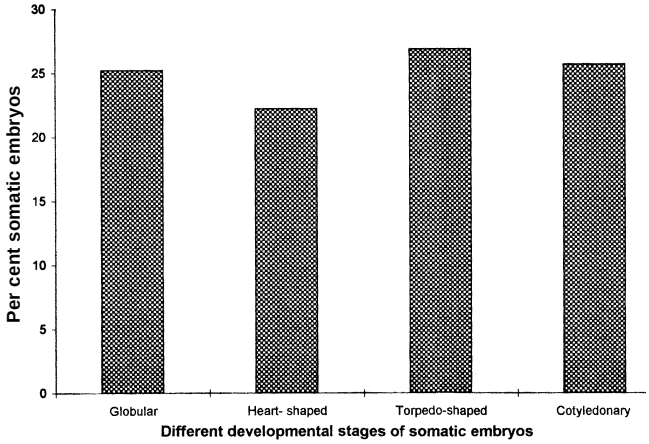

Figure 2. Per cent somatic embroys at different developmental stages after three weeks of culture initiation.

The present work depicts the SEM ontogeny of somatic embryo in safflower. It reveals normal development of somatic embryos from globular to heart-shaped, torpedo-shaped and cotyledonary-stage embryos. Also, precise identification of incipient globular embryo and early heart-shaped embryos, i.e. initiation of the development of two cotyledons was possible. Thus, the present work confirmed the direct development of somatic embryos from the cotyledon surface.

1. Narayanswamy, S., *Plant Cell and Tissue Culture*, Tata McGraw-Hill, New Delhi, 1994, pp. 263–272.
2. Dos Santos, A. V. P. and Machado, R. D., *Ann. Bot.*, 1989, **64**, 293–296.
3. Nakamura, T., Taniguchi, T. and Maeda, E., *Jpn. J. Crop Sci.*, 1992, **61**, 476–486.
4. Mandal, A. K. A., Chatterji, A. K. and Dutta Gupta, S., *Plant Cell Tissue Org. Cult.*, 1995, **43**, 287–289.
5. Mandal, A. K. A. and Dutta Gupta, S., *In Vitro Cell. Dev. Biol. P.*, 2001, **37**, 50–54.
6. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 473–497.
7. Lazzeri, P. A., Hildebrand, D. F. and Collins, G. B., *Plant Cell Tissue Org. Cult.*, 1987, **10**, 197–208.
8. Lazzeri, P. A., Hildebrand, D. F. and Collins, G. B., *ibid*, 1987, **10**, 209–220.
9. Sellars, R. M., Southard, G. M. and Phillips, G. C., *Crop Sci.*, 1990, **30**, 408–414.
10. Hazra, S., Sathaye, S. S. and Mascarenhas, A. F., *Biotechnology*, 1989, **7**, 949–951.
11. McKently, A., *In Vitro Cell. Dev. Biol. P.*, 1991, **27**, 197–200.
12. Chengalrayan, K., Sathaye, S. S. and Hazra, S., *Plant Cell Rep*., 1994, **13**, 578–581.
13. Finer, J. J., *ibid*, 1987, **6**, 372–374.
14. Fambrini, M., Cionini G. and Pugliesi C., *Plant Cell Tissue Org. Cult*., 1996, **114**, 205–214.
15. Sarvesh, A., Reddy, T. P. and Kavi Kishor, P. B., *In Vitro Cell. Dev. Biol. P*., 1994, **30**, 104–107.
16. Maheswaran, G. and Williams, E. G., *Ann. Bot*., 1984, **54**, 201–211.
17. Liu, W., Moore, P. J. and Collins, G. B., *In Vitro Cell. Dev. Biol. P*., 1992, **28**, 153–160.
18. Mandal, A. K. A., Dutta Gupta, S. and Chatterji, A. K., *Biol. Plant*., 2001, **44**, 503–507.

ACKNOWLEDGEMENTS. The first author is grateful to IIT Kharagpur, for providing financial assistance.

Received 24 June 2002; revised accepted 2 September 2002

Role of biological preparations in enhancement of rice seedling growth and grain yield

Preeti Vasudevan[†], M. S. Reddy[#], S. Kavitha[†], P. Velusamy[†], R. S. David PaulRaj[†], S. M. Purushothaman[‡], V. Brindha Priyadarisini[†], S. Bharathkumar[†], J. W. Kloepper[#] and S. S. Gnanamanickam[†,*]

[†]Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai 400 025, India
[#]Department of Plant Pathology and Entomology, Auburn University, AL 36809, USA
[‡]Regional Agricultural Research Station, Kerala Agricultural University, Pattambi 679 306, India

The application of five commercial chitosan-based formulations of carefully chosen plant growth-promoting rhizobacteria developed at Auburn University, USA has previously shown demonstrable increase in the growth of nursery-raised plants such as cucumber, pepper and tomato among others. The present study evaluates the beneficial effects of the formulations on the growth of rice seedlings. Seedlings of three indica rice cultivars, IR24, IR50 and Jyothi raised in rice field soil amended with each of the formulations in a 1 : 40 (formulation : soil) ratio have shown significant two-fold increase in root and shoot length, and grain yield. The observations do suggest that application of such commercial bacterial formulations can serve as microbial inoculants for the improvement of rice growth.

AS public opinion against the use of chemical pesticides on food crops grows, more and more pesticides have been removed from agricultural use. Hence, establishing new and effective pest control measures is a concern. One such alternative pest control strategy is the use of microorganisms. This strategy has the potential to reduce or eliminate chemical pesticides on agriculturally important crops, and thereby reduce the risk associated with pesticide residues in the environment. Biofertilizers and

*For correspondence. (e-mail: gmanick@vsnl.com)

biofungicides include many types of bacteria and fungi. One group that has been extensively investigated is plant growth-promoting rhizobacteria (PGPR). PGPR are root-colonizing bacteria beneficial to various agricultural crops. The beneficial effects include plant growth promotion, biological control of various diseases, and the activation of the defence responses of the host plant, which is termed as induced systemic resistance (ISR)[1–9]. Some PGPR not only benefit from the nutrients secreted by the plant root but also beneficially influence the plant in a direct or indirect way, resulting in stimulation of its growth.

Despite the fact that microbial inoculants have long been incorporated into field practices worldwide, most of them have been used and evaluated on the legumes and to a lesser extent on cereal crops[9–14]. Hence, the current study was undertaken to evaluate five biological preparations on rice, the world's most widely grown cereal crop.

The five biological preparations LS213, LS254, LS255, LS256 and LS257 were obtained from Auburn University, AL, USA to screen for growth promotion on three rice cultivars. These preparations had previously shown growth promotion and induced systemic protection in tomato, cucumber, pepper and tobacco[6]. Each biological preparation contained industrially formulated endospores of two *Bacillus* strains (Table 1) and chitosan, a formulation carrier. There were 1.0×10^9 colony forming units/l (cfu/l) of each strain in each biological preparation.

Three cultivars of rice, Jyothi, IR24, and IR50 were used. Jyothi is a high-yielding and extremely popular cultivar and is grown extensively in Kerala. Seeds of Jyothi were obtained from the Regional Agricultural Research Station (RARS), Pattambi. Seeds of IR24 and IR50 were obtained from Dr Susan McCouch, Cornell University, USA and the International Rice Research Institute, Manila, Philippines respectively.

To screen the five biological preparations on three cultivars of rice, experiments were conducted in a net-house at RARS. Soil collected from rice fields of RARS was used to grow the seedlings. There were three experiments, one for each cultivar. Each experiment consisted of five biological preparations and an untreated control. Each treatment was replicated ten times. Treatments were arranged in a randomized complete block design on a bench in the net-house. Styrofoam cups (10″ diameter) were used to grow the seedlings. Each of the five biological preparations was thoroughly mixed with field soil at a rate of 1 : 40 (v/v). The soil containing the biological preparation was placed into styrofoam cups (330 g of soil/cup). Non-amended soil served as untreated control. Five seeds of each rice cultivar were sown into each cup. Seedlings were watered regularly.

The growth of the seedlings was monitored at weekly intervals for a period of three weeks. Shoot lengths of the seedlings were recorded at the end of the first, second and third week. At the end of the second week, four seedlings were carefully pulled from each cup with adequate precautions to prevent any damage to the roots, and the root lengths of these seedlings were also recorded. One seedling was retained in each cup in order to record the shoot length at the end of the third week. Each experiment was conducted three times. The data were analysed separately for each experiment and subjected to analysis of variance. The significance of effect of biological preparation was determined by the magnitude of the *F* value ($P = 0.05$). Treatment means were separated by Fisher's protected least significant difference (LSD). Grain yields recorded as 1000-grain weights were compared after the rice cultivars IR24, IR50 and Jyothi, received the same biological treatments (mainplot) as described above with an untreated control, in a separate split-plot design experiment in the net-house. In this experiment, cultivars/varieties were factors used as the subplot in making the relative comparison. Grain yield data were subjected to test of significance (*F*-test) (see Tables 2 and 3).

Rice seedlings germinated in soils amended with the five bacterial preparations exhibited increased shoot lengths in all the three rice cultivars used in this study (Table 4). It generally took 14 days after seeding (DAS) to observe significant root length increases, although root length at 7 DAS was enhanced by LS254 on cv. IR50 and LS255 on cv. Jyothi. Also, seedlings raised in soils amended with the formulation LS256 showed lesser growth compared to the untreated control. At 21 DAS,

Table 1. Biological preparations used in the study

Biological preparation		
Control	Strains in preparation	Identification
LS213	GB03 + IN937a	*Bacillus amyloliquefaciens*
LS254	GB03 + SE34	*B. pumilus*
LS255	GB03 + IN937b	*B. subtilis*
LS256	GB03 + INR7	*B. pumilus*
LS257	GB03 + T4	*B. pumilus*

GB03, *Paenobacillus macerans*.

Table 2. Effect of biological formulations on grain yield of rice cultivars

	1000-grain weights (g) recorded in rice cultivar		
Treatment	IR24	IR50	Jyothi
Untreated control	11.88	12.20	11.35
LS254	15.79	17.42	21.41
LS255	16.40	18.15	23.13
LS256	16.46	18.90	22.80
LS257	15.70	16.60	18.04
LS213	15.90	17.96	19.50

Net-house experiment (split-plot design), Regional Agricultural Research Station, Pattambi, July–November 2001.
Each value is a mean of four replications.

root length increases compared to the untreated control were observed on cv. IR24 with four biological preparations, on IR50 and Jyothi with five preparations.

Table 3. Analysis of variance on 1000-grain weight data in Table 2

Source of variation	Degrees of freedom	Sum of squares	Mean square	Observed *F*	Tabular *F* 5%	Tabular *F* 1%
Block	3	12.599				
Cultivar	2	8.062	4.026	3.48*	3.22	5.15
Biological treatment	5	41.234	8.247	68.26**	2.59	3.80
Cultivar × treatment	10	8.292	0.829	1.89^{ns}	2.17	2.96
Error	51	6.353	0.124			
Total	71	76.530				

*Significant at 5% ($P = 0.05$); **Significant at 1% ($P = 0.01$) level; ^{ns}Not significant.

However, most of the treatments seemed to effect statistically significant increases in shoot lengths when compared to that of their respective untreated controls at the end of the second week (Figure 1 *a–c*; Table 4). Germinating seeds derive most of their nutrients from the reserve food material available in the endosperm. Therefore, the role of PGPR at this stage of development may be less critical. However, when this reserve begins to diminish, the seedlings depend more on their own machinery for nutrient uptake[1]. It is probably at this stage that the seedlings may benefit by the association of a beneficial bacterium. Indeed, PGPR are known to enhance plant growth by different mechanisms[15].

Similar results were observed when shoot lengths were recorded at the end of the third week. All the formulations afforded appreciable increases in shoot length of the three rice cultivars (Table 4). It is interesting to note that the formulation LS213 resulted in significant shoot

Table 4. Effect of biological preparation on shoot length of rice seedlings grown in field soil

	Rice cultivar								
	IR24[a]			IR50[a]			Jyothi[a]		
Treatment	7 DAS	14 DAS	21 DAS	7 DAS	14 DAS	21 DAS	7 DAS	14 DAS	21 DAS
Untreated control	3.10	18.34	30.91	4.20	20.23	29.52	5.39	21.20	28.93
LS254	3.33	24.54**	35.63**	5.22**	24.54**	34.20**	4.10	25.55**	35.32**
LS255	3.72	23.13**	39.83**	4.08	24.49**	35.21**	6.40**	27.90**	35.49**
LS256	1.29	16.25	46.20**	1.84	22.54**	36.50**	3.41	23.36**	33.74**
LS257	2.58	22.99**	35.85**	4.33	22.42**	35.28**	3.52	21.85*	32.21*
LS213	1.88	17.45	36.68**	3.33	22.01**	30.87	3.22	21.41	32.75*

[a]Values are mean of ten replications and one seedling per replication; Values followed by *, ** are significantly different from untreated control based on Fisher's protected least significant difference at $P = 0.05$ and $P = 0.01$ respectively.

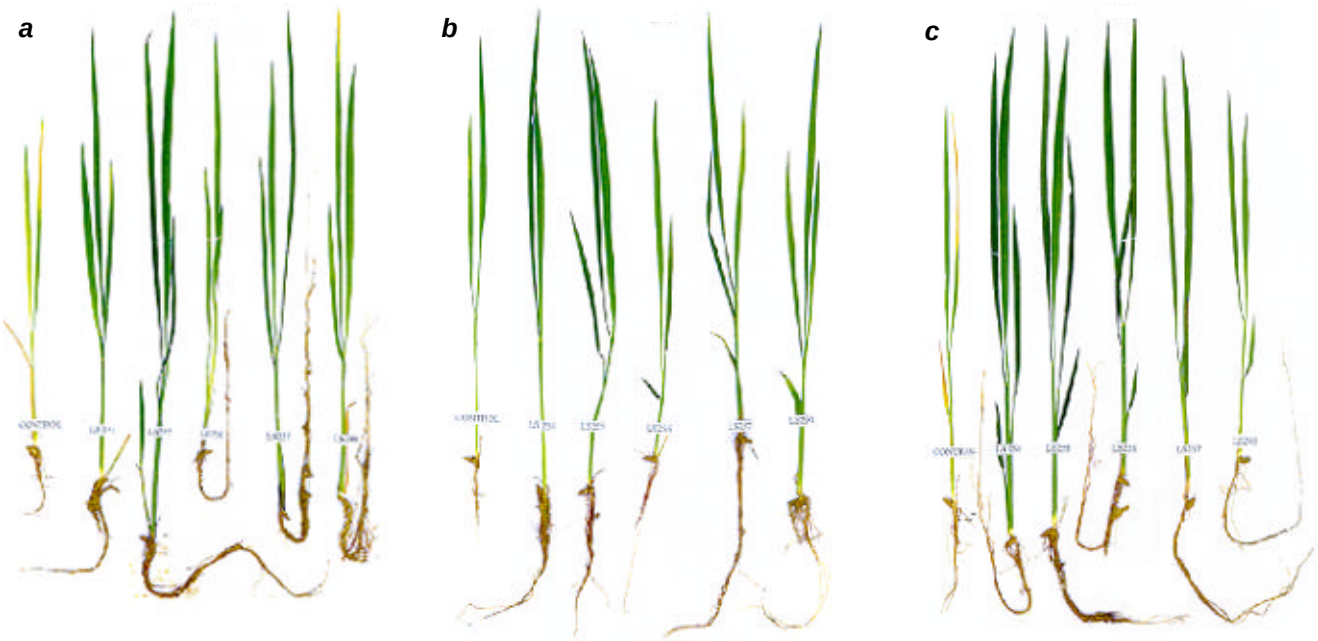

Figure 1. Enhancement of shoot and root length by bacterial formulations LS254, LS255, LS256, LS257 and LS213 on rice cultivars. ***a***, IR50; ***b***, IR24; and ***c***, Jyothi.

Table 5. Effect of biological preparations on root length of rice seedlings grown in field soil

Treatment	Rice cultivar[a]		
	IR24	IR50	Jyothi
Untreated control	6.83	7.31	9.20
LS254	12.20**	12.20**	14.72**
LS255	8.41**	12.21**	15.62**
LS256	6.47	9.83**	13.76**
LS257	13.09**	13.00**	14.28**
LS213	7.54**	13.78**	12.95**

[a]Values are mean of ten replications and one seedling per replication; Values followed by *, ** are significantly different from untreated control based on Fisher's protected least significant difference at $P = 0.05$ and $P = 0.01$ respectively.

length enhancements only at the end of the third week. A possible reason for the delay in growth enhancements by this particular bacterial product may be due to the time taken by the introduced bacterium to establish itself in the rhizosphere. Establishment of a threshold population of viable inoculants is an important prerequisite for plant–microbe interactions like growth enhancement and biocontrol by bacteria[16,17]. Data from 1000-grain weights reveal that among the two factors (biological formulations and cultivars) compared, the bacterial formulations are a lot more significant to the grain yields obtained. Cultivars, and interactions between cultivars and formulations appear to have a limited significance or no significance to rice yield(s) (Tables 2 and 3), although the response of Jyothi to bacterial formulations is more pronounced than in the other two rice cultivars.

Root length increases were recorded only at the end of the second week and the results have been summarized in Table 5. Almost all the formulations resulted in significant increases in root length of the three rice cultivars (Figure 1 *a*–*c*). Two-fold increases in root length could be observed in seedlings raised in soil amended with some formulations. Many reports have shown bacterially-induced enhancements of root lengths[2,17–22]. Such increases in root length can confer many advantages to the host system with respect to its health and growth. While extensive development of the adventitious root system helps increase surface area and consequently increase the efficiency of nutrient absorption by plants, an increased root length improves the survival of young seedlings, especially at the initial stages of development[14].

It appears that the application of biological preparations to cereal crops like rice can bring about appreciable levels of growth and yield enhancements. However, this is only a preliminary attempt to determine if the commercial product, whose efficiency in enhancing plant growth in other host systems has been well established, has similar beneficial effects on a cereal crop like rice. A thorough investigation, taking into consideration certain parameters such as increases in biomass and yield of treated rice plants in field plots and at multi-locations, is necessary to provide unequivocal evidence for the usefulness of biological preparations in sustaining rice yields.

1. Glick, B. R. and Bashan, Y., *Biotechnol. Adv.*, 1997, **15**, 353–378.
2. Glick, B. R., *Can. J. Microbiol.*, 1995, **41**, 109–117.
3. Tang, W. H., in *Improving Plant Productivity with Rhizosphere Bacteria* (eds Ryder, M. H., Stephens, P. M. and Bowen, G. D.), CSIRO, Adelaide, 1994, pp. 267–278.
4. Frommel, M. I., Nowak, J. and Lazarovits, G., *Plant Physiol.*, 1991, **96**, 928–936.
5. Lambert, B. and Joos, H., *Trends Biotechnol.*, 1989 **7**, 215–219.
6. Kloepper, J. W., Lifshitz, R. and Zablotowicz, R. M., *ibid*, 1989, **7**, 39–43.
7. van Peer, R. and Schippers, B., *Can. J. Microbiol.*, 1989, **35**, 456–463.
8. Kloepper, J. W., Leong, J., Teintze, M. and Schroth, M. N., *Nature*, 1980, **286**, 885–886.
9. Gnanamanickam, S. S., Vasudevan, P., Reddy, M. S., Kloepper, J. W. and Defago, G., in *Biological Control of Crop Diseases* (ed. Gnanamanickam, S. S.), Marcel Dekker, New York, 2002, pp. 1–9.
10. Bashan, Y., *Biotechnol. Adv.*, 1998, **16**, 729–770.
11. Hedge, S. V. and Brahmaprakash, G. P., *Plant Soil*, 1992, **144**, 309–311.
12. Fages, J., in *Azospirillum/Plant Associations* (ed. Okon, Y.), CRC Press, Boca Raton, Fl., 1994, pp. 87–110.
13. Backman, P. A., Brannen, P. M. and Mahaffer, W. F., see ref. 3, pp. 3–8.
14. Tang, W. H. and Yang, H., in *Plant Growth-Promoting Rhizobacteria – Present Status and Future Prospects* (eds Ogoshi, A. *et al.*), OCED, Paris, 1997, pp. 4–9.
15. Glick, B. R., Patten, C. L. Holguin, G. and Penrose, D. M., in *Biochemical and Genetic Mechanisms used by Plant Growth-Promoting Bacteria*, Imperial College Press, London, 1999, p. 267.
16. Parke, J. L., in *The Rhizosphere and Plant Growth* (eds Keister, D. L. and Cregan, P. B.), Kluwer Academic Publishers, Boston, MA, 1991.
17. Glick, B. R. and Skof, Y. C., *Biotechnol. Adv.*, 1986, **4**, 261–277.
18. Lifshitz, R., Kloepper, J. W., Kozlowski, M., Simonson, C., Carlson, J., Tipping, E. M. and Zaleska, I., *Can. J. Microbiol.*, 1987, **33**, 390–395.
19. Dubeikovsky, A. N., Mordukhova, E. A., Kochetkov, V. V., Polikarpova, F. Y. and Boronin, A. M., *Soil Biol. Biochem.*, 1993, **25**, 1277–1281.
20. Shah, S., Li, J., Moffatt, B. M. and Glick, B. R., see ref. 14, pp. 320–324.
21. Caron, M., Patten, C. L., Gosh, S. and Glick, B. R., *Plant Growth Regulators*, Society of America Quarterly, 1995, pp. 297–302.
22. Xie, H., Pasternak, J. J. and Glick, B. R., *Curr. Microbiol.*, 1996, **32**, 67–71.

ACKNOWLEDGEMENTS. We thank D. Kenney and Gustafson Limited, McKinney, Texas for supplying the chitosan-based formulations. We also thank the Associate Director, Regional Agricultural Research Station, Kerala Agricultural University, Pattambi for providing rice field soil and experimental space for this study.

Received 16 February 2002; revised accepted 9 August 2002