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# Distribution of Ribonucleic Acid Coliphages in Korea

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To determine the geographical distribution of ribonucleic acid (RNA) coliphages in Korea, we collected sewage samples from domestic drainage in densely populated urban areas in July through August, 1979. Of 132 samples, 74 (56%) contained RNA phages (106 strains). They were classified into groups I, II, and III (4:47:55) by serological analysis. Based on previous data for Japan (groups II and III [3:1]) and Southeast Asia (mostly group III), the distribution pattern of RNA phages in Korea was of an intermediate type between those of Japan and Southeast Asia.

In the course of our systematic surveys on ribonucleic acid (RNA) phages, we initially checked sewage from domestic drainage and raw sewage from treatment plants as sources for the isolation of RNA phages and found them to be suitable materials. We thus focused on sewage from domestic drainage, isolated many RNA phages from sewage samples (3, 5, 7), and classified them into four groups (I through IV) on the basis of various biological and physicochemical properties (4, 8, 9, 11).

To elucidate the ecology of bacteriophages, we made extensive efforts to determine the geographical distribution of RNA phages in sewage collected from domestic drainage in several countries. Group II RNA phages predominated in mainland Japan (3) and in islands in the seas adjacent to Japan (Rebun, Rishiri, Hachijojima, Miyakejima, and Niijima Islands) (2), whereas the most prevalent RNA phages in Amamiohshima, mainland Okinawa, Ishigakijima, Iriomotejima Islands, Taiwan, the Philippines, Singapore, and Indonesia were those of group III (5). Furthermore, stability and continuity of RNA phages in domestic drainage was revealed from a 5-year continuous survey in Japan (1). We therefore proposed a border line between Kyushu and Amamiohshima Islands in the geographical distribution of RNA phages in the domestic drainage of Southeast Asia. Moreover, Iki and Tsushima Islands (groups II and III [1.5:1]), which are located between Japan and Korea, showed a somewhat different distribution pattern from that of mainland Japan (groups II and III [3:1]).

In this connection, it is interesting to determine the distribution pattern of RNA phages in Korea, especially in relation to that of Iki and of Tsushima Islands. We therefore report here the distribution pattern of RNA phages isolated from 132 sewage samples collected from domestic drainage in Korea during the summer of 1979.

## MATERIALS AND METHODS

Peptone-glucose (5 g of NaCl, 20 g of peptone, 2 g of glucose, 1,000 ml of water, pH 7.4) medium was used for the collection of sewage samples, isolation of RNA phages, and dilution of phage and antiphage sera. Peptone-glucose medium supplemented with 0.25% yeast extract and 0.01 M $CaCl_2$ was used for the propagation of RNA phages. For phage assay, the usual agar layer method was employed.

*Escherichia coli* K-12 strains A/λ ($F^+$), Q13 (ribonuclease $I^-$, Hfr), and W3110 ($F^-$) were used as host strains for the isolation and preparation of RNA phages.

Antiphage sera of groups I (MS2, B01, and JP501), II (GA and JP34), III (Qβ and VK), and IV (SP) were employed for the serological grouping of newly isolated RNA phages.

The collection of sewage samples, preparation of original phage samples, and records of collection were as described previously (5). Original phage samples were prepared at Seoul (W. H. Chang, Department of Microbiology, College of Medicine, Seoul National University) and Busan (H. D. Yang, Department of Microbiology, College of Medicine, Busan National University) and transported back to Japan with us under normal temperature conditions for further examination.

Isolation and grouping of the RNA phages by the serological method were carried out as described previously (3, 5,10). We usually picked up 10 to 20 plaques per sample and analyzed the material serologically. The RNA phages so obtained were designated serially as KR1 through KR106 according to the sample number collected in Korea.

## RESULTS

**Frequency of isolation of total coliphages and RNA phages.** A sample which contained one or more coliphages (or RNA phages) per 0.1 ml of original sample was judged to be a coliphage-positive (or RNA phage-positive) sample. As shown in Table 1, coliphages were detected in all of the sewage samples collected from sewage treatment plants and in almost all of the sewage samples from domestic drainage. The amounts of total coliphages in these sewage samples were fairly high, ranging from 10 to $10^6$ plaque-forming units per ml of original phage sample. The relative amounts of RNA phages in the sewage samples were also high, representing from 10 to 90% of the total coliphages. The isolation frequencies of total coliphages and RNA phages in the sewage samples obtained from Korea were 95 and 56%, respectively. These values were comparable to previous data reported for other Asian countries: Taiwan (89 and 28%) (7), Japan (69 to 79% and 41 to 51%) (2, 3), the Philippines (100 and 48%), Singapore (69 and 35%), and Indonesia (88 and 21%) (5). No significant differences in sewage pH (most values were between 5.5 to 6.5), sewage temperature (25 to 30°C), volume of sewage water, or apparent environmental conditions were detected between the domestic drainage in the four cities of Korea examined and that in typical cities of the other Asian countries mentioned above.

**Serological grouping and geographical distribution of RNA phgaes.** When two or more RNA phages isolated from the same original sample showed similar inactivation patterns serologically, they were considered as the same strain. We isolated 106 RNA phage strains and examined their susceptibility to six standard antisera (MS2 [group I], GA [group II], JP34 [group II], Qβ [group III], VK [group III], and SP [group IV]) by the spot test method or by the plating method (3) (Table 2). All of the RNA phages tested were inactivated by one of the six standard antisera and were classified into one of the four known groups. The numbers of RNA phage strains which belonged to groups I, II, III, and IV were 4, 47, 55, and 0, respectively. We failed to detect any significant differences in distribution patterns of RNA phages among the four cities examined. It can be said, therefore, that group II and group III phages were isolated in almost equal proportions in Korea. Insofar as domestic drainage was concerned, the frequencies of isolation of group I and group IV phages were negligible in Korea, as in Japan (2, 3) and Southeast Asia (5).

## DISCUSSION

The distribution pattern of RNA phages in Korea (groups II and III [1:1]) was clearly different from those in Japan (north of Kyushu) (groups II and III [3:1]) (2, 3) and Southeast

TABLE 2. *Serological grouping of RNA phages isolated in Korea*

| Group | Subgroup | Phage | No. of strains |
|---|---|---|---|
| I | a | KR17, 32, 69, 87 | 4 |
| II | a | KR3, 6, 8, 10, 11, 13, 14, 15, 25, 27, 29, 31, 33, 35, 36, 37, 39, 40, 42, 44, 46, 50, 51, 53, 55, 59, 61, 63, 65, 67, 70, 72, 73, 75, 77, 79, 81, 83, 85, 88, 90, 93, 98, 101, 103, 105, 106 | 47 |
| III | a | KR1, 4, 5, 7, 9, 12, 16, 18, 19, 20, 21, 22, 23, 24, 26, 28, 30, 34, 38, 41, 43, 45, 47, 48, 49, 54, 56, 57, 62, 64, 66, 68, 71, 74, 76, 78, 80, 82, 84, 86, 89, 91, 92, 94, 95, 96, 99, 100, 102, 104 | 50 |
| | b | KR2, 52, 58, 60, 97 | 5 |

TABLE 1. *Frequencies of isolation of total coliphages and RNA phages in collected materials*

| City (treatment plant) | No. of samples collected | Coliphage positive | | RNA phage positive | | | No. in RNA phage group: | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | No. of samples[a] | % | No. of samples[a] | % | No. of strains[b] | I | II | III | IV |
| Seoul (north sanitation plant) | 1 | 1 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Seoul (Eui-Jung Bu slaughter house) | 2 | 2 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Seoul (Woo Sung Nong Yock slaughter house) | 1 | 1 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Seoul | 46 | 45 | 98 | 34 | 74 | 45 | 2 | 20 | 23 | 0 |
| Kwangju | 35 | 31 | 89 | 11 | 31 | 12 | 0 | 5 | 7 | 0 |
| Busan | 40 | 38 | 95 | 28 | 70 | 48 | 2 | 21 | 25 | 0 |
| Kyongju | 11 | 11 | 100 | 1 | 9 | 1 | 0 | 1 | 0 | 0 |

[a] Number of samples containing one or more coliphages or RNA phages per 0.1 ml of original phage sample.
[b] Number of RNA phages exhibiting different serological properties in the same original phage sample.

Asia (south of Amamiohshima Island) (mostly group III) (5). It rather showed an intermediate character between them. In this respect, it is worth noting the existence of a gradual increase in group III phages over group II phages with progressive movement away from Japan proper towards the south as follows; Rebun and Rishiri (mostly group II) → Japan (north of Kyushu) (groups II and III [3:1]) → Iki and Tsushima (groups II and III [1.5:1]) → Korea (groups II and III [1:1]) → Southeast Asia (south of Amamiohshima) (mostly group III).

Although it is not yet possible to elucidate the underlying causes of this gradual change in distribution pattern of RNA phages in the global Asian area, it seems that it may depend mainly on the general climate (i.e., integrating or maximum-minimum temperatures, or both) surrounding the domestic drainage, since (i) sewage samples were obtained exclusively from domestic drainage whose physical conditions appeared to be controlled directly by the local climate, (ii) domestic drainage seems to suffer little influence from contamination by animal sources such as human or domesticated animal feces, insofar as we have been able to observe, and seems to construct, as itself, one of the stable and favorable natural habitats for this phage, and (iii) group II RNA phages, which are the most prevalent RNA phages in Japan proper, have the lowest optimal temperature for growth among the four known groups of RNA phages in in vitro experiments. They can propagate even at 20°C, which is a limiting temperature for group I, III, and IV phages. Furthermore, group I, III, and IV phages can propagate almost normally at 40°C, whereas group II phages cannot produce any progeny at that temperature (unpublished data).

Another characteristic of the isolation pattern of RNA phages in Korea is the high frequency of concurrent occurrence of RNA phages of different groups or subgroups in the same sewage sample. About 41% of the sewage samples contained two or more RNA phage strains concomitantly which belonged to different groups or subgroups, as opposed to only 4 to 16% in other countries such as Japan, Taiwan, the Philippines, Singapore, and Indonesia. It should be added that not only the frequency of isolation of group II and III phages but also the amount of both phages in a sample were roughly the same in all of the cities in Korea examined. Similar patterns were also observed in Choshi City (Japan proper) in the survey from 1972 through 1977 (1). These high frequencies of appearance of RNA phages in sewage samples from domestic drainage in Asian countries (at least in Southeast and East Asia) (3, 5) may reflect the overall pattern of environmental conditions surrounding the domestic drainage, incorporating the modes of life, living conditions, racial traits, etc.

Considering that the RNA phage which specifically infects bacteria harboring F or R factor is one of the predominant constituents, or at least a common bacteriophage species, of the sewage floras (1, 6), the natural hosts which support the propagation of this phage in the domestic drainage must be determined.

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