tio, however, 2 times difference between the highest and the lowest was found. The 5-HIAA/5-HT ratio was highest in n. caudatus putamen, higher in n. ventromedialis and lowest in n. suprachiasmaticus. These results suggest that the turnover rate of serotonin, reflected by the ratio 5-HIAA/5-HT, is different in each brain area examined. Gaudin-Chazal et al.[15] showed negative correlation between 5-HIAA/5-HT ratio and 5-HT level in different structures of cat brain. In discrete rat brain areas examined, however, such a correlation was not found. Our highly sensitive method for simultaneous measurement of serotonin and 5-HIAA by HPLC-ECD made it possible to study the serotonin neuron function even in discrete hypothalamic nuclei.

1 Reinhard, J.F. Jr, and Wurtman, R.J., Life Sci. *21* (1977) 1741.
2 Saavedra, J.M., Brownstein, M., and Axelrod, J., J. pharmac. exp. Ther. *186* (1973) 508.
3 Cattabeni, F., Koslow, S.H., and Costa, E., Science *178* (1972) 166.
4 Mefford, I.N., and Barchas, J.O., J. Chromat. *181* (1980) 187.
5 Koch, D.D., and Kissinger, P.T., Life Sci. *26* (1980) 1099.
6 Reinhard, J.F. Jr, Moskowits, M.A., Sved, A.F., and Fernsrom, J.D., Life Sci. *27* (1980) 905.
7 Lacković, Z., Parenti, M., and Neff, N.H., Eur. J. Pharmac. *69* (1981) 347.
8 Johnson, M.D., and Crowley, W.R., Life Sci. *31* (1982) 589.
9 Oomagari, K., Uchimura, H., Matsumoto, T., Yokoo, H., Hirano, M., Shimomura, M., and Nakahara, T., Neurosciences, Jap. *8* (1982) 64.
10 König, J.F.R., and Klippel, R.A., The Rat Brain. Williams and Wilkins, Baltimore 1963.
11 Lindvall, O., Björklund, A., and Divac, I., Brain Res. *142* (1978) 1.
12 Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
13 Saavedra, J.M., Palkovits, M., Brownstein, M., and Axelrod, J., Brain Res. *77* (1974) 157.
14 Saavedra, J.M., Fedn Proc. *8* (1977) 2134.
15 Gaudin-Chazal, G., Daszuta, A., Faudon, M., and Ternaux, J.P., Brain Res. *160* (1979) 281.

0014-4754/84/111288-03$1.50 + 0.20/0

Genome size variation of rhizobia

S.K. Chakrabarti, A.K. Mishra and P.K. Chakrabartty

Department of Microbiology, Bose Institute, Calcutta 700009 (India), 22 August 1983

Summary. Slow-growing species of *Rhizobium* have a genome size almost double that of the fast-growing species. This provides additional support in favor of their being placed in separate genera.
Key words. *Rhizobium* genus; genome size; rhizobia, slow-growing; rhizobia, fast-growing; taxonomic classification.

The genus *Rhizobium* (family Rhizobiaceae) comprises the bacteria responsible for infecting roots of members of the plant family Leguminosae and inciting the production of morphologically defined nodules in which they fix nitrogen in symbiosis. The existing classification of rhizobia recognizes 6 species, i.e., *Rhizobium leguminosarum, R. phaseoli, R. trifolii, R. meliloti, R. japonicum* and *R. lupini,* based on cross-inoculation between the bacteria and their legume hosts, in the single genus *Rhizobium*[1]. Of these 6 species, the first 4 mentioned are fast growers as judged by in vitro growth on yeast extract media. However, the existing classification is considered inadequate and can be treated as only tentative. This is mainly because of mutual symbiotic promiscuity and the entire exclusion of other groups, such as the 'Cowpea miscellany' group[2]; these are slow growers and also symbiotically promiscuous.

The distinction between the slow-growing and fast-growing rhizobia was recognized in the 8th edition of Bergey's Manual[1], where they are placed in 2 separate groups under the genus *Rhizobium*. Studies on DNA base composition[3], nucleic acid hybridization[4], internal antigens[5] and numerical taxonomy[6] provide additional support in favor of their taxonomic spearation. Based on these studies it has been further argued[7] that these 2 groups of rhizobia should be placed in separate genera, considering their differences in properties. The name *'Brachyrhizobium'* has been proposed by Jordan[8] for a new genus to include the slow growers.

Knowledge of bacterial genome size has taxonomic implications[9] and is known to be useful in assessing the evolutionary relationships[10]. In a previous study, the genome sizes of 3 slow-growing species of rhizobia were estimated[11]. In the present study the genome sizes of 5 species of fast growing and slow-growing rhizobia were measured for a comparison to probe the proposal for splitting the genus *Rhizobium* into 2. Our study further supports the justification of the proposal for the separation of the 2 groups of rhizobia into separate genera.

Materials and methods. The bacterial strains: *R. meliloti* (SU216), *R. trifolii* (T19), *R. japonicum* (SB16) obtained from Dr K.R. Dadarwal, Haryana Agricultural University; and *R. phaseoli* (CC365), *R. leguminosarum* (SU391) acquired from Dr J. Brockwell, CSIRO, Australia, were used in this study. The bacteria were grown in yeast extract mannitol medium at 28 °C and harvested during the stationary phase of growth. DNA was extracted and purified by a modification of Marmur's method[12]. The final DNA preparation had absorbance ratios 260 nm/280 nm and 260 nm/230 nm of about 2 and greater than 2, respectively. The preparation had an amount of RNA contamination of less than 4% as mesured by solubility in cold perchloric acid[13]. DNA was heared by partial depurination and alkaline cleavage[14]. Hybridization was performed at 60 °C in 0.12 M phosphate buffer, pH 6.8 ($Na^+ = 0.18$ M) to the desired C_0t values. C_0t was calculated as: optical density$_{260\,nm}$ × time of incubation in h/2. Hybrid DNA was fractionated by chromatography on hydroxyapatite columns[15-17]. The concentration of single stranded and reassociated DNA was estimated from the column eluates and the percentage of reassociation was calculated. The data were plotted on a log-C_0t plot to estimate the $C_0t_{½}$ values of hybridization.

Results and discussion. Our previous study[11] showed that strains of slow-growing *Rhizobium* had a 2.5 to 3.5 times bigger genome size than that of *Escherichia coli.* The genomes of fast-growing rhizobia may well be smaller than those of slow-growing rhizobia. For a mesure we compared the genome size of a few fast- and slow-growing strains of rhizobia, by comparing the $C_0t_{½}$ of hybridization of rhizobial DNA with that of *E. coli* B DNA. It has been shown that there is an apparent proportionality between $C_0t_{½}$ of hybridization and the genome size of DNA[18]. The figure shows the reassociation kinetic profiles of DNA isolated from *R. meliloti* (SU216), *R. phaseoli* (CC365), *R. trifolii* (T19) and *R. leguminosarum* (SU391), all of which are fast-growers, and of *R. japonicum* (SB16) which is a slow-grower. All the DNAs hybridized to the extent of 90% or more at a C_0t of 100. No significant hybridization was observed below a C_0t of 0.1. All the curves followed a second order kinetic pattern typical of bacterial DNA reasso-

ciation. The DNA used in this study was total cellular DNA and therefore may have contained plasmid DNA, if present. However, plasmid DNA being only a small fraction of total DNA should not appreciably affect the data presented here. $C_0t_{½}$ values were calculated from the C_0t corresponding to the 50% of observed maximum hybridization.

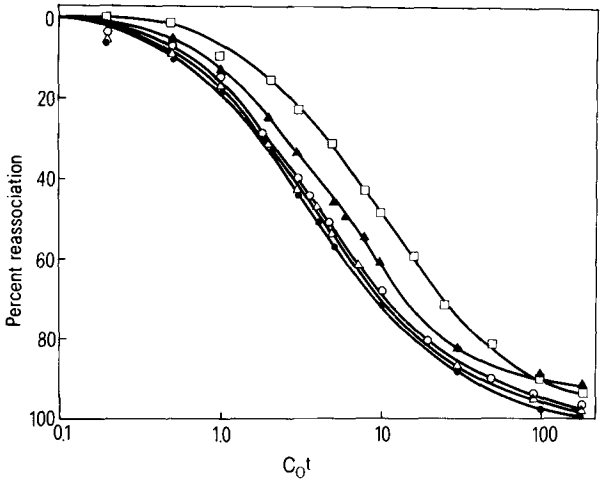
The $C_0t_{½}$ of DNA reassociation of the fast-growing species of *Rhizobium; R. meliloti* (SU216), *R. phaseoli* (CC365), *R. trifolii* (T19), *R. leguminosarum* (SU391), were found to be 4.1, 4.4, 3.9 and 4.9, respectively. These values are considered to be very close to each other. On the other hand the DNA of *R. japonicum* (SB16), a slowgrower, has a $C_0t_{½}$ of 9.8 which is about double that of the fastgrowing species studied, indicating that it has a genome size almost twice that of the fast-growing ones. The DNA of *E. coli* B was found to have a $C_0t_{½}$ of 2.8. The table presents the calculated genome size of the strains of fast- and slow-growing rhizobia. *E. coli* B has a molecular weight of 2.2×10^9 daltons for the genome size[19]. The value has been used as a primary molecular-weight standard for measurement of the rhizobial DNA genome by proportionality relationship of $C_0t_{½}$ of reassociation. The values for the genome size of *R. japonicum* (CC409), *R. lupini* (RL3001) and *R. sp.* Cowpea (U_8) were obtained from our previous study[11] and have been used for comparison (table). The genome sizes of the fast-growing and the slow-growing species fall in the range of 3.1 to 3.9×10^9 and 5.4 to 7.7×10^9 dalton, respectively. The fast-growers and the slow-growers, therefore, have genome sizes in 2 distinct ranges of values and the 2 groups differ by a factor of approximately 1.5 to 2. If this is confirmed in general for the 2 groups of rhizobia, this difference would support the proposal[7] to establish 2 distinct genera.

2 classes, distinct as regards their genome size, have already been found in mycoplasma[20]. On the basis of that, and of the frequency distribution of prokaryotic genome sizes, an evolutionary scheme by genome duplication has been suggested[10]. If increase in genome size is a primary step of evolution, we feel that slow-growing strains of rhizobia having a larger genome size are to be considered more advanced in the evolutionary scale than their fast-growing counterparts.

Genome size estimates for strains of *Rhizobium*

Strain	Calculated genome size in daltons $\times 10^{-9}$
R. meliloti (SU216)	3.22
R. phaseoli (CC365)	3.46
R. trifolii (T19)	3.06
R. leguminosarum (SU391)	3.84
R. japonicum (SB16)	7.69
R. japonicum (CC409)	7.62*
R. lupini (RL3001)	5.42*
R. sp. Cowpea (U8)	6.91*

*Values are quoted from our previous study[11].



The kinetics of reassociation of DNA of *Rhizobium* species. The samples were sheared by partial depurination and alkaline cleavage, made up to 0.12 M in phosphate buffer, pH 6.8, denatured by heat and incubated at 60 °C. Kinetic points were then taken. Each point was analyzed by fractionation on hydroxyapatite. *E. coli* B DNA was determined to have a $C_0t_{½}$ of 2.8 (not shown here). *R. trifolii* (T19), ●—●; *R. meliloti* (SU216), △—△; *R. phaseoli* (CC365), ○—○; *R. leguminosarum* (SU391), ▲—▲; *R. japonicum* (SB16), □—□.

1 Jordan, D.C., and Allen, O.N., Family Rhizobiaceae, in: Bergey's manual of determinative bacteriology, 8th edn, p. 261. Eds R.E. Buchanon and N.E. Gibbons. Williams and Wilkins, Baltimore 1974.
2 Lange, R.T., J. gen. Microbiol. *26* (1961) 351.
3 De Ley, G., and Rassel, A., J. gen. Microbiol. *41* (1965) 85.
4 Gibbins, A.M., and Gregory, K.F., J. Bact. *111* (1972) 129.
5 Vincent, J.M., and Humphrey, B., J. gen. Microbiol. *63* (1970) 379.
6 Graham, P.H., J. gen. Microbiol. *35* (1964) 511.
7 Crow, V.L., Jarvis, B.D.W., and Greenwood, R.M., Int. J. syst. Bact. *31* (1981) 152.
8 Jordan, D.C., Int. J. syst Bact. *32* (1982) 378.
9 Imaeda, T., Kirchheimer, W.F., and Barksdale, L., J. Bact. *150* (1982) 414.
10 Wallace, D.C., and Morowitz, H.J., Chromosome *40* (1973) 121.
11 Chakrabarti, S.K., Mishra, A.K., and Chakrabartty, P.K., Curr. Sci. *52* (1983) 779.
12 Marmur, J., J. molec. Biol. *3* (1961) 208.
13 Savitsky, J.P., and Stand, F., Nature, Lond. *207* (1965) 758.
14 Grouse, L., Chilton, M.D., and McCarthy, B.J., Biochemistry *11* (1972) 798.
15 Chakrabartty, P.K., J. Hered. *66* (1975) 213.
16 Chakrabartty, P.K., J. gen. appl. Microbiol. *23* (1977) 77.
17 Chakrabartty, P.K., Chattopadhyay, S.K., and Schneider, W.C., Cancer Res. *42* (1982) 421.
18 Britten, R.J., and Kohne, D.E., Science *161* (1968) 529.
19 Eigner, F., in: Methods in Enzymology, vol. 12, p. 386. Eds L. Grossman and K. Moldave. Academic Press, New York 1968.
20 Bak, A.L., Black, F.T., Christiansen, C., and Fraundt, E.A., Nature, Lond. *224* (1969) 1209.

0014-4754/84/111290-02$1.50 + 0.20/0

Bacterial growth in artificial capillary spaces

M.T. Bomar[1]

Federal Research Center for Nutrition, Engesserstrasse 20, D-7500 Karlsruhe 1 (Federal Republic of Germany), 16 November 1983

Summary. In experiments on the influence of microscopic capillaries on the growth of *Escherichia coli, Brevibacterium linens, Micrococcus flavus* and *Bacillus cereus*, a distinct delay in growth was observed. The difference in counts was greatest after 8 h. Later a slight equilibration of counts was noted. With *B. cereus*, only slight or no spore formation was observed under microcapillary conditions.

Key words. Bacterial growth; capillary space; spore formation; microcapillary conditions; ecological niche.