CORYNEBACTERIUM RENALE PHAGE-TYPES AND THEIR EPIDEMIOLOGICAL SIGNIFICANCE

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INTRODUCTION

Corynebacterium renale was recently found to be lysogenic by YANAGAWA et al. (1968). They observed that about 2/3 strains of *C. renale* type I were lysogenic, and many of them produced phages similar in host range. The representative phage, designated RP 6, was studied in detail. It was found to be morphologically similar to the *C. diphtheriae* phage, had a slow absorption rate, a latent period of 50 min, and a small burst size, and the nucleic acid was double stranded DNA (YANAGAWA & SHINAGAWA, 1968).

Pyelonephritis due to *C. renale* has been reported in Japan (HIRATO, 1933; KUBOTA et al., 1958; KUME et al., 1959; HIRAMUNE et al., 1968[2]). Distribution of this microorganism has been observed in apparently healthy cattle (HIRAMUNE et al. 1967). YANAGAWA et al. (1967) reported that *C. renale* could be classified into 3 types, serologically and biochemically. Recently, HIRAMUNE et al.[3] investigated the distribution of *C. renale* in cattle of Japan, relating to their types. The results indicated that type I strains were found, at various rates ranging from 13.3 to 26.9%, in the urine of apparently healthy cattle in herds where clinical pyelonephritis had been reported, while type II strains were isolated, at the average rate of 5.5%, from the urine of healthy cattle, regardless of whether they were raised in herds where clinical pyelonephritis occurred or not. Type I strains collected during the above investigations were used in this study in order to isolate bacteriophages and to determine bacteriophage-types of these strains. The results presenting a new phage type and the epidemiological significance of *C. renale* phage types are described in this paper.

MATERIALS AND METHODS

Strains used Sixty-seven strains belonging to *C. renale* type I were used. Number

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and the origins of these strains are shown in table 1. Most of the strains were isolated from cows in 10 herds in Hokkaido between 1962 and 1968. Eight of these herds have had cases of clinical pyelonephritis, while the rest, T and H herds, have had no such cases but neighbored to one of these herds. Four strains (R-4, R-1301, R-3175 & FS113-63) were provided by Dr. J. E. PHILLIPS of the Royal (Dick) School of Veterinary Studies, Summerhall, Edinburgh, and one (CR 56) was sent us for identification from Dr. P. KRISTAL, City of Montevideo, Uruguay.

TABLE 1 *Number of strains used and their origins*

ISOLATED FROM	ISOLATED IN												TOTAL
	Hokkaido										Scotland	Uruguay	
	N*1	G	T	H	K	Z	U	NA	M	F			
Urine of cows with pyelonephritis symptoms	5*2	2	0	0	1	1	1	1	1	1	4	1	18
Urine of apparently healthy cows	21	6	18	4	0	0	0	0	0	0	0	0	49

*1 Name of herd
*2 Number of strains

Induction and propagation of phage The methods reported by YANAGAWA et al. (1968) were used. The preliminary survey was done for detecting phages with the combination of specimens of 67 strains irradiated with ultraviolet light and all these strains as indicators. From the results, attempts to propagate phages were made with the following combinations: RP H4 (phage)/*C. renale* H25 (propagating strain), RP H39/71, RP H46/7, RP FS113-63/R4 and RP 6/71. The propagation procedure, done in soft agar, was repeated respectively more than 10 times, in order to obtain the phage titer of more than 100× routine test dilution (RTD). The phages thus propagated were, after removing the bacterial cells by centrifugation at 6,000 rpm for 20 min, kept in −20°C, without adding chloroform.

Spot test Cultures of logarithmic phase of each strain, 6 hr incubation at 37°C, were spread on nutrient agar plate and then 100× RTD of each phage was spotted on it. The result was observed after incubation overnight at 37°C. The RTD was assayed by making serial tenfold dilutions of phage in nutrient broth. A plate of nutrient agar was spread with a logarithmic phase culture of the respective propagating strain. One drop of each phage dilution was placed on the inoculated plates, incubated overnight and then read. The RTD is the highest dilution that caused confluent lysis.

RESULTS

The rate of lysogenic strains is shown in table 2. In total, 11 of 67 strains (16.4%) were lysogenic. The lysogenic strains were *C. renale* H4, H38, H39, H40, H42, H43, H44,

TABLE 2 *Number of lysogenic strains*

ISOLATED FROM	ISOLATED IN												SUBTOTAL	TOTAL
	Hokkaido										Scotland	Uruguay		
	N*	G	T	H	K	Z	U	NA	M	F				
Urine of cows with pyelonephritis symptoms	1/5	0/2			0/1	0/1	0/1	1/1	0/1	0/1	1/4	0/1	3/18	11/67 (16.4%)
Urine of apparently healthy cows	8/21	0/6	0/18	0/4									8/49	

* Name of herd

Numerator : Strains lysogenic

Denominator : Strains tested

H46, H47, No. 6 and FS113-63. Of these, H38, H39, H40, H42, H43, H44, H47 and No. 6 produced phages which were identical in host range pattern. Therefore, from them, the phages produced from H39 and H46 were selected and used as representatives. *C. renale* H4 produced a phage showing different host range pattern. These 3 phages (designated RP H39, RP H46, and RP H4), with RP 6 and RP FS113-63 which were previously reported by YANAGAWA et al. (1968), were then propagated. Propagating strains used were indicated in MATERIALS AND METHODS.

Strains which were lysed by 100× RTD of these phages are indicated in table 3. Phage RP H4 completely lysed 18 strains and partially lysed 3 strains. Phages RP H39, RP H46 and RP 6 lysed another five common strains. Phage RP FS113-63 lysed only R-4. Therefore, 3 phage-types were distinguishable from these results.

TABLE 3 *Strains lysed by the 3 groups of phage*

PHAGES (100× RTD)	NAME OF STRAINS LYSED (HOST RANGE)
RP H4	H20/H21/H22/H23/H24/H25/H26/H27/H28/H29/H30/ H31/H32/H33/H34/H35/H36/H37/ H13*/H14*/H15*
RP H39 RP H46 RP 6	H55/7/71/73/76
RP FS113-63	R-4

* Partial lysis

The results of the phage typing applying the 3 phage-types in relation to herds, are shown in table 4. Phage RP H4, produced by strain H4 isolated in NA herd (Sapporo),

TABLE 4 *Strains lysed by the 3 groups of phages in relation to herds*

PHAGES	ORIGIN OF PHAGES	STRAINS ISOLATED IN											
		Hokkaido										Scotland	Uruguay
		N*1	G	T	H	K	Z	U	NA	M	F		
		Number of strains tested											
		26	8	18	4	1	1	1	1	1	1	4	1
RP H4	NA herd	0	3*2	18	0	0	0	0	0	0	0	0	0
RP H39 RP H46 RP 6	N herd	5	0	0	0	0	0	0	0	0	0	0	0
RP FS 113-63	Scotland	0	0	0	0	0	0	0	0	0	0	1	0

*1 Name of herd *2 Lysed only partially

lysed all the strains isolated from cows in T herd (Hayakita). It partially lysed 3 strains from G herd (Sapporo). But this phage did not show lytic activity against the strains isolated from other herds. Five of the 26 strains isolated from N herd (Hidaka) were all commonly lysed by phages RP H39, RP H46 and RP 6, which were respectively produced by the strains isolated from the same herd. Phage RP FS113-63 produced from strain FS113-63 (origin Scotland), lysed only R-4, another Scottish strain. Thus, a distinct relation was found between *C. renale* phage-types and their sources (herds).

N herd consisted of several barns while other herds consisted of only one barn. The strains isolated in each barn of N herd were classified according to their response to phage RP 6. The response was classified as productive of, susceptible and resistant to phage RP 6, respectively. All the strains isolated from A barn were resistant to phage RP 6, all the strains isolated from B and C barns, except one, produced the same phage, and all the strains from F barn were resistant to RP 6, except one (tab. 5). Therefore, relations were also found in N herd between lytic pattern and sources (barns) of *C. renale*.

TABLE 5 *Response of strains isolated in each barn of N herd to phage RP 6*

BARNS	RESPONSE TO PHAGE RP 6		
	Resistant	Susceptible	Productive
A	3/3	0/3	0/3
B	0/2	0/2	2/2
C	1/7	0/7	6/7
F	5/6	1/6	0/6

Numerator : Response of strains
Denominator : Strains tested

DISCUSSION

Lysogeny of *C. renale* was reported recently by YANAGAWA et al. (1968). They reported that about 2/3 of *C. renale* type I strains were lysogenic and that a phage-type of Japan was different from a phage-type of Scotland. According to the results described in the present paper, the rate of lysogenic strains was 16.4%, lower than in the previous work. The reason is not clear.

The herds examined in the present study were 10, which was more in number than those studied by YAMAGAWA et al. (1968). We obtained, as described above, a new phage-type. The new phage-type (RP H4) was produced by strain H4 isolated from the urine of a cow with pyelonephritis symptoms in NA herd (Sapporo) and lysed all strains isolated from cows in T herd (Hayakita). On the other hand, phags RP 6, RP H39 and RP H46 which were produced by strains

isolated in N (Hidaka), lysed 5 strains all obtained from the same herd. Phage RP FS113-63 which was produced by a strain from Scotland lysed only another strain of Scottish origin. Therefore, the existence of 3 types of *C. renale* phage was clarified in this paper. In addition, the lytic pattern of these 3 phage-types showed distinct relations with the sources of strains, herds and barns.

These results suggest that *C. renale* type I of a phage-type is distributed among cows of very limited area, such as a herd or a barn. According to the authors' other work on the distribution of *C. renale* types in Japan, type I has been isolated also from apparently healthy cows, but only those raised in the herds where clinical pyelonephritis occurred. From the herds where no clinical case of pyelonephritis was known type I was not isolated from apparently healthy cows. This fact supports the above suggestion that *C. renale* type I originally spread only within a limited area. Accordingly, phage typing should be useful in studying the ecology of this organism and the epidemiology of this disease.

Since the distribution of a phage type of *C. renale* is restricted to a very limited area, it will be necessary in future to obtain more phages from the strains collected from many parts of the world. A complete set of phage-types of *C. renale* will thus be obtained and it will serve to clarify the complete epidemiological picture of *C. renale* infection.

SUMMARY

Three phage types of *Corynebacterium renale* were obtained from 67 strains of *C. renale* type I, isolated mostly in Hokkaido, Japan, and Scotland and Uruguay. Two of the 3 phage-types were identical with those already reported by YANAGAWA et al. (1968).

A new type phage (RP H4) was obtained. This phage was produced from a strain isolated from a cow with pyelonephritis symptoms in NA herd (Sapporo) and lysed all the strains isolated from cows in T herd (Hayakita). Phages RP H39 and RP H46, produced by the strains isolated in N herd (Hidaka) and similar in host range pattern to the previously reported phage RP 6, lysed 5 strains all derived from the same herd. In this herd, a relation was found between the lytic pattern and the sources of strains (barns). All the strains isolated from cows in A and F barns were resistant to phage RP 6, except one; all the strains except one of B and C barns produced the same phage. These findings suggest that *C. renale* type I is distributed among cows of a very limited area, such as a herd or a barn. From the findings obtained, the importance of *C. renale* phage-types in relation to ecology of *C. renale* and epidemiology of *C. renale* infection was discussed.

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