*Indian Journal of Microbiology Vol 44, No. 2, March 2004, pp 95-100*

# Spore-forming bacteria, a major group among potential antagonists isolated from natural sources such as termitaria soil and composts used by organic farmers

**M. Sriveni, O. P. Rupela*, S. Gopalakrishnan, M. Krajewski[1]**
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, 502 324, India

**A recently published method allowed counting of both total number of microorganisms and those antagonistic to a disease causing fungus in naturally occurring materials such as composts. Using this method and *Fusarium solani*, cause of black root rot of chickpea, as the target fungus, five of the nine materials [termitaria soil, compost from leaf-liter and kitchen waste, debris in the leaf-axil of plants of *Billbergia* sp. and six composts used by organic farmers and called as biodynamic *(BD) prep*arations – BD500, BD502, BD503, BD504, BD505, BD506] were found to have high population (4.28 $log_{10}$ *to 6.98* $log_{10}$ $g^{-1}$ material) of antagonistic bacteria. Fifty five (82%) of the 67 isolates were Gram-positive spore-forming rods and the rest non-sporulating. All the 55 spore-forming antagonistic bacteria survived for at least 108 days in a desiccator; 20 survived for at least 17 months. Antagonistic bacteria surviving desiccation for long periods can potentially be used in dry formulations as biocontrol agents. Presence of large number of antagonistic spore forming bacteria in these different materials appears to be responsible for the reported low pest incidence at the farms using BD preparations. Thirty-five of the 67 isolates showed commensal behaviour towards groundnut rhizobia, under in-vitro conditions. Eleven of the 55 spore-forming bacteria were studied using Biolog kit. Only one, BCB 103, was identified as *Bacillus thermoglucosidasius*, others did not match any of the 23 spore-forming species (including *B. thuringiensis*) in its database.**

**Key words**: Antagonists, biocontrol, biodynamic agriculture, desiccation tolerance, organic farming, spore-forming bacteria.

Fungal diseases are one of the major factors limiting crop production. Protection of plants against fungal infection is dependent mainly on chemical pesticides, many of which are known to be hazardous to the environment. Some chemical pesticides are known to cause adverse effects on humans and natural enemies of pests, and have been banned[1]. This concern has led researchers to develop safer and more environment-friendly pest-control alternatives.

Farmers growing organic food use alternatives such as biopesticides and biofertilizers. There are claims that such crops suffer less damage from diseases and insect-pests than the neighboring fields dependent on the mainstream agricultural practices[2,3]. In some cases such alternatives have been developed and promoted by farmers themselves. Several farmers practicing organic farming in Karnataka and Tamil Nadu states of India were noted using six different materials (popularly known as Biodynamic or BD preparations), when visited in 1998. Most of the ten farmers, visited during the travel, reported lower incidence of diseases and insect-pests than the neighboring "mainstream" farmers. Further queries revealed that each of the six materials were prepared using a different protocol and raw material (e.g. cowdung and herbs) proposed in 1924 by Rudolf Steiner[4]. These preparations are available commercially in India (Kurunji Organic Foods, Genguvarpatti, 625203, Tamil Nadu; Supa Biotech, Pvt. Ltd., Mallital, Nainital, Uttranchal). Because the protocol of preparing each of the six materials differed due to material or environment during preparation, we called the different BD preparations as composts. Farmers invariably used small quantities (generally <50g $ha^{-1}$), by suspending in water, stirring in a specific manner before applying to crops and land by spreading the liquid, generally by brooms used as brush. It was hypothesized that if the reported low incidence of pests at the organic farms is associated with the BD preparations, these may be having high numbers of antagonistic microorganisms. The recently developed method, called 'two layer method', allowed counting of antagonistic bacteria[5] in the presence of several

*Corresponding author; E-mail: o.rupela@cgiar.org
[1]Present address: Forschungszentrum Juelich GmbH, ICG-IV (Agrosphere), 52425 Juelich, Germany.

types of microorganisms and was used to verify this hypothesis. In the study reported here, *Fusarium solani*, causing black root rot of chickpea, was used as the target fungus for counting antagonists in the different organic materials [termitaria soil, compost from leaf-litter and kitchen waste, debris in the leaf-axil of plants of Billbergia sp. and six composts or biodynamic (BD) preparations (BD500, BD502, BD503, BD504, BD505, BD506)]. From the plates having highest dilution of a given material, 67 potentially antagonistic bacteria representing different colony types were isolated and purified. This paper reports diversity of the antagonistic isolates and discusses their potential value as biopesticides.

**Materials and Methods**

*Isolation of antagonists*: The two-layer method[5] was used for identification and isolation of microorganisms antagonistic to *Fusarium solani*. Approximately 30 ml of ¼ PDA[6] was poured into a sterilized petri-plate (95 mm diameter) and allowed to dry for 45 minutes, in a laminar flow. A 4 to 5-day-old sporulating culture of *F. solani* grown on ¼ PDA plate was scraped, suspended in 5 mL sterile water using a Tissumizer Mark II (from Tekmar, Cincinnati, USA) blender. The fungal suspension (0.1 ml) was spread plated on the previously prepared ¼ PDA plates. The plates were surface-dried in a laminar flow for 4 h. A second layer of ¼ PDA (20 ml) was then poured on top of the first layer. Care was taken that the temperature of the molten PDA was 45°C: The plates were again surface-dried in a laminar flow for 45 minutes. Appropriate dilution of the likely source of antagonistic microorganisms was spread plated on top of the second layer. The plates were incubated at 28±2°C and observed for different types of microorganisms.

Colonies of microorganisms showing a halo were isolated and purified on ¼ PDA and assigned a number prefixed with BCB (Biological Control Bacteria) or BCF (Biological Control Fungi). Care was taken to pick up those colonies which showed distinct difference in morphological characters on the top layer of ¼ PDA. Sixty seven bacterial and 10 fungal colonies were isolated. Studies on bacteria are only reported in this paper. Most of the 67 potential antagonists grew very rapidly on full strength potato dextrose agar (PDA); consequently purification and observations for colony morphology were difficult. Therefore, all the isolates were purified by dilution streaking and characterized on ¼ PDA[6], for cultural and microscopic (Gram staining, spore staining) characteristics. Strains of *Escherichia coli* (Gram negative) and *Bacillus megaterium* (Gram positive) were used as standards.

*Desiccation studies*: Twenty-four h old cultures on ¼ PDA were placed in a desiccator containing blue silica gel at the base. The studies were carried out for about 17 months. Temperature and humidity in the air-conditioned lab was measured for the first 12 of the 17 months of the study, using a HOBO® Pro Series data logger (model H08-032-08, from ONSET Computer Corporation, USA). Low humidity inside the desiccator (not measured) was ensured by frequently replacing the hydrated (indicated by pink color) silica gel with fresh lot (blue). Viability of 65 of the 67 isolates was examined periodically (once in 15 d for the first 3 months, once a month for the next 9 months, and thereafter once in 3 months) during a total period of 17 months. For this purpose, a heat sterilized wireloop was dipped in sterile water before picking the inoculum from the desiccated plate, and streaked on fresh ¼ PDA plates. The plates were incubated at 28°C.

*Microscopic observations*: Cultures stored in a refrigerator for 40d were stained for spores[7] and observed under 400 and 1000 magnification using a Zeiss microscope (model HBO 50/Ac). Growth from plates desiccated for 80 days, described above, were subjected to boiling test. A loop full of inoculum of a strain was aseptically transferred into a tube containing 10 ml of sterile distilled water, placed in boiling water for about 15 min, and allowed to cool and streaked over ¼ PDA. The plates were incubated overnight at 28±2°C and observed for growth.

*Interaction studies*: Thirty five of the 67 isolates were characterized for interactions with rhizobia. Five rhizobial strains of groundnut (IC 6006, -7001, -7017, -7029, -7114) were obtained from the microbial culture collection at ICRISAT[8]. In a previous unpublished study, we have established that both groups of bacteria grew on yeast extract manitol agar (YEMA[9]). Six different bacteria (one antagonist and five rhizobia) were streaked on YEMA in 10 cm dia plate in a specific pattern that was first drawn on a paper. A 5 cm long vertical line at a distance of 5 mm from the margin was drawn on one side of the circle. Five lines, each 5 mm away and as perpendicular to the vertical line, were drawn. Each perpendicular line was 5 cm long and 1 cm apart from each other. Keeping this as a template below the petridish, the antagonistic cultures were streaked over the vertical line and five rhizobial cultures over the perpendicular lines. Care was taken that the cultures did not touch wall of the pertiplates. Rhizobia were inoculated 36 h prior to the inoculation of a potential antagonist because of slow growth

of rhizobia. The plates were further incubated for 24 h and observed for "interaction distance" and "spreading capacity". The distance of growth of a potential antagonist from center of growth towards the rhizobial streak, was termed "interaction distance", and the distance of its growth on the side opposite to rhizobial streaks was termed "spreading capacity".

*Species identification*: Eleven (BCB 69, BCB 74, BCB 75, BCB 85, BCB 97, BCB 103, BCB 106, BCB 111, BCB 116, BCB 117a and BCB 117b) spore-forming isolates were used in identification of species by using commercially available microbial identification kit from BIOLOG, Hayward, USA[10]. After purification, Gram straining and other characterization, a special procedure advised for spore formers[10] was followed. Sixteen h old cultures were inoculated to GP2 microplates, each having 96 wells including one control (i.e. involving 95 biochemical tests for a given isolate) as per manufacturers instructions, in duplicate. The colour in each well of a given plate was read manually and using a microplate reader (from Lab System, Finland) at $OD_{590}$. The data was analysed by a MicroLog software (BIOLOG), for species identification.

**Results and Discussion**

*Diversity in growth traits*: The features common to most isolates were, fast growth (18 to 24 h), spreading tendency, circular colonies (79%) with smooth margins (85%), raised and sticky surface (79%), and dull white colonies (57%) on ¼ PDA. Surfaces of all the fast growing circular colonies dried and flattened after 48h and in some cases were associated with irregular margins. Colonies of isolates BCB 25 and BCB 113 were non-spreading, non-sticky and milky white in colour. Whereas those of BCB 124, 125, 127, 129 and 151 were light yellow. A yellow pigment, which fluoresced under UV light, was found around the colonies of BCB 122 and 123. Data on relative frequency of occurrence of each of these traits is given in Table 1.

Population of antagonists in the BD preparations (composts used by the organic farmers) and in the other three materials ranged from 3.24 $\log_{10}$ (BD 502) to 6.90 $\log_{10}$ (BD 500) $g^{-1}$ of material[5]. Of the 67 isolates used in this study, 17 were antagonistic to at least one more disease-causing fungus[5]. This indicated that the use of BD preparations were at least one of the factors responsible for reduced incidence of pests on the farms following organic agricultural practices. This would, however, need confirmatory data.

**Table 1.** Colony characters of the bacteria (on ¼ potato dextrose agar at 1) with potential to suppress the fungus causing black root rot of chickpea.

| Colony character | Range/Type | Relative frequency (± SE) | Example |
|---|---|---|---|
| Size (mm) | <1 | 4.5±0.06 | BCB 135 |
| | 1-2 | 19.4±0.23 | BCB 20a |
| | 2-3 | 59.7±0.36 | BCB 7>3 |
| 16.4±0.20 | BCB 98 | | |
| Shape | Circular | 79.1±0.25 | BCB 7 |
| | Irregular | 19.4±0.23 | BCB 20a |
| | Oval | 1.5±0.02 | BCB 60 |
| Margin | Smooth | 73.1±0.29 | BCB 114 |
| | Irregular | 26.9±0.29 | BCB 115 |
| Elevation | Flat | 14.9±0.19 | BCB 135 |
| | Raised | 85.1±0.19 | BCB 117a |
| Surface | Dry | 26.9±0.29 | BCB 115 |
| | Wet | 7.5±0.10 | BCB 14 |
| | Smooth | 56.7±0.37 | BCB 117a |
| | Smooth and wet | 3.0±0.04 | BCB 58 |
| | Smooth and dry | 1.5±0.02 | BCB 62b |
| | Wrinkled | 4.5±0.06 | BCB 64 |
| Consistency | Sticky | 79.1±0.25 | BCB 117a |
| | Viscous | 10.4±0.14 | BCB 127 |
| | Dry | 10.4±0.14 | BCB 135 |
| Optical features | Opaque | 71.6±0.30 | BCB 117a |
| | Transparent | 28.4±0.30 | BCB 116 |
| Colour | Colorless | 19.4±0.23 | BCB 98 |
| | White | 9.0±0.12 | BCB 63 |
| | Dull white | 56.7±0.37 | BCB 115 |
| | Milky white | 3.0±0.04 | BCB 25 |
| | Yellow | 9.0±0.12 | BCB 151 |
| | Yellow colonies with pigment diffusing into agar | 3.0±0.04 | BCB 122 |

*Diversity in microscopic traits*: Ten of the 67 isolates were Gram-negative and the remaining 57 (85%) were Gram-positive rods. BCB 14 had Gram-positive rods in pairs generally aligned in parallel rows. Gram-positives, BCB25 and BCB113 were pleomorphic while BCB 115 had long chains with broad cells. BCB135 was Gram-negative and unique, with spindle shaped rods that stained lightly. With respect to size, most bacterial isolates had cell size that ranged between 2 to 3 µm. Relative frequency of occurrence of these traits is given in table 2. Size and arrangement of the cells of spore-formers varied. About 70% of these were single rods, 22% had rods in chains or as pairs; some had rods in bundles or as long

**Table 2.** Microscopic characters of the bacteria with potential to suppress black root rot of chickpea.

| Character | Type | Relative frequency (± SE) | Example |
|---|---|---|---|
| Gram reaction | Gram positive | 85.1±0.19 | BCB 98 |
| | Gram negative | 14.9±0.19 | BCB 135 |
| Size[a] | Small | 19.4±0.23 | BCB 58 |
| | Medium | 76.1±0.27 | BCB 20a |
| | Large | 4.5±0.06 | BCB 7 |
| Shape | Rods | 59.7±0.36 | BCB 114 |
| | Small rods | 11.9±0.16 | BCB 122 |
| | Rods in chains and pairs | 22.4±0.26 | BCB 14 |
| | Rods in bundles | 1.5±0.02 | BCB 34 |
| | Pleomorphic rods | 3.0±0.04 | BCB 25 |
| | Rods in long chains | 1.5±0.02 | BCB 115 |

Only four representative bacteria of each type were measured using micrometer. Small size bacteria measured about 1 to <2 micron (µm), medium ranged from 2 to <3µm, and large ones were >3µm.

chains (1.5% each), and 3% were pleomorphic. Figure 1 has photographs of Gram stained BCB 14 and spore stained BCB 60.

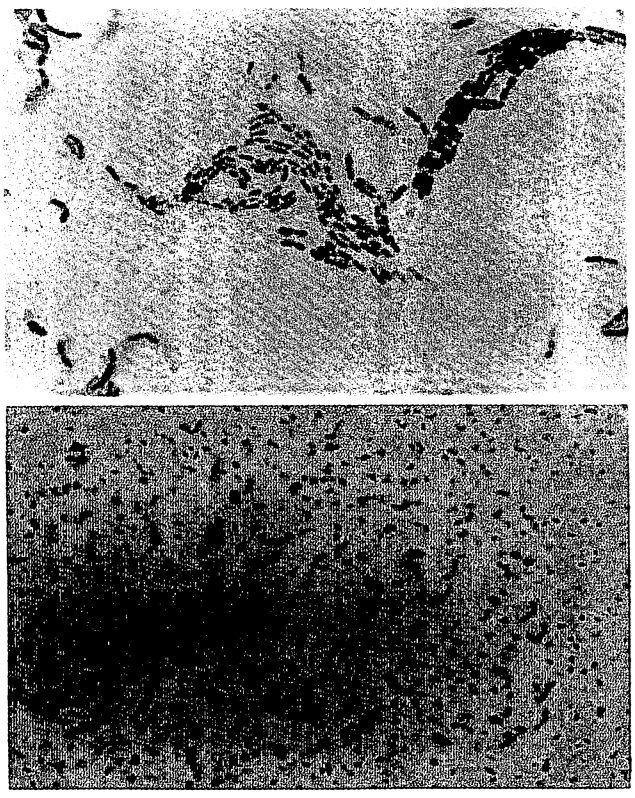

**Fig 1. a)** Gram positive rods (deep blue color) in pairs, aligned in rows, strain BCB14, b) spore staining of strain BCB60, note the green color spores and pink color vegetative cells.

*Desiccation and boiling tolerance*: Microorganisms, when applied to crops/soil, are expected to face desiccation. In nature, microorganisms variously adapt to overcome desiccation, through spore formation or by production of extracellular polysaccharides[11]. All isolates were grown on ¼ PDA and evaluated for survival in artificially created low moisture conditions in a desiccator containing self-indicating silica gel. Mean lab temperature and humidity for the first 12 of the 17 months of the study was 24.4°C (range 19.9 to 29.5°C) and 64.7% (range 38.2 to 89.4%). Humidity inside the

**Table 3.** Interactions between rhizobia nodulating groundnut and the potential antagonists.

| Isolate number | Spreading capacity (mm) Mean | Range | Interaction distance (mm)[a] Mean | Minimum | Maximum |
|---|---|---|---|---|---|
| BCB 014 | 12.0 | 12-12 | 16.25 | 13 (IC 7017) | 19.5 (IC 7001) |
| BCB 020a | 13.5 | 12-15 | 10.75 | 7.0 (IC 7001) | 14.5 (IC 7029) |
| BCB 020b | 14.0 | 13-15 | 16.75 | 8.5 (IC 7017) | 25.0 (IC 7029) |
| BCB 021 | 13.0 | 11-15 | 14.25 | 11.5 (IC 7017) | 17 .0 (IC 7029) |
| BCB 025 | 4.50 | 4-5 | 3.50 | 2.0 (IC 7017) | 5.0 (IC 7001) |
| BCB 029 | 5.50 | 5-6 | 7.25 | 4.0 (IC 7001) | 10.5 (IC 7114) |
| BCB 031 | 20.0 | 18-22 | 20.00 | 8.5 (IC 7017) | 31.5 (IC 7001) |
| BCB 044 | 21.0 | 20-22 | 24.00 | 13.0 (IC 6006) | 35.0 (IC 7001) |
| BCB 045 | 16.5 | 15-18 | 37.00 | 14.0 (IC 6006) | 60.0 (IC 7001) |
| BCB 046a | 15.0 | 15-15 | 38.00 | 16.0 (IC 6006) | 60.0 (IC 7001) |
| BCB 046b | 14.5 | 14-15 | 15.75 | 10.0 (IC 6006) | 21.5 (IC 7029) |
| BCB 047 | 5.50 | 4-7 | 5.75 | 3.0 (IC 7029) | 8.5 (IC 6006) |
| BCB 054 | 10.0 | 8-12 | 21.75 | 8.5 (IC 7114) | 35.0 (IC 7001) |
| BCB 056 | 14.0 | 13-15 | 13.50 | 12 (IC 7114) | 15.0 (IC 7029) |
| BCB 058 | 9.0 | 8-10 | 5.50 | 4.5 (IC 7001) | 6.5 (IC 7029) |
| BCB 063 | 18.5 | 17-20 | 16.50 | 10.5 (IC 7017) | 22.5 (IC 6006) |
| BCB 065 | 13.5 | 12-15 | 17.00 | 8.0 (IC 7017) | 26.0 (IC 7029) |
| BCB 066 | 15.0 | 15-15 | 14.00 | 10.5 (IC 7001) | 17.5 (IC 7029) |
| BCB 069 | 10.5 | 10-11 | 10.00 | 6.5 (IC 7017) | 13.5 (IC 6006) |
| BCB 083 | 16.5 | 15-18 | 31.25 | 12.5 (IC 7001) | 50.0 (IC 7029) |
| BCB 087 | 16.0 | 15-17 | 35.75 | 11.5 (IC 7017) | 60.0 (IC 7001) |
| BCB 089 | 14.5 | 9-20 | 24.25 | 16.5 (IC 6006) | 32.0 (IC 7029) |
| BCB 098 | 6.0 | 5-7 | 12.50 | 10.5 (IC 6006) | 14.5 (IC 7001) |
| BCB 099 | 17.5 | 17-18 | 29.50 | 15.5 (IC 6006) | 43.5 (IC 7001) |
| BCB 100 | 14.0 | 13-15 | 18.75 | 10.0 (IC 7017) | 27.5 (IC 7029) |
| BCB 104 | 14.0 | 13-15 | 26.50 | 8.0 (IC 6006) | 45.0 (IC 7029) |
| BCB 106 | 19.0 | 18-20 | 25.5 | 4.0 (IC 7017) | 47.0 (IC 7029) |
| BCB 107 | 8.5 | 7-10 | 12.00 | 10.0 (IC 7001) | 14.0 (IC 7114) |
| BCB 108 | 5.5 | 4-7 | 18.25 | 3.5 (IC 7001) | 33.0 (IC 7029) |
| BCB 109 | 10.0 | 8-12 | 8.50 | 8.0 (IC 7017) | 9.0 (IC 7001) |
| BCB 115 | 8.0 | 8-8 | 6.25 | 5.0 (IC 7017) | 7.5 (IC 7114) |
| BCB 116 | 7.5 | 7-8 | 5.00 | 3.5 (IC 7001) | 6.5 (IC 6006) |
| BCB 117a | 8.0 | 7-9 | 8.75 | 7.5 (IC 7001) | 10.0 (IC 6006) |
| BCB117b | 7.5 | 7-8 | 6.25 | 4.5 (IC 7017) | 8.0 (IC 7001) |
| BCB 118 | 4.5 | 4-5 | 14.75 | 8.0 (IC 7029) | 21.5 (IC 7114) |

[a]. In parentheses, name of the rhizobial strain (among the 5 evaluated) with that data value.

desiccator was apparently very less due to the silica gel. Within the first 40 days, agar in all the plates was apparently very dry as indicated by presence of agar layer as flakes in all plates. Sixty-five of the 67 isolates were studied and all survived desiccation for 108 days; isolate BCB 135 was an exception and survived for 80 days. Twenty isolates survived desiccation for at least 17 months when the plates were last examined.

Staining and boiling protocol revealed that 55 of the 67 isolates formed spores, and 11 were non-spore formers. Most of the sporulation-positive strains sporulated abundantly as evident by microscopic observations. Antagonistic bacteria surviving desiccation for long periods could be used in dry formulations as biocontrol agents. BCB 122 and 123 were Gram-negative rods, non-spore formers, and produced fluorescent pigment on ¼ PDA, suggesting them to belong to the group fluorescent pseudomonads.

*Interactions between antagonists and rhizobia*: The suppressive effect of the 67 isolates may not be confined to pathogenic fungi and they may adversely affect beneficial organisms living in the soil. The later was tested on groundnut rhizobia, in plate culture. Among 35 antagonistic isolates studied, none interfered with rhizobial growth in culture. At least 19 of the 35 BCBs grew better where rhizobia were present, compared to the opposite side (Table 3). Since the experiment was performed in conditions different from the natural root rhizosphere and the soil environment, the results can at best be indicative. Analysis of the data on "interaction distance" and "spreading ability" indicated existence of two clusters, one of eight BCBs (BCB15, -58, -69, -107, -109, -117a and -117b) and the other of nine BCBs (BCB14, -20a, -20b, -21, -46b, -56, -65, -66 and -100, Fig. 2). This suggested similarity of the strains within a given group for their response to rhizobia, other strains were widely scattered, indicating heterogeneity in their response to rhizobia. Interestingly, there was no apparent sign of suppression of rhizobia by any of the 35 antagonistic bacteria that were studied.

Gram-positive endospore-forming aerobic bacteria reported in the literature are either *Bacillus* or *Amphibacillus* spp.[12]. The Biolog kit identified only one of the 11 spore-forming isolates as *Bacillus thermoglucosidasius*. Obviously, the other 10 did not match the 23 species of spore-forming bacteria in the software used (including *B. thuringiensis*). This also means that the 10 isolates belong to the species other than *thuringiensis*. It is also likely that most of the isolates represent a new species, however, this would require further analyses.

Eighty two percent of the 67 isolates were spore-formers. Previous observations[5] have shown that all 67 bacterial isolates were antagonistic to at least one disease-causing fungus (*Fusarium solani*) and 17 suppressed at least two. Sixteen of these isolates killed larvae of *Helicoverpa armigera* (legume pod-borer or cotton boll worm). Six (BCB 106, -111, -114, -117a, -117b, -122) of the 16 killed 50.0 to 71.7% larvae in the lab study[13]. Thus, same strains besides being able to suppress the disease causing fungus could also kill an insect-pest. Recovery of these isolates from the highest dilution would suggest that such spore formers are in large numbers (4.28 $\log_{10}$ to 6.98 $\log_{10} g^{-1}$ material) in at least five of the nine materials. In the absence of availability of biopesticides in remote villages, materials such as termitaria soil having 5.01 $\log_{10}$ antagonists $g^{-1}$ soil can potentially serve as a biopesticide. This needs further examination. Farmers in Africa use termitaria soil as an amendment to their agricultural fields and believe that it helps their crops[14]. Increased water infiltration in applied fields was reported as one of the advantage of this practice[15]. Termitaria soil has been reported to be rich in microbial activity and nitrogen[14]. This study suggested that the likely role of termitaria soil in pest management may also be an important contribution to the reported good crop growth in the fields using termitaria soil.

Overall, these studies revealed that the spore-forming bacteria (*Bacillus* spp.) were a major group among the antagonists present in the organic materials. There was a large diversity for cultural and microscopic traits in the 67 isolates, and no definite pattern in the different traits and extent/type of antagonism was noticed. None of the 11

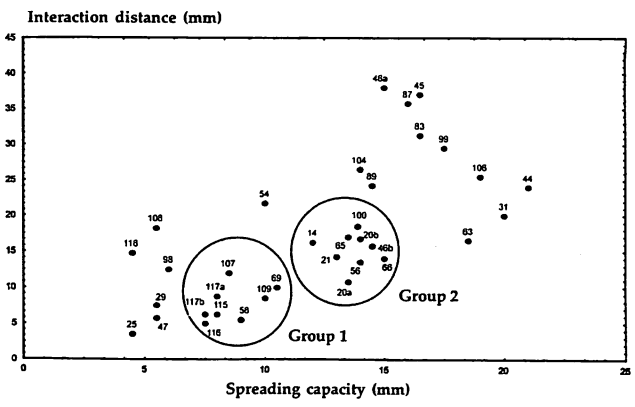


**Fig 2.** Cluster analysis of growth of potential biocontrol bacteria (spreading capacity) and interactions between rhizobia and potential biocontrol bacteria. Digits near the dots are strain numbers.

isolates studied using BIOLOG was *B. thuringiensis* (Bt) suggesting that majority of the isolates are different than Bt, a bacterium widely used by biopesticide industry. It also suggests the richness of the 67 isolates that can be explored as biopsticides. The study suggested that use of the organic materials may be playing an important role in protecting crops of organic farmers from pests and supports their belief of lower pest-incidence at their farms than their neighbor 'mainstream' farmers. More studies will be needed to confirm this belief.

**Acknowledgment**

We thank Dr M. L. Prasad of Sujay Agri Labs, Vijayawada, Andhra Pradesh, India for providing reference cultures used in the study, Mr S Veera Reddy and Dr D V R Reddy, ICRISAT for help on using microplate reader; Dr S Chandra, ICRISAT for verification of statistical aspects and suggestions to improve presentation of data; and Dr S D Singh and Dr R P Thakur of ICRISAT, and Dr. P. Tauro, Professor Emeritus, Mangalore, India for comments on the manuscript.

**References**

1. Chet I & Inbar J (1994) Biological control of fungal pathogens. Appl Biochem Biotech 48:37-48.
2. Fukuoka M (1993) The Theory and Practice of Green Philosophy. In: The Natural Way of Farming, (translation form Japanese by F.P. Metrand), Bookventure, Madras, India, 284 p.
3. USDA (1980) Report and recommendations on organic farming. United States Department of Agriculture, Study Team on Organic Farming, 94 p.
4. Proctor P, Cole G & Lyons T (1997) Making Biodynamic Farming and Gardening Work. In: Grasp the Nettle, Random House, Ltd., New Zealand, 176 p.
5. Rupela OP, Krajewski M, Gopalakrishnan S & Sriveni M (2003) A novel method for the identification and enumeration of microorganisms with potential for suppressing fungal plant pathogens. Biol Fertil Soils 39: 131-134.
6. Johnston A & Booth C (1983) Plant Pathologist's Pocket Book, Commonwealth Agricultural Bureau, England, 439 p.
7. Pelczar Jr MJ, Bard RC, Burnett GW, Conn HJ, Jennison MW, Mc Kee AP, Warren J, Weeks OB & Weiss FA (1957) Manual of Microbiological Methods. Society of American Bacteriologists and Mc Graw Hill Book Company, New York, pp 10-36.
8. Rupela OP, Kumar Rao JVDK, Sudarshana MR, Usha Kiran, M & Anjaiah V (1991) *Rhizobium* germplasm resources at ICRISAT Center. Research Bulletin no.15. Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics. 32 p.
9. Dalton H (1980) The cultivation of diazotrophic microorganisms. In: Methods for Evaluating Biological Nitrogen Fixation (ed. F. J. Bergersen), John Wiley & Sons Ltd. Brisbane, pp 13-64.
10. www.biolog.com. Microlog microbial identification system. BIOLOG, Hayward, USA.
11. Robertson EB & Firestone MK (1992) Relationships between desiccation and exopolysaccharide production in a soil Pseudomonas spp. Appl Env Microbiol 58:1284-1291.
12. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST & Williams W (1994) Endospores forming rods and cocci. In: Bergey's manual of determinative bacteriology, (Kreig NR & Holt JG eds) 9th edn. Baltimore/London, pp 559-561.
13. Sushma YVN (2000) Biological control of *Helicoverpa armigera* (Hubner) using microorganisms and plant products. M.Sc. thesis submitted to the Nagarjuna Univeristy, Andhra Pradesh. 47 p.
14. www.bangor.ac.uk/~azs80f/556_Land-Husbandry/2002-Assessments/termites.doc, dated 22.10.2003. Influence of termites on soil properties, Franco Cube Kalisto.
15. Mando A, Stroosnijder L & Brussaard L (1996) Effects of termites on infiltration into crusted soil. Geodema 74: 107-113.

*Received 28 October 2003; final revision 11 June 2004 and accepted 15 June 2004.*