

The geographical features like beach morphology of Colva, the absence of beach cusps and the absence of extensive beach rocks¹ indicate that the site of the fossil wood, was not occupying a palaeo-tidal channel. This leads to the inference that the Colva beach is prograding at a rapid rate.

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1. Davies J. L., *Geographical variation in coastal development*. Longman, New York, 1977.

GENOME SIZE OF SLOW GROWING SPECIES OF RHIZOBIUM

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KNOWLEDGE of bacterial genome size which is the total molecular weight of haploid unreplicated bacterial genome DNA is known to be useful to assess the average number of genes and the phenotypic potential of an organism as well as the evolutionary relationships¹. This knowledge also has taxonomic implications and the values are indispensable to calculate possible DNA sequence between organisms². Britten and Kohne³ established an apparent proportionality between the genome size and its $C_{0t_{1/2}}$ corresponding to the C_{0t} (moles nucleotides/lit \times sec) at which a given DNA sample attains 50% renaturation. Bacterial genome size can thus be measured from the $C_{0t_{1/2}}$ of its DNA by comparing it to that of a standard bacteria whose genome size is precisely known. This method has been successfully used for many organisms⁴⁻⁸. *Escherichia coli* B has a genome of about 1100μ in length⁹ and its genome size has been estimated to be 2.2×10^9 daltons¹⁰. As such *E. coli* B DNA can serve as a primary molecular weight standard in DNA renaturation studies. In our endeavour, as a prelude to the studies of evolutionary relationships of rhizobia, the genome sizes of three slow-growing species of *Rhizobium*, e.g. *R. lupini* 3001, *R. sp.* Cowpea U₈ and *R. japonicum* CC409 were measured.

E. coli was grown in nutrient broth and rhizobia were propagated in Ashby's Mannitol Medium at 28°C and harvested during the stationary phase of growth. DNA was extracted and purified by a modification of Marmur's method¹¹. The final DNA preparation had an $A_{260\text{nm}}/A_{230\text{nm}}$ value of about 2 and

$A_{260\text{nm}}/A_{230\text{nm}}$ value of more than 2. The preparation had an RNA contamination of less than 4% as estimated by solubility in cold normal perchloric acid¹². DNA was sheared by partial depurination and alkali cleavage¹³ and hybridizations were performed at 60°C in 0.12 M phosphate buffer, pH 6.8 (0.18 M Na⁺). For the reassociation kinetics, fractionation of reassociated DNA was carried out by chromatography on columns of hydroxyapatite as described earlier¹⁴⁻¹⁷ at the desired C_{0t} values. The C_{0t} values were conveniently calculated by use of the formulae—optical density at 260 nm \times time of incubation in hr/2. The concentration of single-stranded and reassociated DNA was determined from the eluates by their absorbance at 260 nm and the percent of reassociation was calculated.

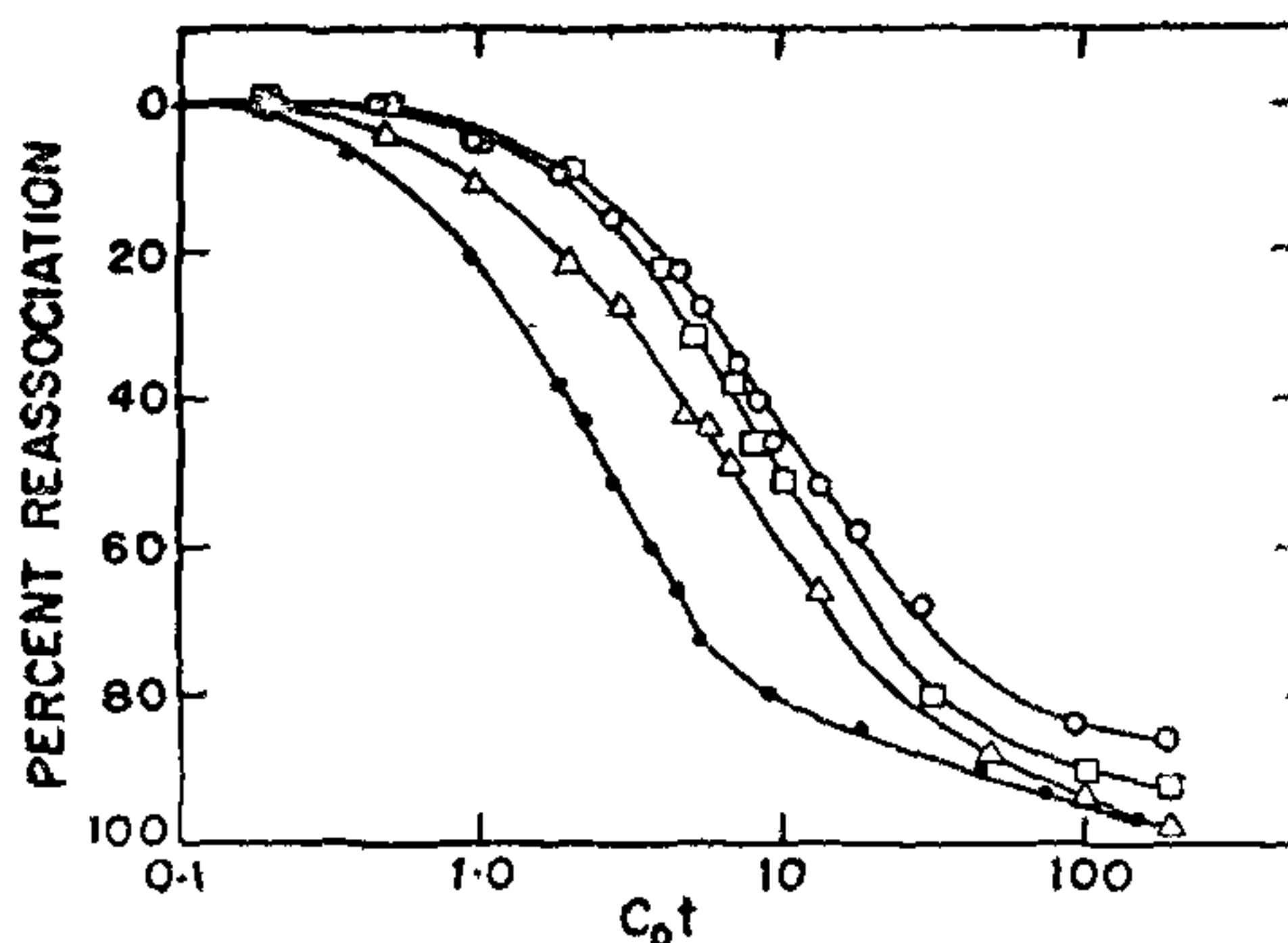


Figure 1. The kinetics of reassociation of the DNAs of *Rhizobium* species and of *E. coli*. The samples were sheared by partial depurination and alkaline cleavage, made to 0.12 M phosphate buffer, pH 6.8, denatured by heat and incubated at 60°C. Kinetic points were then taken. Each point was analysed by fractionation on hydroxyapatite. *E. coli* B, (closed circle) *R. lupini* 3001, (open triangle) *R. sp.* Cowpea U₈, (open square) *R. japonicum* CC 409, (open circle).

Figure 1 presents the reassociation kinetic profiles of DNAs obtained from the three species of rhizobia. The kinetics followed a second order pattern and all the DNAs hybridized to the extent of about 90% at a C_{0t} of 100. No significant hybridizations were observed below a C_{0t} of 0.1. From the figure it is apparent that DNAs from *E. coli* and the three species of rhizobia attained 50% of maximum hybridization at a C_{0t} of 2.8, 6.9, 8.8 and 9.7 respectively. This suggests that the genomes of *R. lupini* 3001, Cowpea U₈ and *R. japonicum* CC 409 are respectively almost 2.46, 3.14 and 3.46 times greater than that of *E. coli* B and, therefore, renatured as many times slower. The molecular weight of *E. coli* B DNA is 2.2×10^9 daltons

and those of *R. lupini* 3001, *R. sp.* Cowpea U₈ and *R. japonicum* CC 409 are 5.42×10^9 , 6.91×10^9 and 7.62×10^9 daltons respectively. In this method eventual presence of plasmid was measured as part of the total genome. However, being only a small percent of the total chromosomal DNA, it should not affect the data significantly. The size of *R. lupini* 3001 genome agrees with that of *Chromobacterium violaceum*², a related species (4.8×10^9 daltons). However, the genome sizes of *R. sp.* Cowpea U₈ and *R. japonicum* are greater. The genome size of *Pseudomonas aeruginosa*² has been reported to be 6.9×10^9 daltons which is in close proximity of the genome size of *R. sp.* Cowpea U₈, while the genome size of *R. japonicum* CC 409 is still larger. All the rhizobial strains studied here belong to the slow-growing group and have genome sizes much larger than those of *E. coli*.

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1. Gillis, M., DeLey, J. and DeCleene, M., *Eur. J. Biochem.*, 1970, 12, 143.
2. Lethbak, A., Christiansen, C. and Stenderup, A., *J. Gen. Microbiol.*, 1970, 64, 377.
3. Britten, R. J. and Kolene, D. E., *Science*, 1968, 161, 529.
4. Brooks, R. R. and Huang, P. C., *Biochem. Genetics*, 1972, 6, 41.
5. Straus, N. A., *Proc. Natl. Acad. Sci. (USA)*, 1971, 68, 799.
6. Lambert, C. C. and Laird, C. D., *Biochim. Biophys. Acta*, 1971, 240, 39.
7. Dutta, S. K., Penn, S. R., Knight, A. R. and Ojha, M., *Experientia*, 1972, 28, 582.
8. Iyenger, G. A. S., Gaddipati, J. P. and Sen S. K., *Theor. Appl. Genet.*, 1979, 54, 219.
9. Cairns, J., *Cold Spring Harbor Symp. Quant. Biol.*, 1963, 28, 43.
10. Eigner, J., in *Methods in Enzymol.*, 1968, 12, 386.
11. Marmur, J., *J. Mol. Biol.*, 1961, 3, 208.
12. Savitsky, J. P. and Stand, F., *Nature (London)*, 1965, 207, 758.
13. Grouse, L., Chilton, M. D. and McCarthy, B. J., *Biochemistry*, 1972, 11, 798.
14. Chakrabartty, P. K., *J. Gen. Appl. Microbiol.*, 1977, 23, 77.
15. Chakrabartty, P. K., *J. Heredity*, 1975, 66, 213.
16. Chakrabartty, P. K., *Experientia*, 1978, 34, 47.
17. Chakrabartty, P. K., Chattopadhyay, S. K. and Schneider, W. C., *Cancer Res.*, 1982, 42, 421.

ACREMONIUM ZEYLANICUM—A NEW RECORD OF ENTOMOGENOUS FUNGUS FROM INDIA

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DURING the survey for entomogenous¹⁻⁴ fungi, an interesting fungus was recorded on *Aphis brassicae*. These aphids are known to be serious pests of brassicae crops and the Delhi climate is polluted with these aphids during December-January when the temperature range is 15–20°C. The infected aphids with swollen abdomen were picked up from the lower surface of brassica leaves and incubated on moist blotters at $18 \pm 2^\circ \text{C}$ for 4–6 days. The fungus growing on the swollen aphids produced long chains of conidia on incubation. Also on dissecting the swollen abdomen under stereobinocular microscope, it was found full of moniliaceous mycelium. The small abdominal fragments upon culturing on agar media yielded the pure growth of a species of *Acremonium*.

Literature survey⁵⁻¹⁰ indicates that although *Acremonium* spp have been isolated from soil, leaf litter, compost and human mycetoma⁸ etc, the presence of *A. zeylanicum* on *A. brassicae* has not been recorded from other countries and is being reported for the first time from India.

The culture of *Acremonium* has been identified as *A. zeylanicum* due to following morphological characters.

Acremonium zeylanicum (Petch.) W. Gams et Evans in *Trans. Brit. Mycol. Soc.* 64, 393, 1975.

Colonies attaining 10 mm diam, in ten days at 20°C on PDA. No growth observed at 30°C. At first colonies were white, turning pale pink with age, reverse of the colony cream-coloured. Sporulation abundant. Phialides mostly simple, arising from aerial hyphae, 1–2 μm size, thin-walled, slender, smooth, hyaline, 7–25 μm long and tapering from 2–2.5 μm to 1 μm. Conidia cohering in long chains, narrow, oval, 3–6 × 1.25–2.5 μm in size, both ends acute and truncate. Chlamydo-spores absent (figure 1).

On *Aphis brassicae*, Dec., 1980, IARI field, New Delhi, J. L. Varshney, ITCC 3027.

The above isolate varies from the type description in having longer phialides and bigger conidia as well as its very long conidial chain and this isolate is a different strain of the above species. Initially⁹ *A. zeylanicum* has been recorded on insect and spiders from