Topical Studies in Oceanography, 2001, vol. 48, pp. 3353–3361.
13. Mahtani, S. and Mavinkurve, S., *J. Ferment. Technol.*, 1979, **57**, 529–533.
14. De Mora, S. J. and Pelltier, E., *Environ. Technol.*, 1997, **18**, 1169–1177.
15. Chau, Y. K., Maguire, R. J., Brown, M., Yang, F. and Batchelar, S. P., *Water Qual. Res. J. (Canada)*, 1997, **32**, 453– 521.
16. De Mora, S. J., Stewart, C. and Phillips, D., *Mar. Pollut. Bull.*, 1995, **30**, 50–57.
17. Krieg, N. R. and Holt, J. G., *Bergey's Manual of Systemic Bacteriology*, The Williams & Wilkins Co, Baltimore, USA, 1984, vol. 1.
18. Goodfellow, M., Numerical taxonomy. In *CRC Hand Book of Microbiology, Vol-1. Bacteria* (eds Laskin, A. I. and Lechevalier, A. H.), CRC Press, Ohio, USA, 1977, 2nd edn, pp. 579–596.
19. Barkay, T. and Pritchard, H., *Microb. Sci.*, 1988, **5**, 165–169.
20. Dubey, S. K. and Roy, U., *Appl. Organometal. Chem.*, 2003, **17**, 1–6.
21. Singh, K., *Frontiers in Applied Microbiology* (eds Mukherjee, K. G., Singh, V. P. and Garg, K. L.), Publication Print House, New Delhi, 1987, vol. II, pp. 297–316.
22. White, J. S., Tobin, J. M. and Cooney J. J., *Can. J. Microbiol.*, 1999, **45**, 541–554.
23. Gray, B. H., Porvaznik, M., Flemming, C. and Lee, L. H., *Toxicology*, 1987, **47**, 35–47.
24. Wuertz, S., Miller, C. E., Pfister, R. M. and Cooney, J. J., *Appl. Environ. Microbiol.*, 1991, **57**, 2783–2789.
25. Gadd, G. M., *FEMS Microbiol. Rev.*, 1993, **11**, 297–316.
26. Barug, D., *Chemosphere*, 1981, **10**, 1145–1154.
27. Dowson, P. H., Bubb, J. M. and Lester, J. N., *Estuarine Coastal Shelf. Sci.*, 1996, **42**, 551–562.
28. Fukagawa, T., Konno, S., Takama, K. and Suzuki, S., *J. Mar. Biotechnol.*, 1994, **1**, 211–214.
29. Suzuki, S., Fukagawa, T. and Takama, K., *Appl. Environ. Microbiol.*, 1992, **58**, 3410–3412.
30. Suzuki, S. and T. Fukagawa., *J. Ind. Microbiol.*, 1995, **14**, 154–158.

ACKNOWLEDGEMENT. We thank DOD, Govt of India, New Delhi for financial support.

Received 5 March 2003; revised accepted 11 October 2003

Mass multiplication of AM inoculum: Effect of plant growth-promoting rhizobacteria and yeast in rapid culturing of *Glomus mosseae*

S. N. Bhowmik[†,*] and C. S. Singh

Division of Microbiology, Indian Agricultural Research Institute, New Delhi 110 012, India
[†]Present address: Department of Plant Pathology, College of Horticulture and Forestry, Pasighat 791 102, India

The efficiency of plant-growth-promoting rhizobacteria (PGPR), viz. *Azospirillum* sp., *Azotobacter chroococcum*, *Pseudomonas fluorescens*, *Pseudomonas striata* and yeast, viz. *Saccharomyces cerevisiae* was evaluated for maximization of *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe root colonization and spore number in the root zone of Rhodes grass (*Chloris gayana* Kunth). The pot culture experiment was carried out under polyhouse condition and observations were recorded at 45 days and 90 days of plant growth. The PGPR considerably enhanced mycorrhizal colonization compared to yeast, with *Azospirillum* sp. being the most efficient. They not only stimulated AM development, but also accelerated the root growth.

ARBUSCULAR mycorrhizal (AM) fungi are obligate symbionts and are grown in association with living tissues[1]. Several culture techniques based on this constraint are applicable for commercial scale production of the inoculum. The most widely used is pot culture, where the fungi are usually maintained and multiplied in conjunction with suitable host plant roots[2]. Related approaches, viz. soilless culture, hydroponic culture, aeroponic culture, and axenic root organ culture techniques are well reviewed[3]. These are two-member (plant and fungus) systems, technically feasible and hold commercial potential. But importantly, all of them involve extended culture periods of several months, making AM inoculants relatively expensive to produce[4]. As such, development of rapid and more efficient culture systems remains an important challenge for commercialization. Synergistic effects of AM fungi and plant-growth-promoting rhizobacteria (PGPR)[5] and yeast[6] on root colonization and subsequent sporulation have been documented. The term PGPR is now applied to a wide spectrum of strains that have, in common, the ability to promote the growth of plants following inoculation onto seeds and subterranean plant parts[7]. The present investigation has been undertaken with a view to explore the possible use of PGPR and yeast to maximize AM fungal root colonization and sporulation in a short period. Uninoculated culture media and cell-free supernatants of the respective organisms were also included to assess the potential of the microbial whole cell.

A culture of *Glomus mosseae* (Nicol. and Gred.) Gred. and Trappe[8] was obtained from Native Plant Institute, Salt Lake City, Utah, USA and maintained as a pure stock-plant culture in pots containing sterilized soil and sand (3 : 1)[9] using Rhodes grass (*Chloris gayana* Kunth) as a host for four months. Spores were collected by wet-sieving and decanting the root zone soil from ten different pots and were mixed to form a composite sample[10]. Healthy pale yellow-brown coloured spores were selected with the aid of stereomicroscope and were surface sterilized in chloramine-T (2% w/v) for 20 min and rinsed 3–4 times in sterile deionized water[11].

The test microbes included were two diazotrophic and two non-N_2-fixing bacterial strains of PGPR and a yeast. Two diazotrophic bacteria, viz. *Azospirillum* sp. R.v.zae grown on Okon's broth[12] as modified by Lakshmi Kumari *et al.*[13] and *Azotobacter chroococcum* M5 grown on Jensen's N-free broth[14] isolated from sporocarp of the ectomycorrhizal fungus (*Rhizopogan vinicolor*) and rhizosphere

*For correspondence. (e-mail: snb70@yahoo.co.in)

of mustard respectively, were taken for the present study. The two non-N_2-fixing bacterial strains and one yeast culture included were *Pseudomonas fluorescens* (antifungal rhizobacteria) grown on King's B broth[15] and *Pseudomonas striata* (P-solubilizing bacteria) grown on Pikovskaya's broth[16] and *Saccharomyces cerevisiae* grown on malt extract broth isolated from roots of birch, rhizosphere of cowpea and sporocarp of unidentified mycorrhiza respectively. The antifungal fluorescent pseudomonad though detrimental to pathogenic fungi[17], consistently promotes AM establishment[18] and was hence included in our study. *Azospirillum* sp. R.v.zae was obtained by courtesy of J. Dobereiner, Embrapa Agrobiology, Rio de Janeiro, Brazil. *P. fluorescens* and *S. cerevisiae* were kindly provided by C. Y. Li, USDA, Corvallis, Oregon, USA, while *A. chroococcum* M5 and *P. striata* were procured from the culture collections existing in the Division of Microbiology, Indian Agricultural Research Institute (IARI), New Delhi. The organisms were grown in respective media (50 ml) in 250 ml conical flasks in a shaking incubator. To achieve a final concentration of 10^8 cells ml^{-1}, the bacterial cultures were incubated at $28 \pm 2°C$ for 1 day (*Azospirillum*), 3 days (*Azotobacter*), 5 days (*P. fluorescens*), and 7 days (*P. striata*), while *S. cerevisiae* was incubated at $23 \pm 2°C$ for 3 days. A similar set of the microbial cultures was also prepared side by side to get 50 ml cell-free supernatants of respective organisms. The cell-free supernatants were obtained by centrifuging the microbial cultures at 5000 *g* for 30 min and further passing the supernatants through nitrocellulose filter (0.22 μm pore diameter). Fifty millilitre of uninoculated culture media of the microorganisms was attained by autoclaving at 15 psi for 15 min. Three preparations, viz. complete microbial culture (= C), cell-free supernatant (= S), uninoculated culture medium (= U) were thus obtained.

Seeds of Rhodes grass were obtained from the Division of Agronomy, IARI. The grass seeds were surface sterilized with 0.01% $HgCl_2$ for 4 min, washed ten times with sterile deionized water and dried on blotting paper under sterile condition.

AM root colonization and sporulation studies were carried out in sanitized (1.5% Sumabac sanitizer) plastic pots (6″ diameter) containing sterilized soil and sand (3 : 1)[9] @ 2 kg pot^{-1}. Sandy loam soil (2 mm mesh size) from IARI farm having 0.52% total organic carbon, 0.04% total nitrogen, 5 ppm $NaHCO_3^-$ extractable P, 1.5% moisture and 6.5–7.5 pH was used. The potting soil at 1 cm depth was removed from each pot and mixed thoroughly with microbial preparations (50 ml) of different organisms separately and left for light drying. Twenty holes (approximately 0.5 cm wide and 1 cm deep) were made on the surface of the soil in each pot and 50 sterile *G. mosseae* spores were pipetted into each hole followed by sowing of one sterile Rhodes grass seed. The open

Table 1. Effect of microbial preparations of PGPR and yeast (U uninoculated culture medium, S cell-free supernatant, C complete microbial culture) on root volume, shoot and root dry weight in Rhodes grass with and without AM at two harvests. Mean values of three replicates (ten plants per pot) are given. Means sharing a letter are not significantly different according to Duncan's test ($P < 0.05$)

	First harvest						Second harvest					
	Without AM			With AM			Without AM			With AM		
Treatment	Root volume (ml pot^{-1})	Shoot dry weight (g pot^{-1})	Root dry weight (g pot^{-1})	Root volume (ml pot^{-1})	Shoot dry weight (g pot^{-1})	Root dry weight (g pot^{-1})	Root volume (ml pot^{-1})	Shoot dry weight (g pot^{-1})	Root dry weight (g pot^{-1})	Root volume (ml pot^{-1})	Shoot dry weight (g pot^{-1})	Root dry weigh (g pot^{-1})
Control	0.54^{e}	0.12^{e}	0.16^{f}	0.90^{h}	0.23^{i}	0.25^{g}	6.34^{f}	1.11^{h}	1.13^{g}	7.15^{f}	3.19^{g}	2.80^{g}
Azospirillum sp.												
U	2.05^{cd}	0.50^{bcd}	0.40^{cd}	3.10^{g}	0.82^{gh}	0.92^{de}	7.15^{e}	3.80^{f}	3.58^{e}	7.67^{e}	4.91^{e}	4.20^{e}
S	3.36^{b}	0.66^{ab}	0.49^{bc}	4.88^{b}	1.63^{bc}	1.14^{bc}	8.50^{c}	4.78^{b}	4.15^{c}	9.45^{b}	5.60^{c}	5.73^{b}
C	4.01^{a}	0.73^{a}	0.60^{a}	5.55^{a}	1.89^{a}	1.43^{a}	10.15^{a}	5.05^{a}	5.09^{a}	12.05^{a}	6.88^{a}	7.01^{a}
A. chroococcum												
U	2.00^{cd}	0.54^{bcd}	0.44^{bcd}	3.30^{efg}	0.97^{fg}	1.10^{cd}	7.26^{e}	3.86^{ef}	3.92^{d}	8.16^{d}	4.07^{f}	4.88^{d}
S	2.30^{cd}	0.58^{abc}	0.48^{bc}	3.84^{d}	1.48^{cd}	1.14^{bc}	7.89^{d}	4.55^{c}	4.00^{d}	8.99^{c}	5.38^{cd}	5.56^{bc}
C	3.85^{a}	0.63^{abc}	0.50^{abc}	5.36^{a}	1.71^{abc}	1.35^{a}	9.95^{b}	4.85^{b}	4.79^{b}	11.88^{a}	6.69^{ab}	6.85^{a}
P. fluorescens												
U	1.86^{d}	0.39^{d}	0.24^{ef}	3.22^{fg}	0.70^{h}	0.58^{f}	6.38^{f}	3.20^{g}	3.16^{f}	7.28^{f}	3.29^{g}	3.86^{f}
S	2.11^{cd}	0.54^{bcd}	0.40^{cd}	3.51^{e}	1.28^{de}	0.94^{de}	7.86^{d}	4.25^{d}	3.89^{d}	8.85^{c}	5.20^{d}	5.37^{c}
C	3.89^{a}	0.65^{abc}	0.53^{ab}	5.38^{a}	1.74^{ab}	1.36^{a}	9.99^{ab}	4.88^{b}	4.83^{b}	11.91^{a}	6.71^{ab}	6.87^{a}
P. striata												
U	2.07^{cd}	0.48^{cd}	0.32^{de}	3.46^{ef}	0.80^{gh}	0.82^{e}	6.42^{f}	3.78^{f}	3.55^{e}	7.30^{f}	3.35^{g}	3.97^{ef}
S	2.45^{c}	0.52^{bcd}	0.42^{bcd}	4.27^{c}	1.19^{ef}	0.98^{cde}	7.11^{e}	4.00^{e}	3.97^{d}	8.35^{d}	3.82^{f}	4.09^{ef}
C	3.82^{ab}	0.62^{abc}	0.51^{abc}	5.35^{a}	1.70^{abc}	1.34^{a}	9.94^{b}	4.83^{b}	4.79^{b}	11.87^{a}	6.66^{ab}	6.83^{a}
S. cerevisiae												
U	0.55^{e}	0.15^{e}	0.18^{f}	0.92^{h}	0.25i	0.26^{g}	6.36^{f}	1.14^{h}	1.17^{g}	7.18^{f}	3.20^{g}	2.82^{g}
S	0.57^{e}	0.15^{e}	0.19^{f}	0.95^{h}	0.25i	0.27^{g}	6.37^{f}	1.15^{h}	1.17^{g}	7.16^{f}	3.20^{g}	2.83^{g}
C	0.59^{e}	0.16^{e}	0.19^{f}	5.35^{a}	1.70^{abc}	1.33^{ab}	6.40^{f}	1.15^{h}	1.19^{g}	11.86^{a}	6.64^{b}	6.82^{a}

seeded holes were then covered evenly with the microbial-preparation mixed soils to give rise to individual treatments. The pot registering for control treatment was devoid of microbial preparation. The batch of non-AM experimental pots received similar treatments, only without *G. mosseae* spores. After germination, ten plants per pot were maintained. The pot culture experiment was set in a semi-sterile environment in a polyhouse during March–May, where the plants received an average of 8 h daylight with a mean ambient temperature of 30.8°C. The pots were irrigated with sterile tap water throughout the experiment. The experiment was a completely randomized design, each treatment replicated six times, i.e. three replicates for the first harvest [45 days after sowing (DAS)] and three replicates for the second harvest (90 DAS). The data were analysed statistically using ANOVA and significant differences between treatments were separated by Duncan's test ($P \leq 0.05$).

At each harvest the volume of root and dry weight of shoot and root per pot were determined by the procedure mentioned by Singh[19]. Mycorrhizal colonization of roots in terms of per cent infection was measured according to Phillips and Hayman[20]. Spores from 25 g of root-zone soil samples were collected by wet sieving and decanting technique, and their number was counted[10,21].

It is evident from data (Tables 1 and 2) that all the treatments increased the per cent mycorrhizal infection and number of spores, besides the plant parameters (comprising root volume, shoot dry weight and root dry weight) compared to control in AM-inoculated plants at the first harvest. A further increment in all parameters was also noticed in the second harvest. Among the three different preparations used in the investigation, complete microbial cultures (C) increased all the parameters studied, while uninoculated culture media (U) responded the least. It is apparent from Table 1 that all complete microbial cultures except yeast stimulated the root growth in non-mycorrhizal plants. Table 2 reveals that AM culture in conjunction with all the organisms (at 45 DAS) has been able to yield equal or even more amount of infected roots and spores than singly cultured AM (at 90 DAS). This suggests the possible reduction of AM culturing period by 45 days compared to its usual three-month pot culturing[4]. The complete microbial cultures of PGPR considerably enhanced mycorrhizal colonization compared to yeast, *Azospirillum* enhancing it to the maximum followed by *P. fluorescens*, *A. chroococcum*, *P. striata* (Table 2). High root colonization also improved AM spore number, since these two phenomena are often closely related[22]. However, no significant ($P \leq 0.05$) differences between

Table 2. Effect of microbial preparations of PGPR and yeast (U uninoculated culture medium, S cell-free supernatant, C complete microbial culture) on mycorrhizal root colonization and sporulation in Rhodes grass at two harvests. Mean values of three replicates are given. For per cent root infection – fifty root sections scored per pot; spore number – 25 g soil sieved per pot. Means sharing a letter are not significantly different according to Duncan's test ($P < 0.05$)

	First harvest				Second harvest			
	Without AM		With AM		Without AM		With AM	
Treatment	Per cent root infection	Spore number	Percent root infection	Spore number	Per cent root infection	Spore number	Per cent root infection	Spore number
Control	00.00	00.00	22.0^{g}	123.0^{j}	00.00	00.00	45.0^{f}	183.0^{f}
Azospirillum sp.								
U	00.00	00.00	25.0^{fg}	130.0^{i}	00.00	00.00	49.0^{def}	198.0^{e}
S	00.00	00.00	52.0^{bcd}	190.0^{d}	00.00	00.00	59.0^{b}	223.0^{d}
C	00.00	00.00	58.0^{a}	202.0^{a}	00.00	00.00	94.0^{a}	710.0^{a}
A. chroococcum								
U	00.00	00.00	30.0^{ef}	139.0^{h}	00.00	00.00	58.0^{bc}	218.0^{d}
S	00.00	00.00	50.0^{cd}	183.0^{e}	00.00	00.00	55.0^{bcd}	240.0^{c}
C	00.00	00.00	55.0^{abc}	195.0^{bcd}	00.00	00.00	91.0^{a}	696.0^{b}
P. fluorescens								
U	00.00	00.00	24.0^{fg}	128.0^{ij}	00.00	00.00	49.0^{def}	193.0^{ef}
S	00.00	00.00	46.0^{d}	176.0^{f}	00.00	00.00	52.0^{cde}	218.0^{d}
C	00.00	00.00	56.0^{ab}	198.0^{ab}	00.00	00.00	92.0^{a}	701.0^{ab}
P. striata								
U	00.00	00.00	23.0^{g}	126.0^{ij}	00.00	00.00	46.0^{ef}	190.0^{ef}
S	00.00	00.00	32.0e	150.0^{g}	00.00	00.00	50.0^{def}	192.0^{ef}
C	00.00	00.00	55.0^{abc}	196.0^{bc}	00.00	00.00	89.0^{a}	694.0^{b}
S. cerevisiae								
U	00.00	00.00	24.0^{fg}	126.0^{ij}	00.00	00.00	47.0^{ef}	186.0^{f}
S	00.00	00.00	24.0^{fg}	127.0^{ij}	00.00	00.00	47.0^{ef}	188.0^{ef}
C	00.00	00.00	54.0^{abc}	193.0^{cd}	00.00	00.00	89.0^{a}	694.0^{b}

the PGPR complete culture treatments were present at most, if not all instances. A similar observation has been made by earlier workers[23–26].

Supernatants of bacterial strains improved all parameters studied compared to uninoculated treatment (Tables 1 and 2). Tilak *et al.*[27] reported the effect of whole cell culture and cell-free extracts of *Azospirillum* and *Azotobacter* on AM spore germination *in vitro* with similar results. This indicates the involvement of water-soluble, diffusible substances of microbial origin as suggested by Azcon-Aguilar *et al.*[28]. Besides fixing atmospheric nitrogen, PGPR are also known to synthesize considerable quantities of biologically active substances in the rhizosphere[27,29]. Among these are phytohormones (IAA and GA) which increase the root biomass[30], AM spore germination and hyphal elongation[27,29] which in turn accelerates the AM root colonization[31]. Here, we state a plausible explanation that accounts for the superiority of *Azospirillum* sp. complete culture among all organisms in the study. The associative symbiont may have thrived well and performed efficiently within Rhodes grass root. Despite its high potential on AM-inoculated plants, complete cells of *S. cerevisiae* continued to have negligible effect on non-mycorrhizal plants. Thus it is inferred that the yeast specifically stimulated the AM development rather than the host plant, which is consistent with the suggestions of Larsen and Jakobsen[32]. Overall, the foreign cultures had well acclimatized to local conditions and yielded good results. The partially uniform soil, water, temperature and light regime throughout the experiment in an automatically controlled polyhouse may be a plausible explanation for this[19,26].

Promoting high levels of mycorrhizal development on young, rapidly growing plants is an important challenge in commercial use of mycorrhizal inoculants[4]. Thus, from the above findings it can be concluded that PGPR and yeast can be used in the mass production of AM fungal cultures.

1. Al-Raddad, A., Mass production of *Glomus mosseae* spores. *Mycorrhiza*, 1995, **5**, 229–231.
2. Ferguson, J. J. and Woodhead, S. H., Production of endomycorrhizal inoculum. An increase and maintenance of vesicular–arbuscular mycorrhizal fungi. In *Methods and Principles of Mycorrhizal Research* (ed. Schenck, N. C.), APS Press, St. Paul, M.N., 1982, pp. 47–54.
3. Jarstfer, A. G. and Sylvia, D. M., Inoculum production and inoculation strategies for vesicular–arbuscular mycorrhizal fungi. In *Soil Microbial Ecology: Applications in Agricultural and Environmental Management* (ed. Metting, Jr., F. B.), Marcel Dekker, New York, 1992, pp. 349–377.
4. Wood, T. E., VA mycorrhizal fungi: challenges for commercialization. In *Handbook of Applied Mycology, Biotechnology* (eds Arora, D. K., Mukerji, K. G. and Elander, R. P.), Marcel Dekker, New York, 1991, vol. 4, pp. 823–847.
5. Linderman, R. G. and Paulitz, T. C., Mycorrhizal–rhizobacterial interactions. In *Biological Control of Soil-Borne Plant Pathogens* (eds Hornby, D. *et. al.*), CAB International, Wallingford, Oxon, UK, 1990, pp. 261–283.
6. Singh, C. S., Kapoor, A. and Wange, S. S., The enhancement of root colonization of legumes by vesicular–arbuscular mycorrhizal (VAM) fungi through the inoculation of the legume seed with commercial yeast (*Saccharomyces cerevisiae*). *Plant Soil*, 1991, **131**, 129–133.
7. Kloepper, J. W., Lifshitz, R. and Schroth, M. N., *Pseudomonas* inoculants to benefit plant production. In *ISI Atlas of Science*, Institute for Scientific Information, Philadelphia, 1988, pp. 60–64.
8. Gerdemann, J. W. and Trappe, J. M., The Endogonaceae in the Pacific Northwest. *Mycol. Mem.*, 1974, **5**, 1–76.
9. Barea, J. M., Bonis, A. F. and Olivares, J., Interactions between *Azospirillum* and VA mycorrhiza and their effects on growth and nutrition of maize and ryegrass. *Soil Biol. Biochem.*, 1983, **15**, 705–709.
10. Gerdemann, J. W. and Nicolson, T. H., Spores of mycorrhizal *Endogone* extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.*, 1963, **46**, 235–244.
11. Mosse, B., The establishment of vesicular–arbuscular mycorrhizae under aseptic conditions. *J. Gen. Microbiol.*, 1962, **27**, 509–520.
12. Okon, Y., Albercht, S. L. and Burris, R. H., Methods of growing *Spirillum lipoferum* for counting it in pure cultures in association with plants. *Appl. Environ. Microbiol.*, 1977, **33**, 85.
13. Lakshmi Kumari, M., Lakshmi, V., Nalini, P. A. and Subba Rao, N. S., Reactions of *Azospirillum* to certain dyes and their usefulness in enumeration of the organism. *Curr. Sci.*, 1980, **49**, 438–439.
14. Jensen, H. L., Nitrogen fixation in leguminous plants II. In symbiotic nitrogen fixation influence by *Azotobacter*. *Proc. Linn. Soc. N. S. W.*, 1942, **57**, 205–212.
15. King, E. P. and Raney, D. E., A simple media for the demonstration of pyocyanin and fluorescin. *J. Lab. Clin. Med.*, 1954, **44**, 301–307.
16. Pikovskaya, R. I., Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Mikrobiologia*, 1948, **17**, 362.
17. Bakker, P. A., Lamers, J. G., Marugg, A. W., Weisback, P. J. and Schippers, B., The role of siderophores in potato tuber yield increase by *Pseudomonas putida* in short rotation of potato. *NJPP*, 1986, **92**, 249–256.
18. Paulitz, T. C. and Linderman, R. G., Interactions between VA mycorrhizal fungi and fluorescent pseudomonads in the rhizosphere. *New Phytol.*, 1989, **113**, 37–45.
19. Singh, C. S., Mass inoculum production of vesicular–arbuscular (VA) mycorrhizae: I. Selection of host in the presence of *Azospirillum brasilense*. *Zentralbl. Mikrobiol.*, 1992, **147**, 447–453.
20. Phillips, J. M. and Hayman, D. S., Improved procedure for clear staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.*, 1970, **55**, 158–161.
21. Bagyaraj, D. J. and Manjunath, A., Selection of suitable host for mass production of VA-mycorrhizal inoculum. *Plant Soil*, 1980, **55**, 495–498.
22. Hayman, D. S., *Endogone* spore numbers in soil and vesicular–arbuscular mycorrhiza in wheat as influenced by season and soil treatment. *Trans. Br. Mycol. Soc.*, 1970, **54**, 53–63.
23. Oliveira, E., Sieverding, E. and Toro, S., Interaction between three species of VAM fungi and one isolate of *Pseudomonas putida* on cassava.. In Proceedings of the 7th North American Conference on Mycorrhizae (eds Sylvia, D. M., Hung, L. L. and Graham, J. H.), University of Florida, Gainesville, 1987, p. 216.
24. Tilak, K. V. B. R. and Singh, C. S., Response of pearl millet (*Pennisetum americanum*) to inoculation with vesicular–arbuscular mycorrhizae and *Azospirillum brasilense* with different sources of phosphorus. *Curr. Sci.*, 1988, **57**, 43–44.
25. El-Shanshoury, A. R., Hassan, M. A. and Ablel-Ghaffar, B. A., Synergistic effects of VAM and *Azotobacter chroococcum* on the growth and nutrient contents of tomato-plants. *Phyton*, 1989, **29**, 203–212.

26. Singh, C. S., Mass inoculum production of vesicular–arbuscular (VA) mycorrhizae: II. Impact of N_2-fixing and P-solubilizing bacterial inoculation of VA-mycorrhiza. *Zentralbl. Mikrobiol.*, 1992, **147**, 503–508.
27. Tilak, K. V. B. R., Dwivedi, A. and Li, C. Y., Enhancement of spore germination of *Glomus fasciculatum* by cell-free extracts. *Indian J. Exp. Biol.*, 1990, **28**, 373–375.
28. Azcon-Aguilar, C., Diaz-Radriguez, R. M. and Barea, J. M., Effect of soil microorganism on spore germination and growth of the vesicular–arbuscular mycorrhizal fungus *Glomus mosseae*. *Trans. Br. Mycol. Soc.*, 1986, **86**, 337–340.
29. Azcon, R., Germination and hyphal growth of *Glomus mosseae in vitro*: effects of rhizosphere bacteria and cell-free culture media. *Soil Biol. Biochem.*, 1987, **19**, 417–419.
30. Dewan, G. K. and Subba Rao, N. S., Seed inoculation of *Azospirillum brasilense* and *Azotobacter chroococcum* and the root biomass of rice (*Oryza sativa* L.). *Plant Soil*, 1979, **53**, 295–302.
31. Singh, C. S., Enhanced formation of vesicles, arbuscules and spores through inoculation of grass with *Azospirillum brasilense and Glomus macrocarpum*. In Mycorrhiza Round Table, Proceedings of a National Workshop, JNU, Delhi, 1987, p. 334.
32. Larsen, J. and Jakobsen, I., Interaction between a mycophagous collembola, dry yeast and the external mycelium of arbuscular mycorrhizal fungus. *Mycorrhiza*, 1996, **6**, 259–264.

ACKNOWLEDGEMENTS. S.N.B. thanks IARI, New Delhi for the award of research fellowship and the Head, Division of Microbiology, IARI, for providing facilities. Assistance of Mrs Soma Nath Bhowmik during preparation of manuscript is acknowledged.

Received 3 July 2003; revised accepted 6 November 2003

Excavation of a unique sailboat at Kadakkarappally, Kerala

M. V. Nair[†], V. Selvakumar[#,*] and P. K. Gopi[‡]

[†]National Museum Institute, New Delhi 110 001, India
[#]Centre for Heritage Studies, Hill Palace, Tripunithura 682 301, India
[‡]Hill Palace Museum, Tripunithura 682 301, India

Recent archaeological excavations at Kadakkarappally, Kerala have exposed an interesting sailboat in a partly intact condition. The method of construction adopted in the boat, which has no known parallels, and the well-preserved condition of the wood, considering the hostile environmental conditions prevailing in India, make this a unique find. The preliminary findings of the excavations are presented here.

KADAKKARAPPALLY is a sleepy village lying about 1.5 km from the Arabian Sea coast, around 30 km south of Kochi near Taikkal, Cherthala Taluk, Alappuzha District, Kerala (Figure 1). This part of Kerala is well known for backwaters and canals. The Vembanadu Lake, which separates the Alappuzha land-stretch from the mainland, opens to the Arabian Sea close to Kochi (Cochin).

Existence of the boat was first brought to notice by the local people, who dug a paddy field for coconut cultivation in the 1990s. It is reported that the stern end of the boat was destroyed by the local people who dug up this area, expecting some treasure. Subsequent explorations by the Department of Archaeology, Government of Kerala, have ascertained the existence of the boat and excavations were conducted in 2002 and 2003 by the Centre for Heritage Studies, Tripunithura and the Department of Archaeology, Government of Kerala. Two trenches (KPY I and KPY II) were excavated at this site – KPY I (30 m × 12 m) revealed the boat and KPY II (10 m × 5 m) was excavated to study the local stratigraphy (Figure 2). Since the site was completely waterlogged, the excavation progressed slowly and pumps were used to drain the water during the excavations.

The intact portion of the double-mast, flat-bottomed sailboat measures about 18.70 m in length and 4.05 m in width (Figure 3 *a* and *b*). The double-hulled boat, which is divided into 11 compartments, has a pointed bow, and the stern and upper parts of the hull are damaged. The boat has its maximum width amidships. The shape of the stern is not clear, but it appears to have had a transom. The wooden pieces that formed the stern end are no longer in

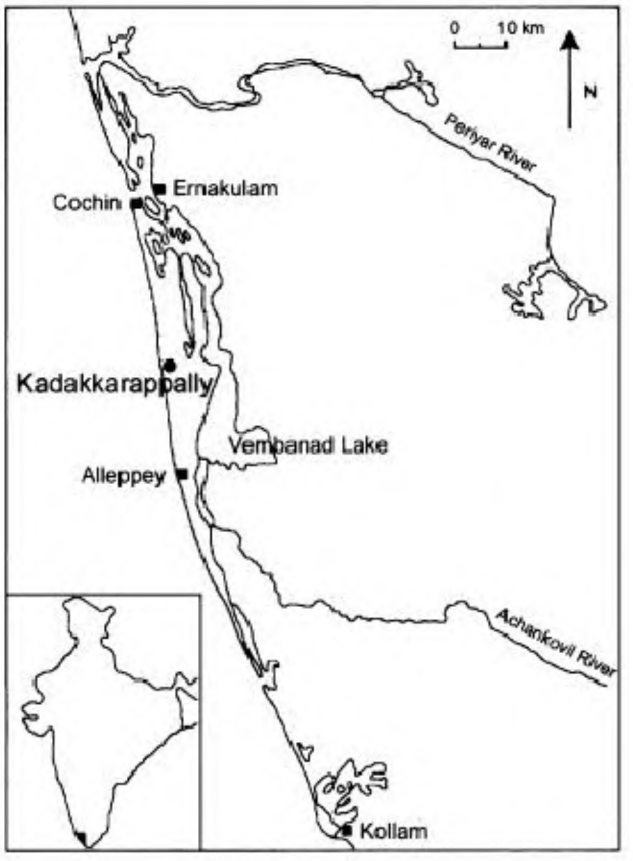

Figure 1. Location of Kadakkarappally.

*For correspondence. (e-mail: selvaveera@hotmail.com)