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PROPERTIES OF DNA FROM VARIOUS LOCAL STRAINS OF *Bradyrhizobium sp.* (*Vigna radiata)*

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*The buoyant density of DNA from various local strains of mung-bean nodule bacteria (*Bradyrhizobium sp.*) was determined by ultracentrifugation in an equilibrium CsCl gradient. The number of GC base pairs (61.1-62.7% mol %) and DNA melting point (94.3-95.0 °C) were calculated from the buoyant density. DNA preparations purified by ultracentrifugation in a CsCl gradient by electrophoresis show a bimodal distribution. The DNA composition varies little (1.6 mol %). The electrophoretic distribution of local and type-strain mung-bean nodule bacteria are identical. This proves that they belong to a single taxonomic group.*

Key words: nodule bacteria, *Bradyrhizobium sp.*, taxonomy, DNA.

The use of nodule bacteria for agricultural applications has broad possibilities. Industrial preparations based on them are used to produce guaranteed harvests of beans. Nodule bacteria represent a unique group of microorganisms that develop an intracellular symbiosis with beans that provides the capability for rhizobia to fix atmospheric nitrogen and supply it to the plant host [1]. Considering these properties of nodule bacteria, we isolated local strains of nitrogen-fixing bacteria from nodules of mung-bean roots and studied their physicochemical properties in order to determine the phylogenetic origin of these bacteria.

This problem was successfully solved by studying the polymorphism of proteins and the length of restrictive fragments of genomic DNA [1, 2]. However, most researchers use classical microbiological methods based on morphobiological and biochemical properties in culture in addition to specifics of the DNA composition to determine the taxonomical classification of bacterial cells [3-5]. This is due to the availability and simplicity of the microbiological method for determining the systematic classification of microorganisms.

The literature contains numerous examples of the taxonomic value of the DNA nucleotide composition [3]. Thus, this feature can be considered to be very valuable for establishing and classifying the phylogenetic origin of bacteria. We investigated the nucleotide composition of DNA from various local strains of mung-bean nodule bacteria to determine the specifics of their species. We studied the DNA distribution in a CsCl density gradient and determined its buoyant density and number of GC base pairs.

Results from equilibrium ultracentrifugation showed that the preparations of nodule bacteria DNA have a unimodal distribution in a linear CsCl density gradient with buoyant density from 1.7192 to 1.7207 g/cm^3 (Fig. 1).

Despite the unimodal distribution in the CsCl gradient, the total DNA from the nodule bacteria studied by us separated into two bands upon electrophoresis (Fig. 2). An analysis of the electrophoregrams indicates that the slowly migrating upper band (R_f 0.27) contains megaplasmids; the lower band (R_f 0.57), chromosomal DNA.

Comparison of Figs. 1 and 2 shows that genomic DNA and megaplasmid DNA from nodule bacteria typically have the same buoyant density but different electrophoretic mobilities.

Based on the buoyant densities, we calculated the number of GC base pairs in the mung-bean nodule bacteria (Table 1).

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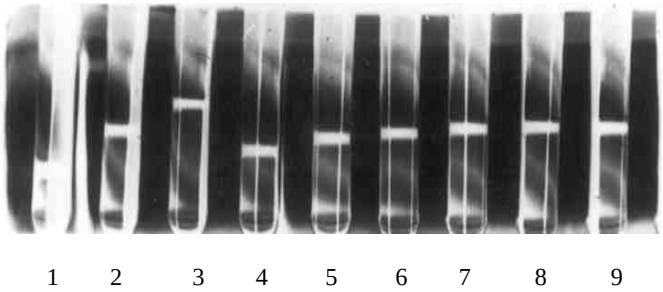
TABLE 1. Physicochemical Properties of DNA from Local Strains of Mung-Bean Tuber Bacteria

Tuber bacterium strains *Bradyrhizobium sp.* (*Vigna radiata* L.)	Buoyant density, g/cm^3	Number of GC base pairs, mol %	mp, °C	G+C / A+T
CIAM 1901	1.7207	62.7	95.0	1.68
M 10	1.7196	61.6	94.5	1.60
M 12	1.7199	61.9	94.7	1.62
M 17	1.7192	61.1	94.3	1.57
M 23	1.7205	62.5	94.9	1.67
M 27	1.7194	61.4	94.5	1.59
M 28	1.7193	61.2	94.4	1.58

CIAM 1901 is the type strain; M10, M12, M17, M27, and M28, local strains of tuber bacteria.



1 2 3 4 5 6 7 8 9

Fig. 1

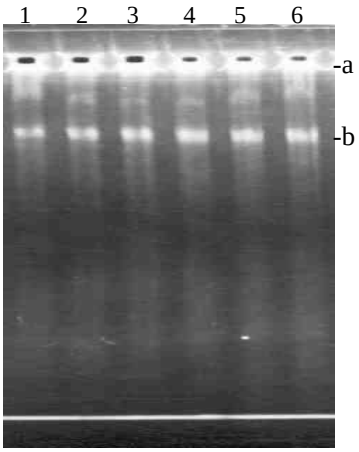


Fig. 2

Fig. 1. Distribution of DNA from local strains of mung-bean tuber bacteria in a CsCl (Hoechst) density gradient. DNA: *Sarcina lutea* (1), cotton nucleus (2), cotton mitochondria (3), CIAM 1901 type strain (4), M10 (5), M12 (6), M17 (7), M23 (8), M28 (9).

Fig. 2. Electrophoregrams of the distribution of total DNA fractions from local mung-bean tuber bacteria strains prepared by ultracentrifugation in a CsCl density gradient. DNA: high-molecular-weight plasmid (a), chromosone (b), CIAM 1901 type strain (1), local strains M10 (2), M12 (3), M17 (4), M23 (5), and M28 (6).

A comparison of the number of GC base pairs has shown that nodule bacteria DNA of the studied strains belongs to the GC-type (61.1-62.7 mol %). The specificity of the DNA composition varies from 1.57 to 1.68. Other researchers observed an analogous trend during a study of the nucleotide composition of DNA from *Rhizobium fredi*, *B. japonicum*, and *Bradyrhizobium sp. cowpea* [5].

We determined the melting point of DNA from the buoyant density and found that it varied in the range 94.3-95.0°C. These data may help the study of the secondary structure of the DNA.

In general, the results indicate that DNA samples from local strains have similar distributions in a linear CsCl density gradient and electrophoresis on agarose gel. The DNA composition from the studied local strains varies very little compared with the type strain CIAM 1901 (1.6 mol %).

The literature on the genetic systematics notes that a bacterial genus should include only related species that differ in nucleotide composition by not more than 5-6 mol % GC and have 30-60% (40-50% under ideal circumstances) DNA homology (also for optimal reassociation conditions) [4].

The number of GC base pairs in DNA is known to be not only a taxonomic indicator but also an aid for studying the

molecular phylogeny of bacteria [4, 6].

Data on the nucleotide composition of DNA are required in research. Therefore, we attempted to obtain data on the content of DNA GC base pairs in order to classify and identify the microorganisms. We used them to determine the taxonomic classification of the new isolated local strains of nodule bacteria.

We have concluded by comparing our results with those in the literature that the isolated local strains belong to the *Bradyrhizobium* genus of symbiotic nitrogen-fixing microorganisms.

EXPERIMENTAL

We used a method based on the determination of DNA composition from its buoyant density to study the nucleotide composition of the DNA [7].

DNA was isolated using spheroplasts of mung-bean nodule bacteria obtained by treating cells with lysozyme (2 mg/mL) in a buffer containing glucose (0.05 M), Tris-HCl (0.025 M), and EDTA (0.01 M) at pH 8.0. The precipitate of spheroplasts was resuspended in a medium of NaCl (0.15 M), Tris-HCl (0.05 M), EDTA (0.02 M), and Na sarcosylate (2%) at pH 8.0. The suspension was stored for 30 min on ice. The spheroplast lysate was treated with dry CsCl until the final density was 1.700 g/cm^3.

Equilibrium centrifugation of DNA in a CsCl density gradient was performed in a Beckman (L-65) ultracentrifuge in a Ti-50 vertical rotor at 44,000 rpm and 18^{o}C for 48 h. The buoyant density of the DNA was determined by measuring the refractive index of the DNA using a refractometer (IRF-22).

The number of GC base pairs in the DNA was calculated by the formula given in the literature [7]. Electrophoresis of the DNA fractions was carried out in agarose gel (1%) by the previous method [8].

A calibration curve for determining the buoyant density of the DNA was constructed using standard DNA samples from *S. lutea* (1.731 g/cm^3) and cotton DNA from cellular organelles (1.692, 1.697, and 1.706 g/cm^3) at 18^{o}C. The gradient was separated after centrifugation in a special fractionating device (Beckman, USA) into 46 fractions. Electrophoresis in agarose gel (1%) determined if DNA was present in the fractions.

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