black pepper vines in Kozhikode and Wyanad districts of Kerala are similar to the symptoms described on black pepper in several South East Asian countries[4] and Brazil[2]. In both these areas, the causal virus has been identified as a badna virus PYMV[4]. PYMV had non-enveloped bacilliform virions and was transmitted by mechanical inoculation and by citrus mealybug, *Planococcus citri*[4]. However, in Brazil it is suspected to be transmitted by another species of mealybug, *Pseudococcus elisae*[2]. Further, PYMV was also serologically closely related to both BSV and ScBV[4]. Our studies based on symptomatology, serological affinities, electron microscopy and mealybug transmissibility, clearly indicate that the virus under investigation is a member of badnavirus. Although the present virus could be transmitted by *F. virgata*, a common foliar mealybug found associated with black pepper in Kerala and Karnataka, it remains to be seen whether this virus could also be transmitted by other species, as nine species of mealybugs have been reported to be associated with black pepper in India[9]. This is a report of the transmission of a badnavirus by *F. virgata* in black pepper. However, it is known to transmit a badnavirus in cacao (Cacao swollen shoot virus, CSSV) in Africa[10].

Badnaviruses have been reported from banana[11], citrus[12], rice[13] and sugarcane[14] in India. In most cases, black pepper is grown as a mixed crop with other badnavirus-susceptible crops like banana and cacao. Hence it is important to study whether these crops are a source of infection to black pepper or vice versa. To unequivocally identify whether badnavirus on black pepper is a strain of an already known badnavirus or a distinct badnavirus, the virus will have to be further characterized at biological and molecular levels.

Occurrence of CMV on black pepper in India is already known[5], and the occurrence of mixed infections by both these viruses cannot be ruled out. As black pepper is clonally propagated, disease spread is rapid through planting material when infected plants are used as a source of planting material. As symptoms alone cannot be used as a criterion for confirming the disease-free nature of the material, it is necessary to develop methods for quick, reliable and sensitive detection (either serological or nucleic-acid based) of the virus for producing and certifying virus-free planting material.

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Oligophilic bacterial diversity of Leh soils and its characterization employing ARDRA

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Oligophiles from uncultivated, mustard-, potato- and wheat-cultivated soils of Leh were studied. The substrate concentration of the standard plate count (SPC) medium was 1.75–0.0035% to facilitate the growth of copiotrophs and oligophiles. The ratio of bacterial counts on 1/20th dilution to full strength SPC was 3.26 in uncultivated and 1.74, 1.83 and 2.7, in wheat-, mustard- and potato-cultivated soil, respectively. ARDRA pattern with *Hae*III of 36 randomly picked isolates showed a dissimilarity coefficient of 3.0. Despite the nature of the soil, seven phylogenetic groups were formed, which consisted of isolates from higher dilutions (1/20 and 1/100). Only three isolates recovered on normal SPC showed similarity with these isolates. This confirms that bacteria growing at higher dilu-

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tions are different. It is also clear that oligophiles are abundant in nature and the need is to develop methodologies to explore their existence.

THE global biodiversity is changing at an unprecedented rate as a complex response to several human-induced perturbations in the environment. The magnitude of this change is large and strongly linked to ecosystem processes and use of natural resources by the society. Though many of the microorganisms, the earliest forms of life on earth, have been commercially exploited, our knowledge of their diversity and key roles in sustaining global life-supporting systems is limited. The axenic culture technique, the stronghold of microbiology itself, has restricted microbiologists within a narrow limit for exploration of the microbial world. As a general rule microbiologists have found that the maximum recovery of heterotrophic bacteria from a variety of oligotrophic to mesotrophic aquatic habitats using cultural methodologies is about 1% of the total direct microscopic count[1].

Theoretically, if an organism grows in nature, it can be cultured if its physiological niche is perceived and duplicated under *in vitro* conditions. It is established that the nutrient concentrations in commonly used laboratory media are several-fold higher than those present in the natural environment, specially the aquatic habitat[2]. It is further revealed that a predominant group, i.e. oligophiles in the natural bacterial population from both aquatic and terrestrial habitats does not grow on conventional media but forms distinct colonies on 100-fold diluted versions of such media[3–8]. Such oligophilic bacteria have been found to show useful properties like N-fixation, denitrification, concentrating micronutrients, bio-control and degradation of xenobiotics[9–12]. There is limited information on oligophilic bacterial diversity, although recent reports suggest their preponderance in various natural habitats. In this study, we describe oligophilic bacterial diversity of the soil from Leh and its characterization employing ARDRA[13].

Four soil samples were collected from Leh (lat. 34°, long. 77.5°), Ladakh, the northernmost district of Kashmir in the rain shadow of the Himalayan ranges. These samples represent uncultivated field, potato-cultivated field, mustard-cultivated field and wheat-cultivated field. About 200 g soil was collected in sterilized plastic bags and transported to the laboratory. Samples were analysed for organic carbon[14], available phosphorus[15] and for microbial population. The pH of the four soils was in the range, 6.62–6.83. Population counts of soil samples were determined by dilution plating with vortexing at every dilution step. A volume of 100 µl of 10^{-4} dilution was spread plated in triplicates on standard plate count (SPC) medium prepared as a series of eight different dilutions, viz. undiluted, 1/10th, 1/20th, 1/40th, 1/60th, 1/100th, 1/200th and 1/500th dilution. One litre of undiluted medium contained: glucose 10 g, tryptone 5 g, yeast extract 2.5 g, NaCl 2.5 g at pH 7. These dilutions provided substrate concentration ranging from 1.75 to 0.0035%. It was anticipated that these dilutions would permit growth of bacterial populations differing in nutritional requirements quantitatively. Plates were incubated at 28°C, 80% RH in a BOD incubator for 15 days. Colonies that appeared on plates were counted visually on the 2nd, 4th and 7th day of incubation. The average of three replicates was used for analysis.

The dominant bacterial forms from each soil plating were isolated by sub-culturing on the corresponding medium. Twelve isolates each from full strength, 1/20th dilution and 1/100th dilution plates were randomly picked up from this collection for phylogenetic analysis to assess the relationship between oligophiles and copiotrophs. The total genomic DNA from actively grown culture of these 36 isolates was extracted. For genomic DNA extraction, cells were lysed with a combination of 0.5% SDS and 0.001% proteinase K followed by treatment with 1% CTAB and washing with phenol : chloroform (1 : 1) and chloroform : isoamyl alcohol (24 : 1). The 16S rDNA fragment of each isolate was amplified by the universal eubacterial primers Gm3f (5′AGAGTTTGATCMTGGC3′) and Gm4r (5′TACCTTGTTACGACTT3′). Gm3f has an alignment with 8–23 bp of 16S rRNA and Gm4r with 1492–1507 bp of 16S rRNA of *E. coli*. These two regions are specific for eubacteria and highly conserved (>90% for a phylogenetically diverse collection of approximately 85 bacterial sequence)[16]. PCR reaction was set up in 50 µl volume and contained 2 mM $MgCl_2$, 0.25 mM dNTP mixture, and 0.25 µM each of primer and one unit *Taq* Polymerase (Genie). Thermal cycler (PTC-100, M. J. Research) was run for initial denaturation at 94°C for 1 min, followed by 30 cycles of 1 min at 94°C, 1 min at 51°C and 1 min 30 s at 72°C, and a final extension at 72°C for 10 min. For 16S rDNA-based phylogenetic analysis, ARDRA was employed using two tetracutter enzymes, viz. *Rsa*I (MBI Fermentas) and *Hae*III (Genei). Amplicons were digested with 1 unit of enzyme for 6 h at 37°C according to manufacturer's protocol. The digested fragments were separated by electrophoresis on 2.5% (w/v) agarose gel and stained with ethidium bromide. The gel was visualized under UV and photographed. A comparative dissimilarity co-efficient based dendrogram was made using the band pattern of ARDRA for the isolates using NT sys P2.0 software program.

The organic C in the soil samples was 1.5, 1.2, 1.4 and 1.47% in uncultivated, wheat-, potato- and mustard-cultivated fields, respectively. Percentage availability of total phosphorus was higher in uncultivated soil (65), moderate in mustard-cultivated (51.5), wheat-cultivated soil (51), and low in potato-cultivated soil (34.0).

Colonies which appeared on SPC medium of 1/20th dilution and onwards were considered as oligophiles according to Watve *et al.*[7], who defined these organisms as those which did not grow or grew with reduced yields

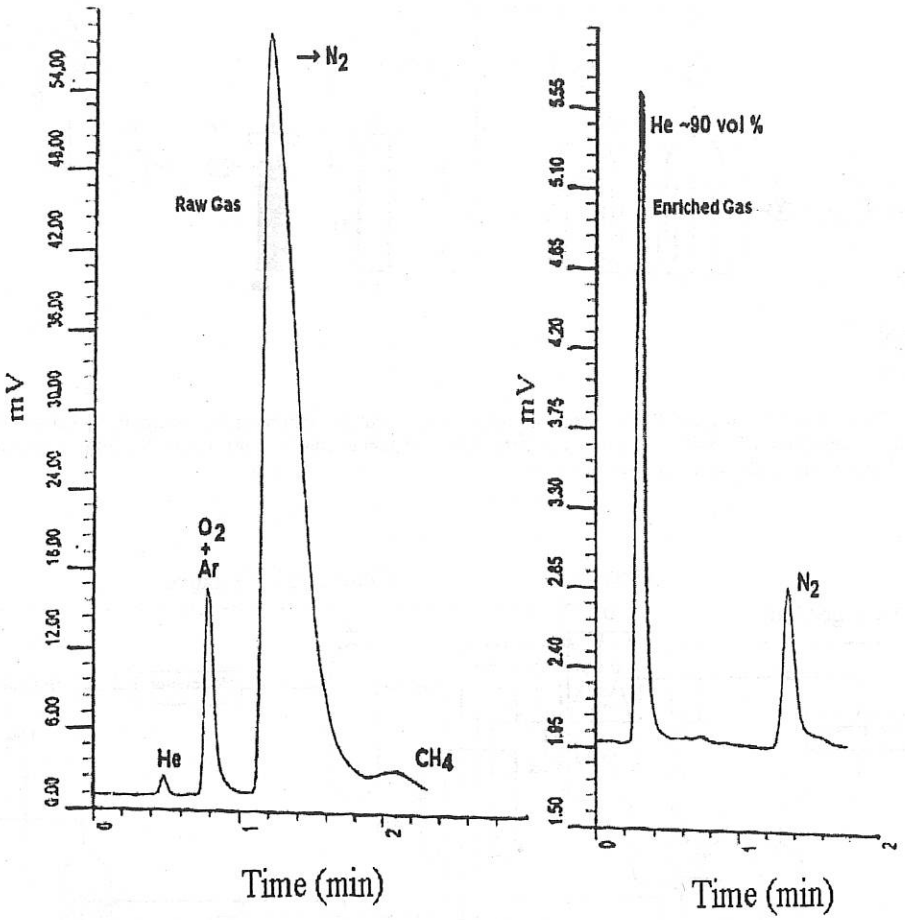


Figure 3. Gas chromatogram of feed gas from Bakreswar and enriched helium.

rial to contain the low temperatures within the condensation module and when the plant is shut down for longer periods, nitrogen atmosphere is maintained by isolating the pipelines and feeding the nitrogen from LN_2 storage container through a vaporizer.

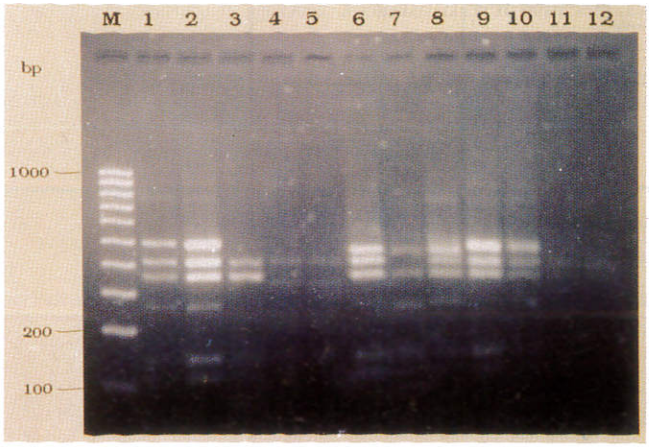
As the basic principle adopted by us for design of the enrichment plant is based on cryogenic condensation technique, balancing the various parameters during fabrication of the plant is rather complex. We have utilized the approximate analytical relationships for different components to make the subsystems consistent with each other, however, it is observed that each process parameter has a direct effect on the level of enrichment. In order to understand the symbiotic associations of the various parameters, a Gaussian Process (GP) computer model has been implemented to study the relevance of these parameters.

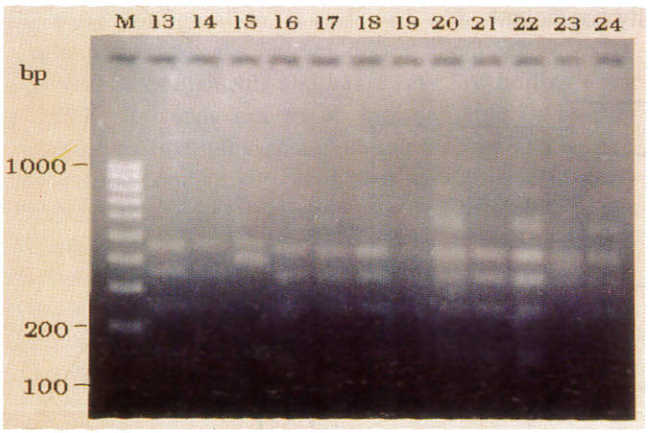
Computer modelling of the enrichment plant

In modelling of complex systems, like the helium-enrichment plant, empirically, we do not know, *a priori*, the parameterized form of the input–output relationship. The Gaussian process model is a way of avoiding having to explicitly parameterize this relationship by invoking a parameterized probability model over the data instead[10,11]. Let the training data set consist of N input vectors $\{x_1, x_2, x_3 \ldots, x_N\}$ each consisting of the input parameters of the plant and the corresponding set of known outputs (or 'targets') $\{t_1, t_2, t_3, \ldots, t_N\}$, the degree of helium concentration. A prediction, t_{N+1}, can then be made at any new input value, x_{N+1}, based on these training data. For brevity, let x_N represent the set of input vectors and T_N be the vector of corresponding outputs. The approach of the Gaussian process model is as follows.

Let $P(T_N| x_N)$ be the joint probability distribution over the N output values in the training dataset, with a set of adjustable parameters called hyperparameters. These hyperparameters explicitly parameterize a probability distribution over the input–output function rather than the function itself. This is a probability distribution in an N-dimensional space. Training of the model is done by maximizing the probabilities of the hyperparameters. Once trained, the model predicts the most probable value of the output for a new set of inputs, together with a measure of uncertainty of the prediction. The degree of correlation achieved by given proximities of the input vectors is dictated by the hyperparameters. There is one of these hyperparameters for each input dimension. The

0.735 similarity value with the 16S rRNA of Gram-negative *Pseudomonas chlororaphis* and *Acinetobacter calocoaceticus*, respectively. Isolates from different dilu-





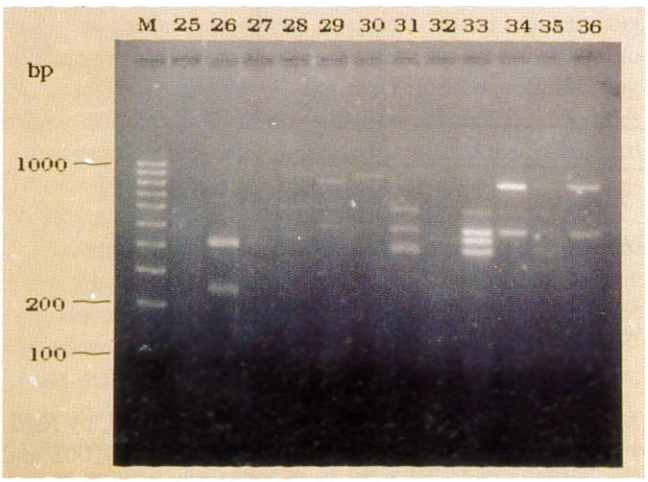
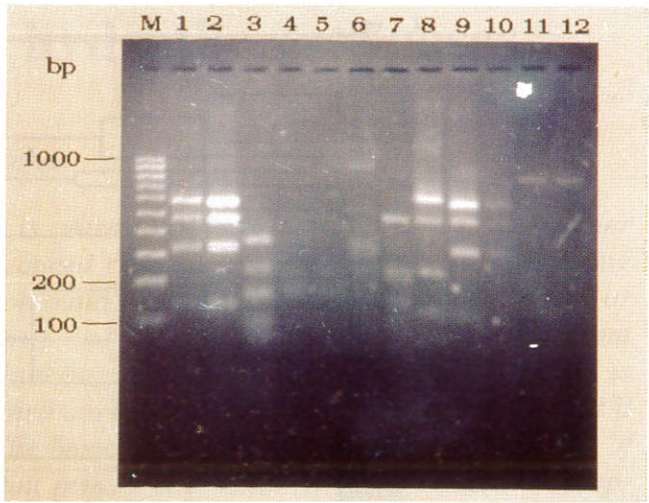
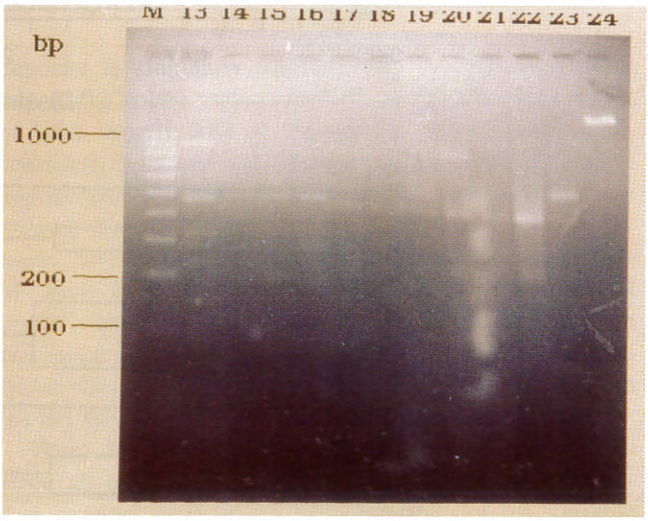


Figure 1. ARDRA pattern of 36 oligophilic isolates with restriction enzyme *Rsa*I. Lanes M-36: M, 100 bp ladder; 1, US1; 2, US4; 3, US203; 4, US1002; 5, US1003; 6, PS5; 7, PS1003; 8, WS201; 9, MS1; 10, MS4; 11, MS205; 12, MS1005; 13, US5; 14, US201; 15, US206; 16, PS1; 17, PS202; 18, PS203; 19, WS3; 20, WS4; 21, WS202; 22, WS1004; 23, WS1005; 24, MS203; 25, US1004; 26, PS4; 27, PS201; 28, PS1002; 29, PS1001; 30, WS2; 31, WS206; 32, WS1003; 33, MS2; 34, MS202; 35, MS1006; 36, MS1002.





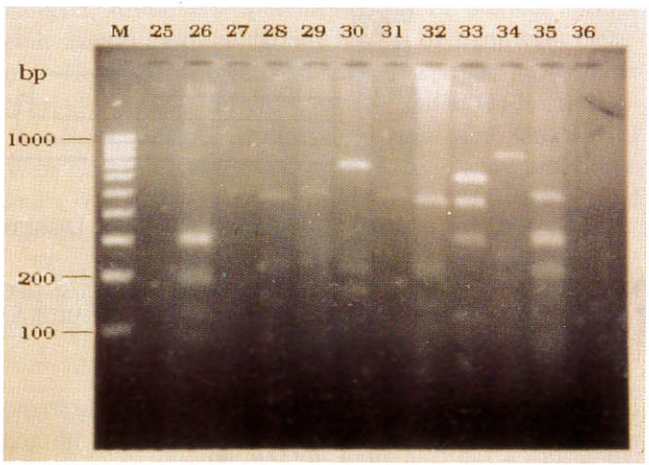


Figure 2. ARDRA pattern of 36 oligophilic isolates with restriction enzyme *Hae*III. Lanes M-36: M, 100 bp ladder; 1, US1; 2, US4; 3, US203; 4, US1002; 5, US1003; 6, PS5; 7, PS1003; 8, WS201; 9, MS1; 10, MS4; 11, MS205; 12, MS1005; 13, US5; 14, US201; 15, US206; 16, PS1; 17, PS202; 18, PS203; 19, WS3; 20, WS4; 21, WS202; 22, WS1004; 23, WS1005; 24, MS203; 25, US1004; 26, PS4; 27, PS201; 28, PS1002; 29, PS1001; 30, WS2; 31, WS206; 32, WS1003; 33, MS2; 34, MS202; 35, MS1006; 36, MS1002.

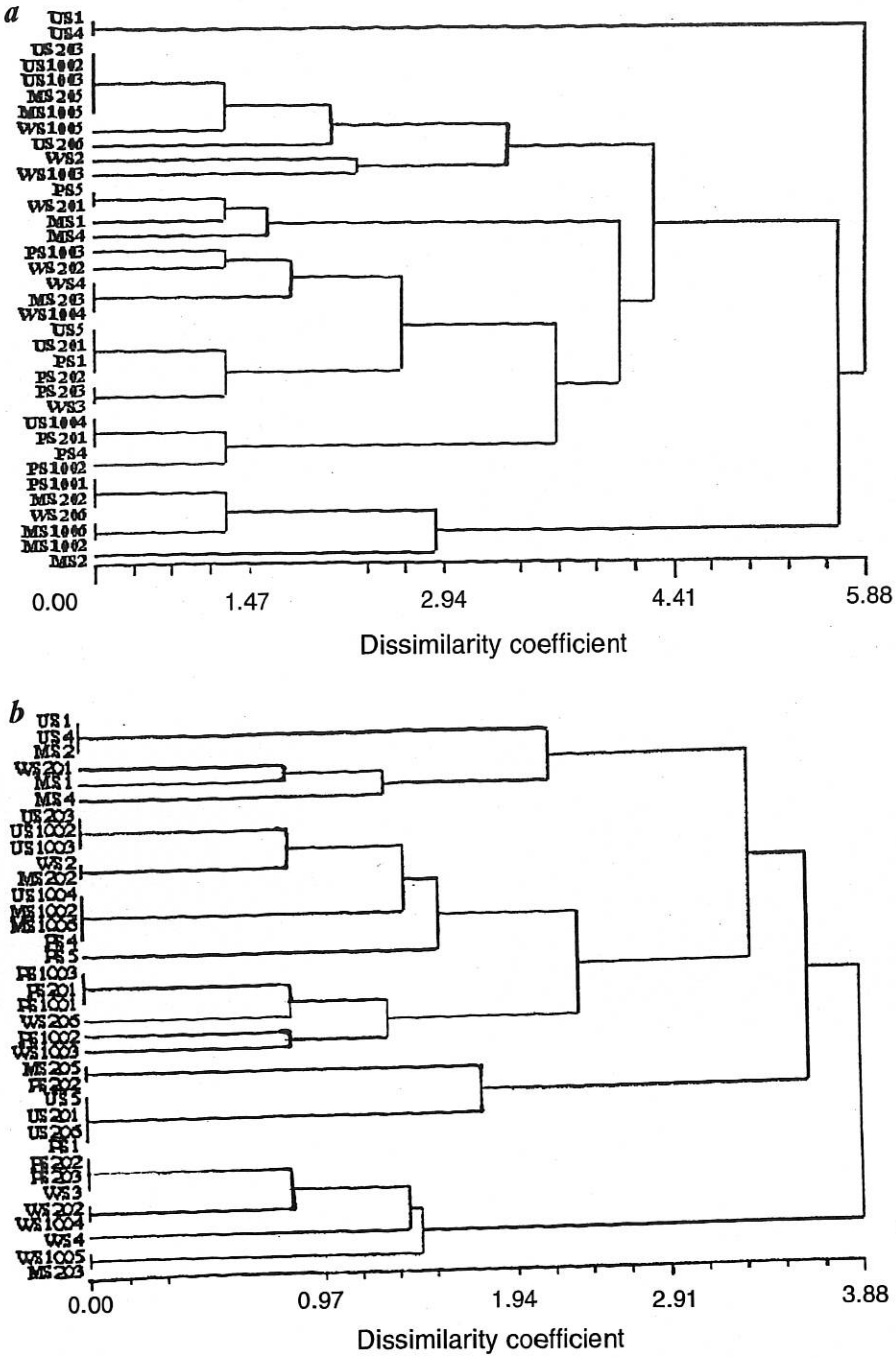


Figure 3. Dice dissimilarity coefficient–UPGMA-based dendrogram showing cluster analysis of the oligophilic isolates on the basis of ARDRA with (*a*) *Rsa*I and (*b*) *Hae*III.

tions but representing the same soil were normally found together in a single set. In only three cases (1st, 5th and 6th sets), isolates from different soils were placed together in the phylogenetic relationship. In only one occasion for *Hae*III and two occasions for *Rsa*I, isolates from full strength medium and those from 1/100th dilution gave similar fragment pattern. Isolate PS4 in the 6th set gave a comparable pattern with US1004, MS1006 and MS1002 on *Hae*III digestion. On the other hand, by *Rsa*I, PS4 and US1004 gave one pattern, whereas MS1002 and MS1006 produced different patterns, which further subdivided this group. On both *Rsa*I and *Hae*III digestions, WS3 gave the same pattern as PS203; PS1 gave a pattern similar to US5 and US201. In these three cases, isolates represent different soils as well as different dilutions of medium. MS1, MS4 and PS1002 each depicted unique

band pattern in both the digestion schedules. MS1 and MS4 were, however, somewhat interrelated phylogenetically, as their dissimilarity coefficient was low, around 1.5 in both the digestions. PS1002 was phylogenetically closer to PS201, although their dissimilarity coefficient was between 1 and 1.5 in the two digestions performed.

From the above observations, it was possible to conclude that bacteria that grew on undiluted SPC and those grown on 1/100th medium were phylogenetically distinct. Oligophiles constitute a different class of microorganisms with typically different metabolic set up than the copiotrophs. It is believed that oligophiles do not arise by simple adaptation of copiotrophs to thrive in low nutrient condition. Rather, they represent a specific line of evolution, enriched by low nutrient conditions which prevail in most of the natural environment. It is evident that a highly heterogeneous system like the soil harbours both copiotrophs and oligophiles independent of the overall nutrient status. Also, there exists a series of microenvironments which differ in nutrient availability both quantitatively and qualitatively. Each microenvironment appears to harbour microorganisms with the typical metabolic set up that suits best for growth and multiplication. Thus, it is nearly impossible to design a single laboratory condition (medium) adapted to the recovery of all types of bacteria. In this study, a class of low nutrient-loving microorganisms could be cultured successfully in the laboratory, however, with slight modification of the classical pure culture techniques. The oligophiles were found to be more abundant than the copiotrophs in a given environmental sample. This opens up the possibility to explore a big part of the uncultured bacterial diversity.

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Asymptotic models of species–area curve for measuring diversity of dry tropical forest tree species

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In a dry tropical forest, we examine the fitness and predictability of two non-asymptotic models (log-linear and power) of species–area curve, and the effect of sample location and scale on their regression-derived coefficients (c, z) for measuring tree diversity. Results indicate that, the log-linear model relatively better fits the data set, and yields better prediction of number of species on a small scale (i.e. predicted number of species for 3 ha using an equation based on 1 ha data). On the other hand, predictions from power function model for a larger area (i.e. predicted number of species for 15 ha using 1 ha and 3 ha equations) were closer to the observed values. The suitability of the model to fit the data was strongly influenced by the site and the scale of the plot size. The equations for the two models derived from data of small area (1 ha plot size) yielded inconsistent results, but those derived from a larger plot size (3 ha) consistently underestimated the number of species for 15 ha. The underestimation by power function model was lower compared to that by log-normal model for predicting the number of tree species. The study also shows that the coefficient z is site- as well as scale-dependent. The coefficient c can be used to predict α-diversity, and the number of species per individual can adequately describe the coefficient z. The results support discrete community concept for the dry tropical forests along a disturbance gradient and indicate that higher the z, greater would be the impact of harvest of individuals on biodiversity.

TROPICAL forests cover only 7% of the earth's land surface, but harbour more than half of the world's species[1],

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