tentatively called KMD 1126) of *Bacillus natto* newly isolated from 'Natto'.

To test the antitumor effect, a conventional screening method was avoided. The bacterial cells were given directly to the established solid type Ehrlich carcinoma cells. The control tumor cells were formed in the same animal, since a direct contact effect of the bacillus on the tumor cells is to be expected, canceling a possible stimulation of defense mechanism in the tumor-bearing animal by this bacillus.

Biological Test: a) Experimental animal: Male inbred mice (20—22.5 g body weight) of ICR-JCL strain were used throughout.

b) Preparation of Ehrlich carcinoma cell suspension: A mouse was transplanted intraperitoneally with approximately 10^7 Ehrlich ascites carcinoma cells. After 7 days, the sedimented cells from the ascitic fluid were diluted with Dulbecco buffer A to the concentration of 2.5×10^7 tumor cells per ml.

c) Preparation of washed *Bacillus natto* KMD 1126 suspension: 10 ml of a 6-hour broth culture of KMD 1126 at 37° by shaking was centrifuged, and the sedimented cells, after

1) Y. Kameda, *et al.*, *Nature*, **169**, 1016 (1952); **170**, 888 (1952); **181**, 1225 (1958); **182**, 453 (1958); **191**, 1122 (1961); **192**, 468 (1961); **197**, 314 (1963).
2) Y. Kemeda, *et al.*, *Chem. Pharm. Bull.* (Tokyo), **15**, 1573, 1578, 1586 (1967); *ibid.*, in press.

washing twice with 10 ml of Dulbecco buffer A, were suspended in 2 ml of the same buffer (Living KMD 1126 suspension).

d) Antitumor experiment on solid tumor of Ehrlich carcinoma in mice: 0.2 ml of the Ehrlich carcinoma cell suspension (5×10^6 carcinoma cells) containing 700 unit of penicillin was subcutaneously implanted in the right and left groins of each mouse. The treatment was started 2 days after tumor implantation, and continued daily for 2 days. The living KMD 1126 suspension (0.2 ml) was injected subcutaneously in the right groin, but not in the left and, instead, 0.2 ml of Dulbecco buffer A was given. The effect of the living KMD 1126 was estimated by the difference in weight of tumors between the treated (right) and the untreated (left) groins on the 11th day of implantation (Table I).

Table I. Effect of *Bacillus natto* KMD 1126 Strain on Solid Tumor of Ehrlich Carcinoma in Mice

Group	Mouse No.	Mouse Body weight (g) at the start	Mouse Body weight (g) after 11 days	Tumor weight (g) right (treated)	Tumor weight (g) left (untreated)	$\frac{\text{right}}{\text{left}} \times 100$
I	1	22.3	29.0	none	0.241	0
	2	21.7	29.5	0.027	0.393	6.9
	3	22.5	28.8	0.051	0.276	18.5
	4	20.4	29.3	0.090	0.436	20.6
	5	20.1	29.3	0.051	0.214	23.8
II	1	20.6	25.8	none	0.222	0
	2	20.6	26.9	none	0.150	0
	3	20.8	27.9	0.031	0.615	5.0
	4	21.3	30.6	0.114	0.294	38.8
	5	21.0	29.6	0.175	0.335	52.2
III	1	21.0	28.6	none	0.474	0
	2	20.2	27.3	none	0.305	0
	3	21.2	25.7	none	0.226	0
	4	21.0	26.7	0.131	0.407	32.2
	5	20.7	25.0	0.131	0.377	34.7

Table I shows the contact antitumor activity of KMD 1126–strain on Ehrlich carcinoma, which has not been reported as yet. The effect is remarkable, but its mode of action remains to be ascertained. It can be said, however, that some excreted substances of the bacillus, including many hydrolytic enzymes, are responsible for the antitumor effect.

Even if KMD 1126 has little curative effect on the tumor cells, there may be an alternative. We may be able to obtain mutants by some mutagenic methods such as transformation and transduction.

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