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DNA BASE COMPOSITION OF DIFFERENT STRAINS OF *RHIZOBIUM* ISOLATED FROM *CICER ARIETINUM* L.

BY

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Base composition of DNA of 4 strains of *Rhizobium* isolated from *Cicer* was determined. The base ratio and GC% calculated from the data were found to lie in two ranges. In strains S_1 and S_3 the base ratio and GC% were similar viz 0.8 and 55% respectively. In S_4 the corresponding values were 0.5 and 66% and in S_6, 0.6 and 61%.

INTRODUCTION

The study of nucleic acid, at the level of similarities of nucleotide sequences, has introduced methods which permit taxonomic studies at the molecular level. Bacteria, which are closely related have many properties in common will also have many identical genes. The DNA base composition will also consequently be very similar. Hoyer and McCullough (1968) have forwarded the view that DNA homology seems to provide aid in taxonomy of newly discovered or incompletely examined microorganisms. The present paper deals with the diferrentiation of the 4 strains of *Rhizobium*, isolated from different varieties of *Cicer arietinum* L., on the basis of DNA base composition.

MATERIALS AND METHODS

Bacterial cultures and host varieties :

Rhizobial strains	*Sources*
S_1	Cicer arietinum L. var. Pb-7
S_3	,, ,, var. Gn-2
S_4	,, ,, var. Ns-4
S_6	,, ,, I.A.R.I. New-Delhi

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Isolation of DNA :

Bacteria were grown in 250 ml of broth having the following compositions (w/v) in a 2-litre Erlenmeyer flasks : Glycerol—0.46%, arabinose—0.1%, yeast extract—0.1%, K_2HPO_4 - 0.1%, $CaSO_4$ - 0.13%, KNO_3 - 0.07%, $MgSO_4$, $7H_2O$ - 0.036% and $FeCl_3$ - 0.0004%.

The inoculum consisted of 10 ml fresh culture and the flasks were incubated for 30 hours at 30°C. The cells were harvested by centrifugation, washed with normal saline EDTA and resuspended in 30 ml of the same solution.

To the homogeneous suspension (30 ml) 0.15 ml of lysozyme solution (100 mg/ml) and 0.3 ml sodium lauryl sulphate solution (25%, w/v) were added and thoroughly mixed. The mixture was incubated with occassional stirring with a glass rod for 60-70 mins at 45°C to allow the bacteria to undergo lysis. When a high degree of lysis was obtained, as evidenced by an increased viscosity, 0.1 ml of sodium deoxycholate solution (25%, w/v) was added and the mixture was allowed to stand for another 15 mins at 30°C. Finally the mixture was taken in a glass stoppered flask, mixed with an equal volume of chloroform isoamyl alcohol mixture (24 : 1, v/v), shaken thoroughly for 30 mins and centrifuged for 5 mins at 500 rpm. Three layers were produced, of which the upper cloudy layer (containing the nucleic acids) was pipetted of in a glass tube. Nucleic acids were precipitated from this layer by careful layering with approximately double volumes of absolute ethanol on it, and then gently mixing the two layers with a glass rod. This resulted in the formation of fibrous precipitate of the nucleic acids which could be easily spooled round the glass rod as threads by twisting it in one direction only. Alcohol was removed as far as possible by pressing the threads against the wall of the tube. The precipitate was transferred to 2.5 ml sterile normal saline citrate (0.15 M NaCl+0.015 M trisodium citrate) in which the nucleic acid precipitates went into solution. To remove RNA, 0.05 mg ribonuclease was added per 10 ml of the solution of nucleic acids and allowed to stand for 15 mins at 30°C. The solution was then mixed with 1.5 volume of ethanol when DNA was precipitated. The fibrous precipitates were collected with a glass rod as before, dried with absolute ethanol and stored at 0°C.

Hydrolysis of DNA :

A weighed quantity of DNA (10 mg) was mixed with 0.2 ml of 72% $HClO_4$ in a small test-tube with a glass marble on the open mouth and hydrolysed at

100°C in a water bath for 60 mins. The hydrolystate was cooled and diluted with water (to 1.0 ml). The solution was centrifuged to separate black residue which was used as such for paper chromatography.

Paper Chromatography :

Descending paper chromatography was used with isopropanol-12 NHCl [65 ml isopropanol+16.7 conc. HCl (12N) and distilled water to make the total volume 100 ml] as solvent. The identification of the spots on chromatograms as dark patches against a back ground of general paper flourescence when viewed in dark room under a ultraviolet lamp, was based on comparison with authentic samples of adnine, guanine, cytosine and thymine.

Elution :

The chromatograms were viewed under an ultraviolet lamp in a dark room and the separated bases which appeared as dark spots were outlined with a pencil. These areas together with identical areas on paper from blank lanes were cut out. Each piece of filter paper was finely subdivided and eluted with 5 ml of O in HCl in a closed tube with constant shaking for 2 hours at 30 c. The elute was decanted and centrifuged to remove shreds.

Ultraviolet spectrophotometry :

The optical density of the elutes taken in matched quartz cuvettes having light path of 1 cm was read against the corresponding elutes of the blank lanes in a Hilger Watts' spectrophotometer. Readings were recorded at 5 μ intervals within the range of 230 and 310 μ. The absorption spectora were plotted for comparison with known bases. From the respective peaks at 262.5 265, 249 and 276 μ the quantities of adenine, thymine, guanine and cytosine were calculated using the formula given by Bendich (1957).

RESULTS

The relative quantities of the four nucleic acid bases in the four isolates of *Rhizobium* from *Cicer* are shown in Table 1.

Table 1. *DNA base composition in 4 strains of* Rhizobium.

Strains	Adenine			Thymine			Guanine			Cytosine		
	O.D.	O.D. unit	mole	O.D.	O.D. unit	mole	O.D.	O.D. unit	mole	O.D.	O.D. unit	mole
S_1	0.112	0.560	0.044	0.081	0.405	0.051	0.120	0.600	0.054	0.122	0.610	0.061
S_3	0.123	0.615	0.049	0.071	0.355	0.045	0.115	0.575	0.052	0.123	0.615	0.062
S_4	0.155	0.775	0.062	0.123	0.615	0.077	0.267	1.335	0.120	0.295	1.475	0.147
S_6	0.143	0.735	0.058	0.120	0.600	0.076	0.215	1.075	0.097	0.227	1.135	0.133

A = Adenine, T = Thymine, G = Guanine, C = Cytosine.

From the data shown in Table 1, quantitative relation between the bases was determined and expressed as adenine + thymine to guanine + cytosine (base ratio) as well as guanine + cytosine content of DNA (GC%). These values are shown in Table 2.

Table 2. *Base ratio and GC% of DNA of 4 strains of* Rhizobium.

Strains	Base ratio $\frac{A-T}{G-C}$	GC%
S_1	0.83	54.76
S_3	0.82	54.81
S_4	0.52	65.76
S_6	0.64	61.05

It may be seen from Table 2 that the GC contents of DNA of the bacteria of S_1 and S_3 are close to each other while the corresponding values for S_4 and S_6 are again significantly different from each other.

Discussion

A modern apprach to the study of taxonomy of bacteria is the application of knowledge emerging from the study of nucleic acids, particularly DNA (Lee *et. al,*

1956 ; Bendich, 1957 and Deley *et. al.* 1966). This new tool has also been applied for the taxonomic study of *Rhizobium* and related genera (Colwell and Mandel, 1964, Deley and Rassel, 1965, Mandel, 1966 and Heberlein, 1967). During the present investigation base composition of DNA of 4 rhizobial straing of *Cicer* was determined (Table 1) and the base ratio and GC% were calculated (Table 2). The GC% was found to be 54.76 and 54.81 for S_1 and S_3 respectively and 61.05 and 65.76 in case of S_6 and S_4 respectively. Deley and Rassel (1965), while studying the DNA base composition of 35 strains of *Rhizobium* observed that the bacteria with peritrichous flagellation and with faster growth rate showed a lower GC-content ranging from 58.6% to 63.1%. The slow growing strains, on the other hand, had a higher GC% varying between 62.8% to 65.5%. Results obtained in the present investigation also indicated the presence of two ranges. The GC% of S_1 and S_3 were somewhat lower than the lowest observed values but the values of S_4 and S_6 tallied well with the known GC-contents of *Rhizobia*. Moreover, the strains S_1 and S_3 having a faster growth rate (Pal and Banerjee, 1968) had a lower GC% and the strains S_4 and S_6 having a slower growth rate had a higher GC% as observed by Deley and Rassel (1965).

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