CHARACTERIZATION OF DNA FROM RHIZOBIUM CELLS AND THEIR BACTERIODS FROM ROOT NODULES*

A.K. Agarwal and S.L. Mehta

Division of Biochemistry/Nuclear Research Laboratory
Indian Agricultural Research Institute
New Delhi-110012, INDIA

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SUMMARY

DNA from two rhizobial strains Cicer and Phaseolus and their bacteroids from root nodules have been isolated, purified and characterized for thermal denaturation temperature and buoyant density. Bacteroid DNA had a lower Tm value and buoyant density compared to Rhizobium cell DNA. The calculated GC content of becteroid DNA was lower than the Rhizobium cell DNA.

INTRODUCTION

The establishment of a effective symbiosis between a strain of Rhizobium and its legume host involves complex physiological and biochemical interactions which are not well understood. The rhizobia are released from the infection thread into tetraploid host cell and change from vegetative rods to the nitrogen fixing bacteroids. In addition to the synthesis of nitrogenase, many other biochemical changes occur during bacteroid formation (1,2). Studies of DNA from Rhizobium cell have been made by many workers (3-7), yet DNA of bacteroids from root nodules has not been characterized. In the present study, DNA of laboratory cultured Rhizobium cells and their bacteroids from root nodules have been isolated, purified and characterised with respect to thermal denaturation temperature and buoyant density.

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MATERIALS AND METHODS

The cultures of Phaseolus rhizobium[1] strain M_1 and Cicer rhizobium[2] strain G_2, obtained from the Division of Microbiology, IARI, New Delhi, were maintained on yeast extract mannitol agar medium (8). The cultures were tested for their ability to nodulate their respective hosts. After 72 hrs growth at 28°C on rotary shaker, the culture broth was centrifuged at 5000 x g for 15 min. The cells were suspended and washed twice with distilled water and finally washed with saline EDTA (NaCl, 0.015 M; EDTA disodium salt, 0.1 M; pH 8.0).

The seeds of green-gram (Phaseolus aureus Rox b.) var. Pusa Baisakhi and Bengal-gram (Cicer arietinum L.) var. C 235 were surface sterilized, soaked in sterile water for 6 hrs, inoculated with 72 hrs old cultures of respective Rhizobium strains by conventional method (9) and sown in earthen pots under bacteriologically controlled conditions.

Isolation of Bacteroids: Washed nodules from the roots (25 days of growth in case of green-gram and 35 days of growth in case of Bengal-gram) were crushed in Tris-Cl buffer (Tris 0.1 M; 2-mercaptoethanol, 4mM; $MgCl_2$, 10 mM; pH 7.6) (1:2 W/V) containing 0.2 M Sodium ascorbate. Polyvinyl polypyrrolidone (high molecular weight) was added (1.5 g/100 ml) to remove the phenolic impurities. The homogenate, after passing through 4 layers of cheesecloth was centrifuged at 500 x g for 10 min at 4°C to remove starch grains and nodule debrie. The supernatant was centrifuged at 27,000 x g for 15 min to sediment bacteroids. The bacteroids were washed twice by suspending in buffer and

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1 An isolate from Phaseolus aureus Rox b.
2 An isolate from Cicer arietinum L.
The name Phaseolus rhizobium and Cicer rhizobium have been used in the text for convenience. These rhizobia have not been included in any of the specified cross-inoculation groups.

finally washed with saline EDTA. All operations unless otherwise stated were performed at 4°C.

DNA Extraction: DNA from rhizobial cells and bacteroids was extracted and purified essentially using Marmur's method (10), excepting that the lysis of rhizobial cells was affected first with 2% Triton X 100, later by addition of 2 ml sodium lauryl sulfate (25% solution) and heating to 60°C for 30 min. Purified DNA had spectral ratios of A_{260}/A_{230}, 1.85-2.00 and A_{260}/A_{280}, 1.89-1.98.

Thermal Denaturation: Thermal denaturation curves were prepared and base composition from Tm values was determined using the method of Marmur and Doty (11).

CsCl Density Gradient Centrifugation: Equilibrium centrifugation of purified DNA in CsCl solution was carried out in L2-75B Beckman preparative ultracentrifuge and GC content calculated according to the method described by Schildkraut, et al. (12).

RESULTS AND DISCUSSION

The thermal denaturation curves of DNA were sigmoid in nature indicating the presence of one type of DNA (Fig. 1). All the four DNA preparations showed a sharp increase in hyperchromicity indicating double stranded nature of DNA of both cultured cells and bacteroids. However, the increase in hyperchromicity in DNA from Rhizobium cells was higher compared to bacteroid DNA (Table 1). The decrease in thermal denaturation temperature indicates a decrease in GC content in bacteroid DNA. The melting profiles of Cicer rhizobium cells and bacteroid DNA are not completely parallel but had different slopes indicative of differences in the GC content of different segments of DNA.

Cicer rhizobium cell DNA showed a single major peak

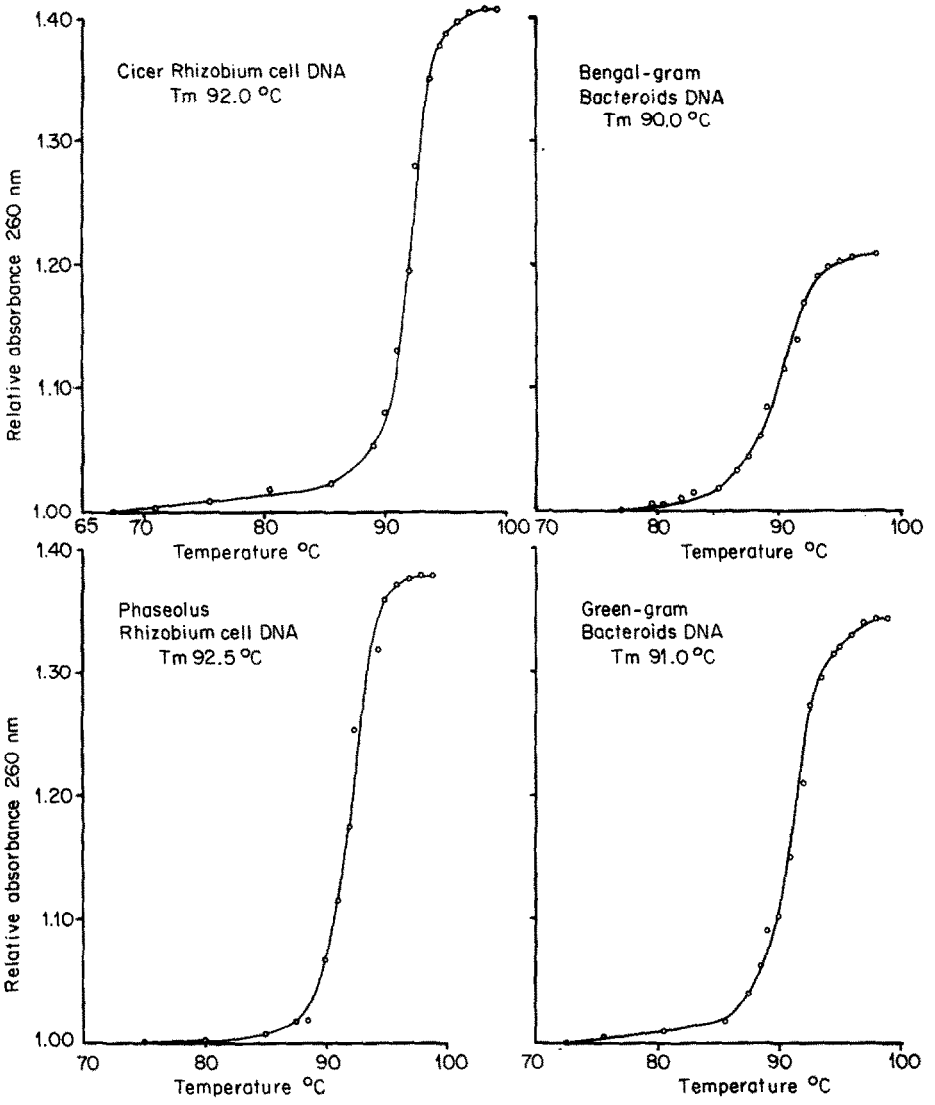


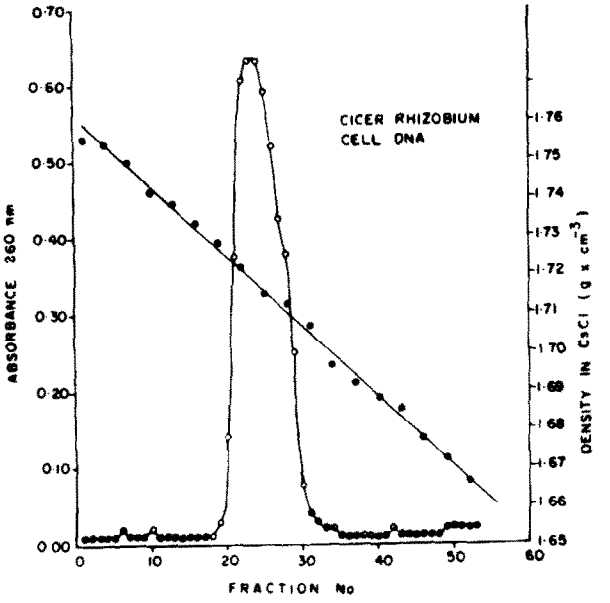
Fig. 1: Thermal denaturation profiles of DNA in standard saline citrate (NaCl, 0.15 M; Sodium citrate, 0.015 M; pH 7.0). DNA (25 ug/ml) was heated in a 3 ml teflon stoppered quartz cuvette placed in a Hitachi Spectrophotometer Model 124 attached with thermostatic cell holder, temperature of which was regulated by circulating hot glycerol : water solution through a lauda constant temperature circulating water bath. The temperature of the chamber was raised quickly to about 5°C below the estimated onset of the melting region after first measuring the optical density at 25°C. The temperature was raised about 1°C at a time, allowing about 20 min for equilibration at each temperature. The optical density read at 260 nm was corrected for thermal expension of the solution. Relative absorbance was plotted as a function of temperature of the sample. The temperature corresponding to half the increase in the relative absorbance was taken as Tm of the DNA.

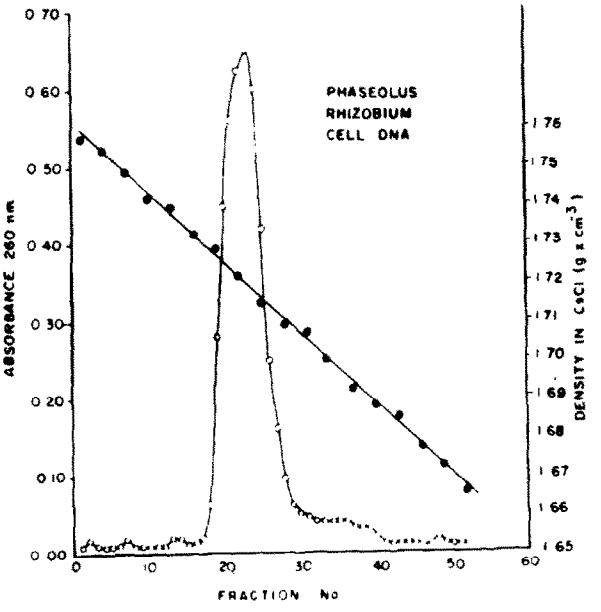
Table 1. Thermal denaturation, buoyant density and mole per cent GC of various DNA.

Source	Hyper-chromi-city %	Tm oC	Buoyant density g/cm^3	Mole per cent GC calculated from Thermal denatura-tion	Buoyant density
Cicer rhizobium cell	40.5	92.0	1.719	55.31	54.08
Bengal-gram Becteroids	33.1	90.0	1.709	50.48	50.00
Phaseolus rhizobium cell	37.6	92.5	1.720	56.58	55.10
Green-gram bacteroids	34.0	91.0	1.717	52.91	52.02

banding at a density of 1.719 g/cm^3 and a minor component banding at 1.710 g/cm^3 which was not well separated. The bacteroid DNA from Bengal-gram root nodules showed a single peak banding at 1.709 g/cm^3. DNA from Phaseolus rhizobium cell as well as its bacteroids showed a single peak banding at 1.720 and 1.717 g/cm^3 (Fig.2). Plasmid DNA has been shown to control the symbiotic properties in Rhizobium (13) but in present study such particles were not observed in cultured cell DNA. Although, Tm values and mole per cent GC obtained in the present study are lower than the values obtained by other workers (3-7) for R.japonicum, R.meliloti and R.leguminosarum, the similarity in GC content based on Tm values and buoyant density data suggest that these species possess lower GC content.

Bacteroid DNA had a lower buoyant density as compared to their rhizobial cell DNA. The decrease in buoyant density of the bacteroid DNA further confirms the decrease in GC content. The lower GC content of the bacteroid DNA indicates changes in


CICER RHIZOBIUM
CELL DNA
ABSORBANCE 260 nm
DENSITY IN CsCl (g x cm⁻³)
FRACTION No


PHASEOLUS
RHIZOBIUM
CELL DNA
ABSORBANCE 260 nm
DENSITY IN CsCl (g x cm⁻³)
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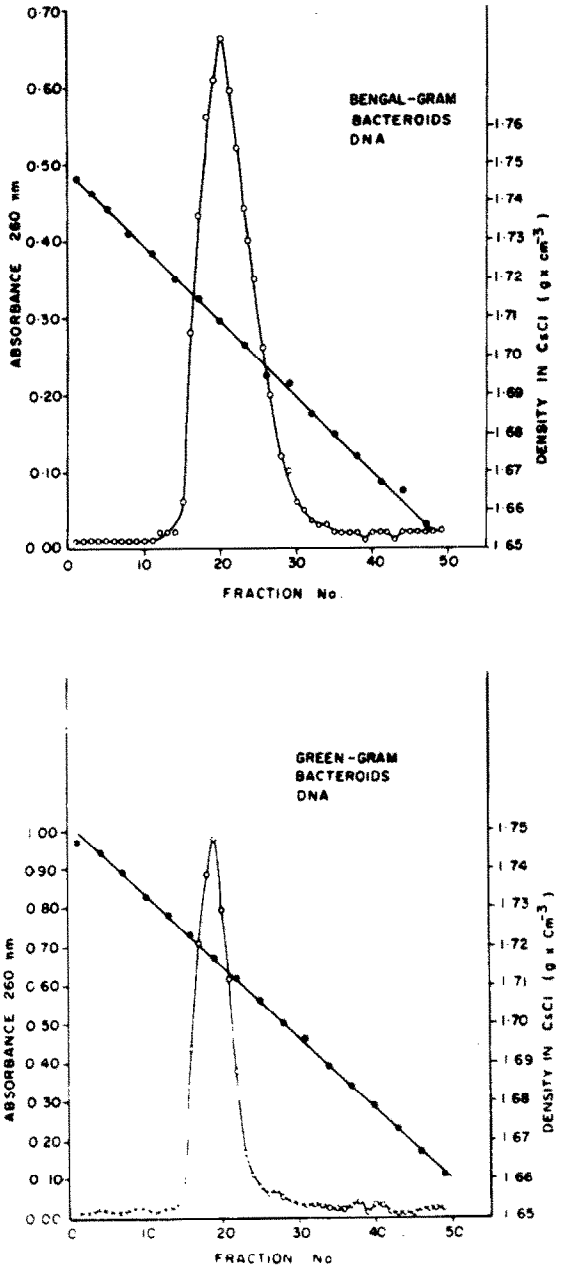

Fig. 2: Sedimentation equilibrium of Rhizobium cell and bacteroid DNA in CsCl. Purified DNA (~ 250 ug) was mixed in 3 ml CsCl solutions having initial density 1.700 g/cm^3 and sedimentation was performed at 33,000 rpm in a SW 65 Ti rotor for 72 hrs at 25°C in Beckman L2-75B ultracentrifuge. 3 drop fractions were collected using Beckman fraction recovery system. Every fourth tube was read for refractive index using Abbe-Zeiss 60 refractometer, maintained at 25°C. The measurement of density was facilitated by use of the linear relationship between refractive index and density (17). The absorbance of different fractions was read after diluting with 0.5 ml of 20 mM Tris-Cl buffer pH 8.5 at 260 nm.

Rhizobium cell DNA during transformation and this could occur either by transformation of Rhizobium DNA where a portion of its DNA that was needed specifically for its nitrogenase system, but which the plant already produced for some other function was lost (14), or a transfer of DNA from host plant to bacterium could occur as the bacteria are budded off the infection thread into the plant cytoplasm (15). Alternatively, GC rich segments of Rhizobium cell DNA could be cleaved during bacteroid transformation and this would result in the loss of DNA content per cell as has been observed (16). This needs further confirmation.

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