DNA BASE COMPOSITIONS OF THE THREE TYPES OF *CORYNEBACTERIUM RENALE*

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(Received for publication, September 1, 1969)

INTRODUCTION

Among bacteria the overall base compositions of DNA, as expressed in mole per cent guanine plus cytosine (GC) of total bases, varies from 25 to 75[4,9]. Different strains of the same well-established species have almost identical DNA base compositions[7]. But within a species, which was not thoroughly classified, the GC contents extended to a considerably wide range. For instance, SAUNDERS et al. showed that the GC content of the DNA of *Desulfovibrio desulfuricans* ranged from 46 to 62% and that they fall into three groups as judged by their DNA base composition. They suggested the need for a taxonomic revision of this group of microorganisms.

Corynebacterium renale is a group of organisms causing pyelonephritis in cattle. The organisms were serologically classified by YANAGAWA et al.[12] into three types. The relations of the serological types to biochemical and cultural behavior were suggested[12]. The three types were also distinguishable by nutritional requirements[5], piliation[13] and lysogeny[14]. However, the DNA base compositions of the three types of *C. renale* have not yet been clarified.

As for the DNA base compositions of *C. renale*, there is only one report of BOUISSET et al. who showed paperchromatographically the GC content of a few strains of this microorganisms to be 53%. An attempt was made by the authors to compare the DNA base compositions of the three types of *C. renale*. The results are described in this paper.

MATERIALS AND METHODS

Bacterial strains Strains of *C. renale* used in the present study were Nos. 6, 8, 9, 71, Fs 113-63 and ATCC 10848 (type I), Nos. 35, 45, 46, RH and 121 (type II), and Nos. 42, 43, 48 and 77 (type III). These strains were isolated from cows with pyelonephritis symptoms except strain Nos. 35 and 121 which were isolated from the urine of apparently healthy cows. Fs 113-63 was isolated in Great Britain and received by the courtesy of J. E. PHILLIPS of the Royal (Dick) School of Veterinary Studies, Summerhall, Edinburgh. ATCC 10848 was isolated in the United States of America and received from American Type

Culture Collections, Rockville, Maryland. Strain RH was isolated in Tottori, Japan, by the department of Veterinary Microbiology, Tottori University. The remaining strains were all isolated in Hokkaido, Japan.

The strains were cultivated on the nutrient agar plate with the addition of 0.5% glucose and 1% bonitoextract (Kishida), pH 7.2~7.4, and incubated at 37°C for 48 hrs with the exception of the strains of type III, which were incubated for 72 hrs.

DNA preparations Organisms were harvested in 0.5 M NaCl solution with the addition of 0.001 M sodium ethylendiaminetetraacetate (EDTA) (NaCl+EDTA solution) (pH 8.0) and collected by centrifugation at 3,000 rpm for 20 min. Six grams (wet weight) of the bacteria thus collected were incubated with 6 mg lysozyme (Sigma) in 6 ml of NaCl+EDTA solution at 37°C for 30~60 min, then frozen at −20°C. 1.5 volume of a solution, containing 0.1 M trisaminomethane +1.5% sodium dodecyl sulfate +0.1 M NaCl (tris SDS) (pH 9.0) was added to the frozen preparation, and was shaken to thaw, then incubated at 60°C for 10 min. The above procedure of freezing and thawing was repeated 5~6 times to complete lysis of bacteria. The lysate was then deproteinized by shaking with an equal volume of phenol saturated with tris SDS (4 : 1 v/v mixture), at a temperature of 0°C. After 20 min shaking, the suspension was separated into 3 layers by centrifugation at 2,500~3,000 rpm for 15 min. The upper layer (water phase) was then collected and centrifuged at 12,000 rpm for 10 min. The supernatant obtained was used for the next procedure of preparing DNA. Two volumes of cooled ethanol was added dropwise to the supernatant and DNA was precipitated fibrously. This was collected by spooling on a glass rod, washed in 70, 80 and 90% ethanol respectively, and dissolved in saline citrate solution (0.015 M NaCl+0.0015 M sodium citrate, hereafter SCS). After dissolving, concentrated saline citrate was added to the DNA-containing SCS so as to obtain a final 10 times concentration of SCS. It was dialyzed against 10 times concentrated SCS overnight. The solution was incubated for 1 hr at 37°C with ribonuclease (Sigma, 100 μg/ml), and then was deproteinized by shaking with phenol saturated with 10 times concentrated SCS. The shaking was done for 5~10 min in an ice bath. After centrifugation at 2,500~3,000 rpm for 15 min, the supernatant (water phase) was collected and centrifuged at 12,000 rpm for 10 min. Fibrous DNA was again collected with 2 volumes of cooled ethanol, washed and dissolved as mentioned above. One-tenth volume of 3.0 M sodium acetate +0.0001 M EDTA (acetate EDTA) (pH 7.0) was added to the DNA solution. While the solution was rapidly stirred, 0.6 volume of isopropyl alcohol was added dropwise into the vortex. The fibrous DNA wound around the glass rod was washed and then dissolved in SCS. After 2~3 more cycles of isopropyl alcohol treatment, DNA was found to be pure by the criteria of ultraviolet spectroscopy.

Calculation of DNA base composition The DNA base compositions of *C. renale* were analysed paperchromatographically. The purified DNA in 10 times concentrated SCS was precipitated with ethanol wound around the glass rod and dried in air. The dried DNA was hydrolysed with 6N HCl. Twenty μl of the hydrolysate was spotted and separated by paperchromatography with the solvent of a mixture of methanol, HCl and distilled water (70 : 20 : 10)[10]. The chromatograms were first dried overnight at room temperature. Then the bases were detected on the chromatograms by ultraviolet radiation. The source of ultraviolet radiation was Manaslu-Light (Manaslu Kogyo) having 2536 Å wave-

length. The spots on the chromatograms were cut out and eluted with 5 ml 0.1 N HCl by standing for 3~4 days at 37°C. Blanks of equal size were cut out at distances corresponding to the Rf value of the bases, from a strip of the same chromatogram. The eluates were read against 0.1 N HCl in a Shimazu spectrophotometer (model QV-50) at 260 (adenine), 265 (thymine), 250 (guanine) and 275 mμ (cytosine) as indicated by TAKEMURA et al. Then the corrections were made on the adenine destruction and the difference in the size of spots on the chromatogram. Adenine destruction during the process of hydrolysis was noticed as described by TAKEMURA et al. The rate of adenine destruction was calculated by using commercial bases (Sigma). After these corrections were made, the amount of bases in the DNA preparations was calculated from the molar absorbancy.

The rate of recovery of the total bases of the DNA preparations was analysed according to the method of phosphorus analysis[10].

RESULTS

The wave-length for maximal absorption of all the DNA preparations used in this experiment was 258 mμ. The ratios for absorption at 260 : 230 : 280 mμ were almost similar to the value reported by MARMUR, and showed that the DNAs were considerably pure.

A preliminary experiment was done to know whether bases were destroyed during hydrolysis. For this purpose each base was subjected to the same procedure of hydrolysis. The results are shown in table 1. About 7% of adenine, but not other bases, were destroyed.

TABLE 1 *Destruction of base during hydrolysis*

BASES	OPTICAL DENSITY *		DESTRUCTION
	Before hydrolysis	After hydrolysis	
Adenine	0.441	0.410	7 %
Thymine	0.301	0.300	0
Guanine	0.254	0.252	0
Cytosine	0.192	0.196	0

* Optical density was determined by absorbancy at 260 (adenine), 265 (thymine), 250 (guanine) and 275 mμ (cytosine).

DNA preparations of strain No. 35 (type II) was determined after necessary corrections were made as described in MATERIALS AND METHODS (tab. 2). The rate of recovery of the total bases was 94.0%. GC content of No. 35 thus calculated was 57.9±0.7%. DNA base compositions of other strains were determined likewise, which were summarized in table 3. The rate of recovery of the total bases of these DNAs was approximately 90%.

As shown in table 3, DNA base compositions of the three types of *C. renale*, as expressed in GC content, ranged from 52.6 to 59.8%. Strains belonging to the same type showed similar GC content. The average of the GC content expressed in percentage were 56.7±1.1 (type I), 57.9±1.9 (type II) and 53.5±0.9 (type III). The GC contents of types I

TABLE 2 *DNA base compositions of C. renale No. 35*

EXP.	BASE	OPTICAL DENSITY *1		mM	MOLE PERCENT	GC PERCENT	GC PERCENT MEAN
		Measured	Corrected*2				
1	T*3	0.298	0.281	0.0353	22.7	57.2	57.9 ± 0.7
	C	0.448	0.431	0.0410	26.4		
	A	0.406	0.406	0.0312	20.1		
	G	0.592	0.525	0.0477	30.7		
2	T	0.284	0.271	0.0341	21.7	58.4	
	C	0.475	0.456	0.0434	27.6		
	A	0.402	0.403	0.0310	19.7		
	G	0.600	0.533	0.0485	30.9		
3	T	0.297	0.280	0.0352	22.5	57.9	
	C	0.462	0.444	0.0423	27.0		
	A	0.400	0.401	0.0308	19.7		
	G	0.590	0.532	0.0484	30.9		

*1 Optical dnesity was determined by the same method as described in table 1.
*2 Corrections were made on the adenine destruction and the difference in the size of spots on the chromatogram.
*3 abbreviations: T (thymine), C(cytosine), A (adenine) and G (guanine)

TABLE 3 *DNA base compositions of C. renale*

TYPE	STRAIN	BASE COMPOSITIONS				
		T	C	A	G	GC
		%	%	%	%	%
I	8	21.1	28.9	21.7	28.3	57.1
	9	21.2	28.4	23.2	27.2	55.6
	ATCC 10848	20.4	27.9	22.3	29.5	57.4
II	35	22.3	27.0	19.8	30.8	57.9
	45	22.9	29.3	20.2	27.6	56.9
	46	20.2	30.1	19.9	29.7	59.8
	RH	22.1	27.1	20.8	29.9	57.0
	121	21.3	29.7	20.5	28.4	58.1
III	42	25.8	26.2	21.6	26.4	52.6
	43	23.5	27.2	22.7	26.6	53.8
	48	22.9	26.9	23.3	26.9	53.8
	77	23.4	27.0	22.9	26.7	53.7

and II were similar. The GC content of type III was distinctly lower than those of types I and II.

The values indicated were the average of at least three repeated calculations. Calf thymus DNA, used as a control, gave a base composition of 40.4 ± 0.6% GC, which is in agreement with the reported value in the literature[2].

Hydrolysates of the DNA preparations of type I strains were colored light brown. The coloring was found in the strains of type I but not in those of other types. Hydrolysates of 3 strains of type I (Nos. 6, 71 & Fs 113-63) were especially colored (dark brown) and this made the calculation of the GC content of these strains difficult.

Discussion

Three types of *C. renale* were reported in the preceding papers from this department. There are several phenotypic differences between the three types, which are considered to be the reflex of the differences of their DNAs. In the present study, we found that strains belonging to the same type showed similar GC content. The GC contents of types I and II were similar. The GC content of type III was distinctly lower than those of types I and II. This adds additional information to the differences between the three types of *C. renale*.

The hydrolysates of all DNA preparations of type I and not other types were colored brown. The coloring was characteristic of the strains of type I. The origin of color was not clear.

Bouisset et al. determined the DNA base compositions of the organisms of genus *Corynebacterium*, and showed the GC content of *C. renale* to be 53%. This value is close to that of our type III. The strain used by Bouisset et al. did not reduce nitrate, so according to Yanagawa et al[12]., it must be either type I or III. However we did not examine the strain used by Bouisset et al.

Methods other than paperchromatographic analysis have been used for the calculation of DNA base composition. The values obtained by chromatographic analysis are reported less reproducible than those obtained by buoyant density or thermal denaturation analysis; the former method provided a wider estimate of GC content for each organism[3]. The use of the physical methods together with paperchromatographic analysis may distinguish more clearly the difference of GC content between the three types of *C. renale*.

There have been reports on the occurrence of rare bases in bacterial DNA. The DNAs of two species of genus *Corynebacterium*, *C. diphtheriae* and *C. vadosum*, contain 6-methylaminopurine[11]. Although rare bases were not detected in the present study, it will be another subject to search rare bases in *C. renale* DNA. Genetic studies of *C. renale* such as transformation, heteroduplex formation are our future projects, to which the present study will be an initial step.

SUMMARY

Corynebacterium renale has been classified into three types. This paper describes a study of the DNA base compositions of the three types of *C. renale*. The DNAs isolated from 12 strains of the three types of *C. renale* were purified by the modified method of phenol deproteinization, combining the method of MARMUR. The purified DNAs were hydrolysed. Purine and pyrimidine bases were then separated and calculated paperchromatographically.

The GC content of the 3 types of *C. renale* ranged from 52.6 to 59.8%. Strains belonging to the same type showed similar GC content. The averages of the GC contents expressed in percentage were 56.7±1.1 (type I), 57.9±1.9 (type II) and 53.5±0.9 (type III). The GC contents of types I and II were similar and distinctly higher than that of type III.

ACKNOWLEDGEMENT

We wish to thank to S. KUBO, Professor of the Department of Biochemistry, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, for his valuable advice.

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