growth from spores was disrupted at less than 90% relative humidity and germination occurred readily in total darkness than under normal conditions[35]. The reason for the isolation of well-documented, highly sporulating species in the present study may be due to the isolation technique used, viz. the dilution plate technique. There is a possibility that many slow-growing fungi belonging to Basidiomycetes might have been eliminated by using this technique.

1. Mohr, C. E. and Poulson, T. L., *The Life of the Cave*, McGraw-Hill, New York, 1966.
2. Poulson, T. L. and White, W. B., *Science*, 1969, **165**, 971–981.
3. Koilraj, J. A. and Marimuthu, G., *Curr. Sci.*, 1998, **75**, 1111–1113.
4. Waltham, T., *Caves*, Macmillan, London, 1974.
5. Marimuthu, G., *Oecologia*, 1984, **61**, 352–357.
6. Maheshwari, R., *J. Genet.*, 1996, **75**, 239–243.
7. Gilman, J. C., *A Manual of Soil Fungi*, State College Press, Ames, 1957.
8. Barron, G. L., *The Genera of Hypomycetes from Soil*, Williams and Wilkins Co., Baltimore, 1968.
9. Paul, E. Q. and Clark, F. E., *Soil Microbiology and Biochemistry*, Academic Press, London, 1996.
10. Paul, M. C. and Mishra, R. R., *Acta Bot. Indica*, 1993, **25**, 16–42.
11. Apinis, A. E., Chesters, C. G. C. and Toligoola, H, K., *Nova Hedwigia*, 1972, **23**, 113–124.
12. Pugh, G. J. F. and Mulder, J. L., *Trans. Br. Acycol. Soc.*, 1971, **57**, 273–282.
13. Panwar, M R. S., Ph D thesis, Meerut University, Meerut, 1979.
14. Mishra, R. C. and Dwivedi, R. S., *Acta Bot. Indica*, 1983, **11**, 28–31.
15. Udaiyan, K. and Krishnamurthy, R., *Acta Bot. Indica*, 1991, **19**, 176–184.
16. Girivasan, K. P., Rajagopal, K., Muruganandam, V. and Suryanarayanan, T. S., *Curr. Sci.*, 1998, **74**, 359–360.
17. Marvanova, L., Kalouskova Hanulakova, D. and Schanel, L., *Mikromycety Zbrasouskych jeskyni*, 1992, **46**, 243–250.
18. Semikolennykh, A. A., in Proceedings of 12th International Congress on Speleology, Switzerland, 1997, vol. 3, pp. 293–296.
19. Ingold, C. T., *The Biology of Fungi*, Anchon Press, London, 1975, pp. 11–33.
20. Usman, K., Ph D thesis, Madurai Kamaraj University, Madurai, 1981.
21. Habersetzer, J., Ph D thesis, Goethe University, Frankfurt, 1983.
22. Garrett, S. D., *Soil Fungi and Soil Fertility*, Pergamon, Oxford, 1963, pp. 1–165.
23. Michael, P., *Ecological Methods for Field and Laboratory Investigation*, Tata McGraw Hill, New Delhi, 1984, pp. 24–45.
24. Vandel, A., *Biospeleology*, Pergamon Press, Oxford, 1965, pp. 57–60.
25. Cubbon, B. D., in *The Science of Speleology* (eds Ford, T. D. and Cullingford, C. H. D.), Academic Press, London, 1976, pp. 423–452.
26. Biswas, J. and Kanoje, R. S., *J. Ravishankar Univ.*, 1992, **45**, 33–41.
27. Cubbon, B. D., *Trans. Cave Res. Group G.B.*, 1970, **12**, 57–74.
28. Tomaselli, R., *Atti. Ist Bot. Lab. Crittogan. Pavia, Ser.*, 1956, **12**, 203–212.
29. Newrick, J. A., *Trans. Cave Res. Group G.B.*, 1957, **5**, 35–51.
30. Walton, G. A., in Proceedings of University of Bristol Speleological Society, Bristol, 1944, vol. 5, pp. 127–137.
31. Ginai, M. A., *J. Indian Bot. Soc.*, 1936, **15**, 269–284.
32. Ramakrishnan, K., *Proc. Indian Acad. Sci.*, 1955, **41**, 110–116.
33. Mehotra, B. S., *Proc. Natl. Acad. Sci.*, 1959, **29**, 94–96.
34. Mehotra, B. S. and Prasad, R., *Mycologia*, 1964, **56**, 905–908.
35. Headlee, T. J. and McColloch, J. W., *Kansas State Agri. College Bull.*, 1913, **191**, 287–353.

ACKNOWLEDGEMENTS. We thank the unknown referee for providing suggestions. The work is supported by a CSIR-SRF to A.J.K. and by a research project of MOEn to G.M.

Received 21 June 1999; revised accepted 16 September 1999

Evaluation of two *in vitro* test systems employing *Brugia malayi* parasite for prescreening of potential antifilarials

P. K. Murthy* and R. K. Chatterjee

Division of Parasitology, Central Drug Research Institute, Lucknow 226 001, India

In the present study, we employed adult worms and microfilariae of the human filarial parasite, *Brugia malayi*, in two *in vitro* systems and evaluated the suitability of the systems as prescreens for identifying potential antifilarials. A total of 38 new synthetic compounds and 19 plant products that were found active or inactive in *Acanthocheilonema viteae–Mastomys coucha* (rodent filariid in rodent host) and/or *B. malayi–M. coucha* (human filariid in rodent host) models, were tested in the *in vitro* systems using inhibition of worm motility (motility assay) and inhibition of MTT reduction potential (MTT assay) of the parasite as test parameters. Two known antifilarials, ivermectin and diethylcarbamazine, were included as standards. All (100%) the synthetic and plant products that were active in *B. malayi–M. coucha* model were also found active in the *in vitro* systems: About 82% and 20% of synthetic and plant products respectively that were active in *A. viteae–M. coucha* system were positive and 87.5% of the synthetic products and 9% of the plant products found inactive in *A. viteae–M. coucha* system were also positive in the *in vitro* systems. The results show that plant products showing LC_{100} in the range of 31.25 μg/ml to 62.5 μg/ml in the *in vitro* systems can be considered as potential antifilarials and followed-up in *in vivo* assay systems. It is concluded that the motility and MTT assays using both the life forms of *B. malayi* are reliable prescreens with high predictive value; both the assays are necessary for screening synthetic compounds whereas the motility assay using adult worms alone appears sufficient for screening plant products.

IN our antifilarial drug development programme, animal models of rodent and human filarial parasites (*Acanthocheilonema viteae* in *Mastomys coucha* and

*For correspondence. (e-mail: root@cscdri.ren.nic.in)

Brugia malayi in *M. coucha*, respectively) are currently employed as primary and secondary screens. However, these *in vivo* models require large quantities of the test compounds, are time-consuming (40–90 days of observation period before end-point determination) and are therefore uneconomical especially for primary screening of large number of new products. Attempts were made by several investigators to develop *in vitro* systems employing rodent filariids and use them as prescreens[1–8]. Among these, the motility assay and the MTT [3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay are two well-characterized *in vitro* systems that employ rodent filariids as targets[8]. Inhibition of worm motility and inhibition of the live parasite's electron transport pathway that catalyses the reduction of MTT to formazan, are the end points used in both the assays.

However, it was found that the responses shown by the rodent filariid *A. viteae* used in the systems were not comparable to those of the human filariid *B. malayi,* since subsequent *in vivo* testing using *B. malayi* did not always yield the predicted result. Therefore, in the present study, with the objective of developing and validating human filarial parasite based *in vitro* systems, we employed adult worms and microfilariae of *B. malayi* in the motility and MTT assays and evaluated the suitability and predictive value of the systems as prescreens. For this, two standard antifilarials ivermectin and diethylcarbamazine citrate (DEC), and several new products identified earlier as positive or negative in *in vivo* assays were tested in the two *in vitro* systems of *B. malayi* and the degree of concordance between the results in these *in vitro* systems and in the *A. viteae–M. coucha* and *B. malayi–M. coucha in vivo* systems was analysed. The new products included 38 synthetic compounds and 19 plant products that showed positive or negative antifilarial activity in *A. viteae–M. coucha* and/or *B. malayi–M. coucha* primary screens.

Adult worms (both sexes) and microfilariae (mf) of *B. malayi* were recovered from peritoneal cavity of the jird, *Meriones unguiculatus* harbouring 5–8-month-old infection, as described elsewhere[9]. The medium in which the adult worms and mf were washed thoroughly before use was RPMI-1640 (Sigma Chemical Co., St Louis) containing 25 mM Hepes, streptomycin (100 µg/ml) and penicillin (100 U/ml) and 10% heat inactivated foetal calf serum (Sigma Chemical Co., St. Louis). The final pH of the medium was adjusted to 7.4.

Synthetic products belonging to different chemical classes (aminothiadiazoles, 3; azalactones, 2; benzimidazoles, 2; benzopyrones, 2; β-carbolines, 16; cyclic guanidines, 2; diethylthiocarbamate, 1; hydramino alkyl ether of carbamate, 1; imidazolone, 1; polyamines, 6, pyrazole, 1 and substituted sugar, 1) and plant products were prepared at the Central Drug Research Institute (CDRI). These included two benzimidazole derivatives 82/437 and 81/504 and one plant product from the stem bark of *Streblus asper* (plant #3234), that have recently been reported to be potent antifilarials[10–12].

Synthetic test compounds and the standard antifilarials, diethylcarbamazine (DEC) and ivermectin, were dissolved in DMSO or distilled ethanol and plant products were dissolved in ethanol. The synthetic compound solutions were made up with the medium to give a final concentration of 100 µM. For plant products, serial two-fold dilutions in the medium starting from 1 mg/ml to 0.001 µg/ml were used. The final concentration of DMSO/ethanol in the incubation medium was less than 0.1%.

The motility assay of Comely *et al.*[13] was used with minor modifications and employing *B. malayi* in place of *A. viteae* as the target parasite. One male and one female adult worm were placed in 1 ml medium into each well of the sterile 24-well flat bottom culture plates (Laxbro, India) and equilibrated to 37°C for 1 h before experimentation. Test/standard substance was added at the final concentration specified above. Control wells received equal amount of vehicle only. Three replicates were set up for each test/standard or control. The plates were incubated at 37°C for 4–24 h. The worms were examined under a stereo zoom microscope (Olympus, Japan) after 1, 2, 3, 4 and 24 h of incubation and the condition of the worms was scored as active (3+), sluggish (2+), paralysed (1+) and dead (0). Female parasites were teased in medium and condition of mf in the uteri was examined to see the effect of compound on mf, if any.

The MTT assay was performed essentially as described by Comley *et al.*[13] except that *B. malayi* was used in place of *A. viteae* as the target parasite. The worms were incubated in medium containing test substance/vehicle as described above for motility assay. After 4 and 24 h of incubation, the medium was removed and the worms were incubated in a fresh medium without the test compounds for 1 h at 37°C. The worms were then gently blotted and transferred to 0.1 ml of 0.5% solution of millipore-filtered (pore size: 0.22 µm) MTT [3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; Sigma Chemical Co., St. Louis] in 0.01 M phosphate-buffered saline (pH 7.2) and incubated for 1 h at 37°C. The formazan formed was extracted in 1 ml of DMSO for 1 h at 37°C and the absorbance of the formazan was measured at 510 nm in a spectrophotometer (Spectronic 3000, Milton Roy, USA) against DMSO blank. The mean absorbance values obtained from 3 replicates were compared with controls. Inhibition in the MTT reduction potential of the parasites was calculated and expressed as per cent of control values following the method of Comley *et al.*[13].

A synthetic compound producing a motility score of 1+ or 0 and more than 25% inhibition in MTT reduction

Table 1. Activity of different categories of synthetic compounds against adult worms and microfilariae of *B. malayi* in *in vitro* assays

In-vitro assay system and *B. malayi* life form(s)	Compounds active against *A. viteae in vivo* ($n = 22$)		Compounds inactive against *A. viteae in vivo* ($n = 8$)		Compounds active against *A. viteae B. malayi in vivo* ($n = 8$)	
	No. of compounds active *in vitro* against *B. malayi**	%	No. of compounds active *in vitro* against *B. malayi*	%	No. of compounds active *in vitro* against *B. malayi*	%
Motility assay						
Male + female + mf	16	72.7	4	50	7	87.5
Male + mf	16	72.7	3	37.5	7	87.5
Female + mf	14	63.6	4	50	6	75.0
mf	6	27.3	0	0	2	25.0
Male + female	14	63.6	4	50	7	87.5
Male	14	63.6	3	37.5	7	87.5
Female	12	54.5	2	25	6	75
MTT assay						
Male + female + mf	10	45.4	4	50	4	50
Male + mf	5	22.7	3	37.5	3	37.5
Female + mf	9	40.9	3	37.5	4	50
mf	2	9.1	2	25	2	25
Male + female	8	36.4	3	37.5	2	25
Male	2	9.1	2	25	1	12.5
Female	7	31.8	2	25	2	25
Motility and/or MTT						
Male + female + mf	18	81.8	7	87.5	8	100
Male + mf	17	77.3	6	75	8	100
Female + mf	15	68.2	6	75	7	87.5
mf	8	36.4	2	25	4	50
Male + female	16	72.7	6	75	7	87.5
Male	15	68.2	4	50	7	87.5
Female	14	63.6	5	62.5	6	71.4

*Parasite forms were incubated in the medium containing 100 µmole of the test compound for 24 h and the antifilarial activity was determined as described in the article.

is considered as a positive antifilarial compound. For plant products, the concentration of the substance which produces 100% worm mortality (LC_{100}) in the motility assay and more than 25% inhibition in MTT reduction, was taken into account to categorize the substance as a positive antifilarial.

In vivo antifilarial activity of the substances was assayed in the *A. viteae–M. coucha* and *B. malayi–M. coucha* models developed and routinely used in our laboratory[14]. Test products were administered to the animals orally at standard dose levels following the protocol described elsewhere[11,12,15]. Evaluation of antifilarial efficacy was done following the method of Chatterjee *et al.*[12] and Lammler *et al.*[16].

In the present study, the choice of motility and MTT reduction potential of parasites as indicators of viability is dictated by the relative ease with which the end-points can be monitored to obtain semi-quantitative (motility assay) and quantitative (MTT assay) information. Rees and Comley[17] and Comley *et al.*[18] have reported that MTT reduction assay could be correlated with biochemical parameters related to viability of parasites. In both the assay systems, a short incubation of 4–24 h was used since prolonged *in vitro* incubation of parasites in the medium affects their viability (data not shown).

Table 1 gives the response of the two *in vitro B. malayi* systems to synthetic compounds that showed either positive or negative antifilarial activity in *A. viteae–M. coucha* and/or *B. malayi–M. coucha* systems. Of the 22 compounds which were active against *A. viteae in vivo*, 12 (54.5%) and 14 (63.6%) were positive in motility assay against female and male *B. malayi*, respectively. In MTT assay, 7 (31.8%) and 2 (9.1%) compounds were positive against female and male parasites, respectively. Against mf of *B. malayi*, 6 (27.3%) compounds were positive in motility assay and 2 (9.1%) in MTT assay.

Table 2. *In vitro* and *in vivo* antifilarial activity of plant products that were active in *A. viteae–M. coucha* model against *B. malayi*

Product code no.	Assay system	LC_{100} (µg/ml) Female worm 4 h	Female worm 24 h	Male worm 4 h	Male worm 24 h	Microfilariae 4 h	Microfilariae 24 h	Activity in *B. malayi–M. coucha* system*
4047	Motility	500	250	500	250	500	125	Negative
	MTT	>500	62.5	>500	62.5	>500	62.5	
4029	Motility	500	250	500	250	250	62.5	Negative
	MTT	>500	250	>500	15.6	>500	250	
4054	Motility	500	125	500	125	500	62.5	Negative
	MTT	>500	125	>500	125	>500	125	
3989	Motility	500	62.5	500	62.5	500	31.2	Negative
	MTT	>500	125	>500	125	>500	62.5	
3912	Motility	62.5	7.8	62.5	7.8	31.2	7.8	Positive
	MTT	>500	125	>500	125	250	62.5	
3975	Motility	500	125	500	125	250	62.5	Positive
	MTT	>500	15.6	>500	15.6	500	31.2	
4034	Motility	500	62.5	250	62.5	250	31.2	Negative
	MTT	>500	15.6	>500	62.5	>500	250	
3966	Motility	125	15.6	125	62.5	125	15.6	Positive
	MTT	250	125	250	62.5	500	62.5	
4050	Motility	500	125	500	62.5	250	62.5	Negative
	MTT	>500	62.5	>500	62.5	>500	31.2	
3234	Motility	62.5	3.9	62.5	3.9	15.6	3.9	Positive
	MTT	250	7.8	125	7.8	62.5	7.8	

*Each product was tested in a group of 4–5 *B. malayi*-infected animals. An equal number of untreated infected animals served as control.

The number of positive compounds picked up by any of the two assay systems increased (to 18 compounds, 81.8%) if adult worms of both sexes along with mf were used as targets, but not when mf was used in combination with either sex alone.

Of the 8 compounds that were inactive against *A. viteae in vivo*, 7 (87.5%) showed positive antifilarial activity in both motility and MTT assays employing *B. malayi* worms of both sexes in combination with mf. However, only 50% of the compounds were positive in either test alone. Therefore, it is clear that the *in vitro B. malayi* assay systems also identify positive activity for 87.5% of the compounds that was not detected by the *A. viteae–M. coucha* system. All the 8 compounds that were positive in the *B. malayi–M. coucha* system were positive (100%) in the *in vitro B. malayi* systems when all the three life forms of the parasite were employed. However, when adult worms of a single sex in combination with mf were employed, 7 of these compounds were positive when motility and MTT assays were considered together. Thus, while the motility assay performed better than MTT assay when adult worm of a single sex and mf were used as targets, the MTT assay could pick up only 50% of the test substances as positive even when both the sexes of worms and mf were used as targets. The standard antifilarial ivermectin showed activity in both MTT and motility assays against both the life forms of the parasite, whereas DEC showed no activity against any parasite stage in these assays. This is as expected since in man, both ivermectin and DEC are known microfilaricides with virtually no effect on adult parasite[19,20]. On the contrary, in the animal model with *B. malayi* infection, a very high concentration of ivermectin was required to kill the adult parasites[21]. In *in vitro* motility and MTT assays, ivermectin exhibited activity against both adult parasites and mf, indicating usefulness of the human parasite in these two *in vitro* systems. The lack of any effect of DEC in the present *in vitro* systems further validates the reliability of the systems, since DEC does not have any direct filaricidal activity and acts through the immune system of the host[22].

Tables 2 and 3 show the *in vitro* effect of plant materials (previously found active or inactive in *in vivo* systems) on the motility and MTT reduction potential of adult parasites (male and female) and mf of *B. malayi*. Among the 10 plant products found active in *A. viteae–M. coucha*, 2 products (#3912 and #3234) killed adult worms and/or mf of *B. malayi in vitro* in the dose range of 15.63–62.5 µg/ml within 4 h of incubation (Table 2). Increasing the incubation time to 24 h produced 4 to 16 times decrease in the LC_{100}. In all instances, the changes induced in female worms by the test substance equally affected the mf in uteri of the worms. These two products showed adulticidal activity in the *B. malayi–M. coucha* model also. Another product (#3966) active in *A. viteae–M. coucha*, however, showed LC_{100} of

Table 3. *In vitro* activity of *A. viteae–M. coucha* inactive plant products against different life forms of *B. malayi*

Product code no.	Assay system	LC_{100} (µg/ml)					
		Female worm		Male worm		Microfilariae	
		4 h	24 h	4 h	24 h	4 h	24 h
4004	Motility	500	250	500	500	500	125
	MTT	>500	>500	>500	>500	>500	500
4001	Motility	500	125	500	125	250	62.5
	MTT	>500	>500	>500	>500	500	500
3961	Motility	>500	250	>500	250	>500	31.2
	MTT	>500	>500	>500	>500	>500	250
4016	Motility	500	250	500	125	500	62.5
	MTT	>500	>500	>500	>500	500	250
4069	Motility	>500	250	500	250	>500	125
	MTT	>500	500	>500	500	>500	500
3952	Motility	500	500	500	500	500	250
	MTT	>500	>500	>500	>500	>500	500
3935	Motility	>500	250	>500	250	>500	62.5
	MTT	>500	125	>500	125	>500	125
3924	Motility	62.5	31.2	500	125	500	125
	MTT	250	125	>500	250	>500	125
3985	Motility	250	62.5	500	125	500	125
	MTT	500	250	500	250	>500	62.5

125 µg/ml at 4 h and 15.6 to 62.5 µg/ml at 24 h *in vitro* for adult parasites or mf. The LC_{100} for the remaining 7 products was between 250 and 500 µg/ml, and they were inactive also *in vivo*. With the exception of product #3234 which inhibited MTT reduction by male worms and mf at 125 µg/ml and 62.5 µg/ml within 4 h of exposure, respectively, none of the plant products showed any effect on MTT reduction potential within 4 h of incubation even at 250 µg/ml concentration. However, the dose level required to produce a 25% inhibition of MTT reduction by 24 h was between 15.6 and 250 µg/ml.

One (#3924) out of 9 products found inactive in *A. viteae–M. coucha* produced inhibition of female parasite motility at 62.5 µg/ml by 4 h and 31.25 µg/ml by 24 h (Table 3). However, male worms and mf required a much higher dose to produce the same effect at 4 or 24 h time point. The motility of worms and mf was not affected at 4 h time point by any of the test substances below 500 µg/ml concentration. At 24 h time point, however, there was reduction in motility at dose levels between 125 and 250 µg/ml. Similarly, the dose requirement for suppression of MTT reduction increased with incubation time, and excepting #3985, which inhibited MTT reduction by mf at 62.5 µg/ml, none of the substances inhibited MTT reduction by males, females or mf at concentrations below 125 µg/ml at 24 h time point. At 4 h time point, MTT reduction by all the parasite forms remained almost unaffected even at 500 µg/ml concentration.

The results show that the use of both sexes of adult parasites as well as mf enhances the utility of the *in vitro* systems and ensures that chances of missing of products with antifilarial potential will be less. Use of mf alone is not suitable since it detected only a small number of products as active. Comley *et al.*[18], using *A. viteae* in an *in vitro* system, found that a larger percentage of compounds could be picked up if both adult parasite motility and mf release from female worms were taken as parameters for evaluation of antifilarial efficacy. However, Mukherjee *et al.*[8] employing *A. viteae* reported that mf alone was sufficient for this purpose, but their comparison was limited to compounds tested in the *A. viteae–M. coucha* system only and therefore, it may not be applicable to the response in the *B. malayi–M. coucha* system. Interestingly, in the present study a very large percentage of *in vivo A. viteae* active synthetic compounds showed positive activity against *B. malayi in vitro* when both the life forms (adults of both sexes and mf) were the targets and when MTT and motility assay results were taken together. This combination of the parasite stages also increased the positivity rate of *in vivo A. viteae*-inactive compounds to a considerable extent.

Thus, the *B. malayi*-based *in vitro* systems reported and evaluated in the present study show robust predictive value as antifilarial prescreens. Both the motility and MTT assays are necessary for reliable prescreening of synthetic agents whereas the motility assay alone appears to be sufficient for plant products. In either case, however, use of both the life-stages of the parasite, namely mf and adults of *B. malayi*, is preferred to a single life-stage. The optimum duration of drug exposure in these test systems was found to be 4 and 24 h for plant and synthetic products, respectively. Very high

percentage of negative response (75–87.5%) was given by MTT assay when male or female worms were used in the test whereas very small number (12.5–25%) of compounds showed negative response in the motility assay with male or female parasites as targets. When male and/or female adults plus mf were used as targets, both the assays increased the detection rate (positivity rate) to a great extent (up to 87.5%). The present study also reveals that those compounds that show LC_{100} at or below 62.5 μg/ml in *in vitro* assay systems can be considered as potential agents for follow-up in *in vivo* assay system.

In conclusion, the predictive value of the *in vitro* systems using the human parasite *B. malayi* was found to be very high as all (100%) the synthetic and plant products that were active in the *B. malayi–M. coucha* model were also found active in the *in vitro* systems. Moreover, 87.5% of the synthetic products and 9% of the plant products which were inactive in the *A. viteae–M. coucha* system were also picked up by the present *in vitro* systems as positive indicating that there are fewer chances of missing the activity of potential antifilarials in the *B. malayi in vitro* systems. These findings clearly show that the *B. malayi*-based MTT and motility assays can be employed as reliable prescreens for evaluating antifilarial activity of new products.

1. Kobayashi, J., Matsuda, H., Fujita, K., Sakai, T. and Shinoda, K., *Jpn. J. Parasitol.*, 1969, **18**, 563–574.
2. Moreau, J. P. J. and Qutin-Fabre, D., *Bull. Soc. Pathol. Exotol.*, 1972, **65**, 93.
3. Moreau, J. P. J. and Qutin-Fabre, D., *Variete Pacifica*, 1972, **65**, 93–98.
4. Denham, D. A., Suswillo, R. R., Rogers, R. and Nelson, G. S., *Trans. R. Soc. Trop. Med. Hyg.*, 1978, **72**, 615–618.
5. Denham, D. A., *J. Helminthol.*, 1979, **53**, 173–187.
6. Jenkins, D. C. and Court, J. P., WHO document TDR/FIL/SWG (5)/80, Working paper No. 8, 1980.
7. Singh, D. P., Mishra, S. and Chatterjee, R. K., *Jpn. J. Exp. Med.*, 1990, **60**, 303–309.
8. Mukherjee, M., Mişra, S. and Chatterjee, R. K., *Acta Trop.*, 1998, **70**, 251–255.
9. Murthy, P. K., Murthy, P. S. R., Tyagi, K. and Chatterjee, R. K., *Folia Parasitol.*, 1997, **44**, 302–304.
10. Abuzar, Sharma, S., Fatma, N., Gupta, S., Murthy, P. K., Katiyar, J. C., Chatterjee, R. K. and Sen, A. B., *J. Med. Chem.*, 1986, **29**, 1296–1299.
11. Fatma, N., Sharma, S. and Chatterjee, R. K., *Acta Trop.*, 1989, **46**, 311–321.
12. Chatterjee, R. K., Fatma, N., Murthy, P. K., Sinha, P., Kulshreshtha, D. K. and Dhawan, B. N., *Drug Dev. Res.*, 1992, **26**, 67–78.
13. Comley, J. C. W., Rees, M. J., Turner, C. H. and Jenkins, D. C., *Int. J. Parasitol.*, 1989, **19**, 77–83.
14. Murthy, P. K., Tyagi, K., Roy Chowdhury, T. K. and Sen, A. B. *Indian J. Med. Res.*, 1983, **77**, 623–630.
15. Tyagi, K., Murthy, P. K. and Chatterjee, R. K., *Indian J. Parasitol.*, 1986, **10**, 195–205.
16. Lammler, G., Herzog, H. and Schultze, H. R., *Bull. WHO*, 1971, **44**, 751–756.
17. Rees, M. J. and Comley, J. C. W., *Trop. Med. Parasitol.*, 1988, **39**, 80–81.
18. Comley, J. C. W., Townson, S., Rees, M. J. and Dobinson, A., *Trop. Med. Parasitol.*, 1989, **40**, 311–316.
19. Ottesen, E. A., *Rev. Infect. Dis.*, 1985, **7**, 341–356.
20. Kumaraswami, V., Ottesen, E. A., Vijayasekaran, V., Umadevi, S., Swaminathan, M. D., Aziz, M. A., Sarma, G. R., Prabhakar, R. and Tripathy, S. P., *J. Am. Med. Assoc.*, 1988, **259**, 3150–3153.
21. Fatma, N., Murthy, P. K. and Chatterjee, R. K., in *Molecular Biology and Control Strategies* (eds Sushil Kumar, Sen, A. K., Dutta, G. P. and Sharma, R. N.), Publications and Information Directorate, New Delhi, 1994, pp. 55–64.
22. Misra, S., Singh, D. P., Murthy, P. K. and Chatterjee, R. K., *Trop. Med.*, 1990, **32**, 33–43.

ACKNOWLEDGEMENTS. We thank Dr C. M. Gupta, Director, CDRI, for providing necessary facilities for carrying out the studies. Thanks are due to Mr V. K. Bose and Mr R. C. Rai for technical assistance.

Received 4 April 1998; revised accepted 27 August 1999

Myxobacterial diversity of Indian soils – How many species do we have?

Milind G. Watve,* Anita M. Shete, Nalini Jadhav, Shilpa A. Wagh, Sheetal P. Shelar, Sudeshna S. Chakraborti, Ashwini P. Botre and Ajit A. Kulkarni

Department of Microbiology, Abasaheb Garware College, Karve Road, Pune 411 004, India

Myxobacteria of tropical soils is an under-explored bacterial group. We report here the results of sampling in Pune district of Western Ghats. A number of novel morphotypes were found in forest as well as urban/semi-urban soils. There was a high level of floral dissimilarity between habitats. The morphotypes detected in Pune district also differed from the northern Indian species recorded earlier. Using a species individual curve on the Pune, Lucknow and pooled data, we try to estimate the number of species that are likely to be present in India. A plausible estimate is several fold higher than the species recorded worldwide so far.

MICROBIAL diversity is one of the difficult areas of biodiversity research. A number of studies[1–8] have made it clear that the number of species of bacteria known to science is only a tip of the iceberg. Majority of bacterial species have not been studied and described, either because they are unculturable by the conventional methods, or because we just have not explored enough. There is evidence that a large number of bacterial species are

*For correspondence (e-mail: watve@pn2'.vsnl.net.in)