Kasetsart J. (Nat. Sci. Suppl.) Vol. 24, : 32-40 (1990)

Antagonistic Bacilli for Biological Control of *Fusarium moniliforme*

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ABSTRACT

Screening of antagonistic *Bacillus* spp. against *Fusarium moniliforme*, the causal agent of sugar cane root and stem rot, was attempted. Nine soil samples containing decomposed plant debris and grasses collected from different places were the sources of the bacteria. As the soil samples were taken during summer, the number of aerobic spore forming bacilli did not exceed 10^5 colony forming units per gram of the soil. However, all of the soil contained antagonistic bacilli. No fewer than 10 different bacillus isolates, with respect to their cultural characteristic, were collected as satisfactory strains for further study on biological control of *Fusarium moniliforme*.

INTRODUCTION

During the past years of teaching practical general microbiology at Kamphaengsaen Campus, Kasetsart University on enumeration of microorganisms in soil samples, it often occurred that there existed antagonistic activity of bacilli against certain molds on agar culture medium. Some bacilli were air-borne contaminants. These observations indicate that antagonistic bacilli may be ubiquitous. Bacilli are spore forming bacteria. They can survive in the soil for many years. Most of them are non fastidious with respect to nutritional requirement. Therefore, they may be ideal organism for biological control. Cook and Baker (1983) reported uses of *Bacillus* spp. in biological control. of plant pathogens. Recently, there were many studies which attempted to employ *Bacillus* spp. for biological control of plant pathogenic fungi, (Singh and Mehrotra, 1980; Gueldner et al. 1988; Handelsman, *et al.* 1988; Ryter *et al.* 1989; Rovertti, 1989; Utkhede, 1989).

Korpraditskul (personal communication, Korpraditskul, V. Department of plant pathology, Faculty of Agriculture, Kasetsart University, Thailand) recently reported that sugar cane farming was severely affected by root and stem rot disease caused by *Fusarium moniliforme*. The disease had become a new problem for sugar cane farmers in many areas in Thailand. Therefore, it was of particular interest to study the antagonism of bacilli against *Fusarium moniliforme*. For the present communication, the number of bacilli on certain soil samples, number of antagonistic species and their antagonistic activity against *Fusarium moniliforme* are reported.

MATERIALS AND METHODS

Soil samples as the sources for the isolation of antagonistic bacilli. Nine soil samples, in which grasses or plant parts were decomposed, were collected from places within kamphaengsaen and Bangkhen Campuses of Kasetsart University.

***Fusarium moniliforme* and its maintenance.** The fungus which was isolated from rot sugar cane was kindly provided by Dr. Vichai Korpraditsakul of Plant Pathology Department, Faculty of Agriculture, Kasetsart University. It was maintained on a corn meal agar at ambient temperature and kept in a refrigerator.

Nutrient agar (N.A.) was prepared in the laboratory. It contained the following composition : Beef extract (Gibco Lab. U.S.A.), 3 g; balanced peptone No.1 (Topley House) 5 g; agar powder, 15 g; water, 1 litre.

Potato dextrose agar (PDA) was of the following composition : The extract of fresh potato of 200 g, 20 g of glucose and 15 g of agar per litre.

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Enumeration of total viable number of bacteria and of aerobic spore forming bacteria in soil samples.

N.A. or Soil extract agar (SEA) was employed for the enumeration of both total viable bacteria and aerobic spore formers. The composition per litre of SEA was as follows; peptone, 1 g; K_2HPO_4,0.5 g; yeast extract, 1.0 g; $(NH_4)_2HPO_4$, 0.5 g; $MgSO_4.7H_2O$, 0.05 g; $FeCl_3$, 0.01 g; $CaCl_2.2H_2O$, 0.1 g; agar, 15 g; soil extract, 250 ml; pH 6.5-7. The preparation of soil extract was as follows : To1,000 g of soil added 1,000 ml of tap water and steamed in an autoclave for 20 minutes. Added approximately 0.5 g of $CaCO_3$ to flocculate colloidal material for an overnight. Clarified liquid was collected for use.

Standard dilution plate count method was followed. The soil samples were ten fold serially diluted. Triplicate aliquots of known volume from each dilution were cultured by shaked plate method. The set of triplicate plates containing 30-300 colony forming units (CFU) were counted for the average.

With respect to the enumeration of aerobic spore forming bacteria, non spore formers and vegetative cells were killed by heating the soil dilutions in test tubes in a boiling bath for 10 minutes prior to plating.

Purification of isolates of spore formers. Cross streak plate technique was employed for the isolation of pure culture of spore forming colonies from plates of highest dilution of each soil sample. Choices of the colonies were randomized. Either soil extract agar or nutrient agar was employed for this purpose.

Maintenance of spore forming isolates. The bacterial isolates were cultivated on slope nutrient agar in test tubes for 3-5 days at ambient temperature. They were then kept in a refrigerator.

***In vitro* test of antagonistic activity of spore formers against *Fusarium moniliforme*.**

Active cultures of both *Fusarium moniliforme* and each bacterium were inoculated on the surface of a PDA plate. The mold was inoculated first at the center of the petri-dish and the bacterial culture was then streaked in two parallel lines 2.5-3 centemeters away from the mold inoculum on each side. With these manners of inoculation, the fungus grew in one large colony if there was no inhibition while the bacterium grew in 2 more or less parallel lines. Zones of inhibition and alteration of the fungal colony were observed after 5-7 days of incubation at ambient temperature when the mold fully grew.

RESULTS AND DISCUSSION

Soil samples and numbers of total viable bacteria and of spore formers are shown in Table 1. It is necessary to note that the soil samples listed in Table 1 were all collected during late March to the first week of May 1989. Most soil samples except number 9 was rather dry. Hence, the total numbers of bacteria (Table 1) seem to be rather low. However, these results are agreeable with that described by Alexander (1977).

The numbers of spore forming bacteria were also low in these soil samples. There were no greater than 2.1 x 10^6 CFU/g and were only .04-6.7% ofthe total bacterial numbers. Albeit, it is interesting to note that the isolates which were found to be antagonistic to *Fusarium moniliforme* could be obtasined in all soil samples tested. More interesting, almost all isolates obtained from the rhizosphere of banyan tree were antagonistic.

Data on the studies on morphological and some cultural characteristics of these spore forming bacteria (though data are not shown herein) indicated that they were spore formers. All produced catalase. Some of them could multiply at 55° C. They grew aerobically. Hence, they were identified as the genus Bacillus after the Bergey's Mannual of Determinative Bacteriology (Buchanan and Gibbons, 1974).

As shown in Table 1 only a portion of colonies of spore formers in the plate of highest dilution of each soil sample were collected and isolated pure for the studies on their antagonistic activity against *Fusarium moniliforme*. Altogether there were 95 isolates. These bacteria and other 55 Bacillus isolates collected some other time from the soil at Kamphaengsaen Campus were studied for their antagonistic activity. Some results are shown in Figure 1. The cultural characteristics of the Bacillus indicates that there may be no fewer than 10 different kinds of Bacillus able to control *Fusarium moniliforme*. Figures 1.1-1.13 indicate that different bacterial isolates may have different mode of action of *Fusarium monilliforme*. The modes of action of the bacteria and the response of the fungus should be further studied for the right Bacillus for biological control of *Fusarium moniliforme*. Identification of the bacilli should also be studied.

Table 1 Total viable bacteria, spore forming bacteria and number of isolates collected for the test of antagonistic activity form nine soil sample

Soil sample			Total viable bacteria/g	Total spore former/g	%spore formers	Number of spore forming isolates	
Number designated	location of collection	Remarks				collected	showing antagonistic activity
1	Bangkhen Campus	Under dry leaf pile, aggregated	$1.9x10^7$	$(2.6\text{-}6.9)x10^4$	0.13-0.36	11	6
2	Bangkhen Campus	Under dry grass pile, aggregated	$6.5x10^6$	$4.6x10^4$	0.70	7	not counted
3	Kamphaengsaen Campus composted plant debris	Banyan tree rhizosphere with	$4.8x10^7$	$7.1x10^4$	0.15	8	7
4	Kamphaengsaen Campus from where natural grasses were decomposed, aggergates looked as if they were earth worm droppongs	Dry aggregated soil collected	$3.1x10^7$	$(0.5\text{-}2.1)x10^6$	1.5-6.7	9	5
5	Kamphaengsaen Campus in small crumps	Banyan tree rhizosphere, dry,	$2.4x10^7$	$(0.3\text{-}1)x10^5$	0.13-0.41	12	11
6	Kamphaengsaen Campus	Containing plant debris	$2.3x10^6$	$6.5x10^4$	2.82	14	2
7	Kamphaengsaen Campus	Mango tree rhizosphere,moist	$4.9x10^7$	$8.8x10^4$	0.18	12	4
8	Kamphaengsaen Campus and decomposed plant debris	Dry soil mixture of aggregates	$2.6x10^7$	$2.2x10^4$	0.08	14	3
9	Kamphaengsaen Campus	Containing decomposed hemp	$7.7x10^8$	$3.2x10^4$	0.04	8	2

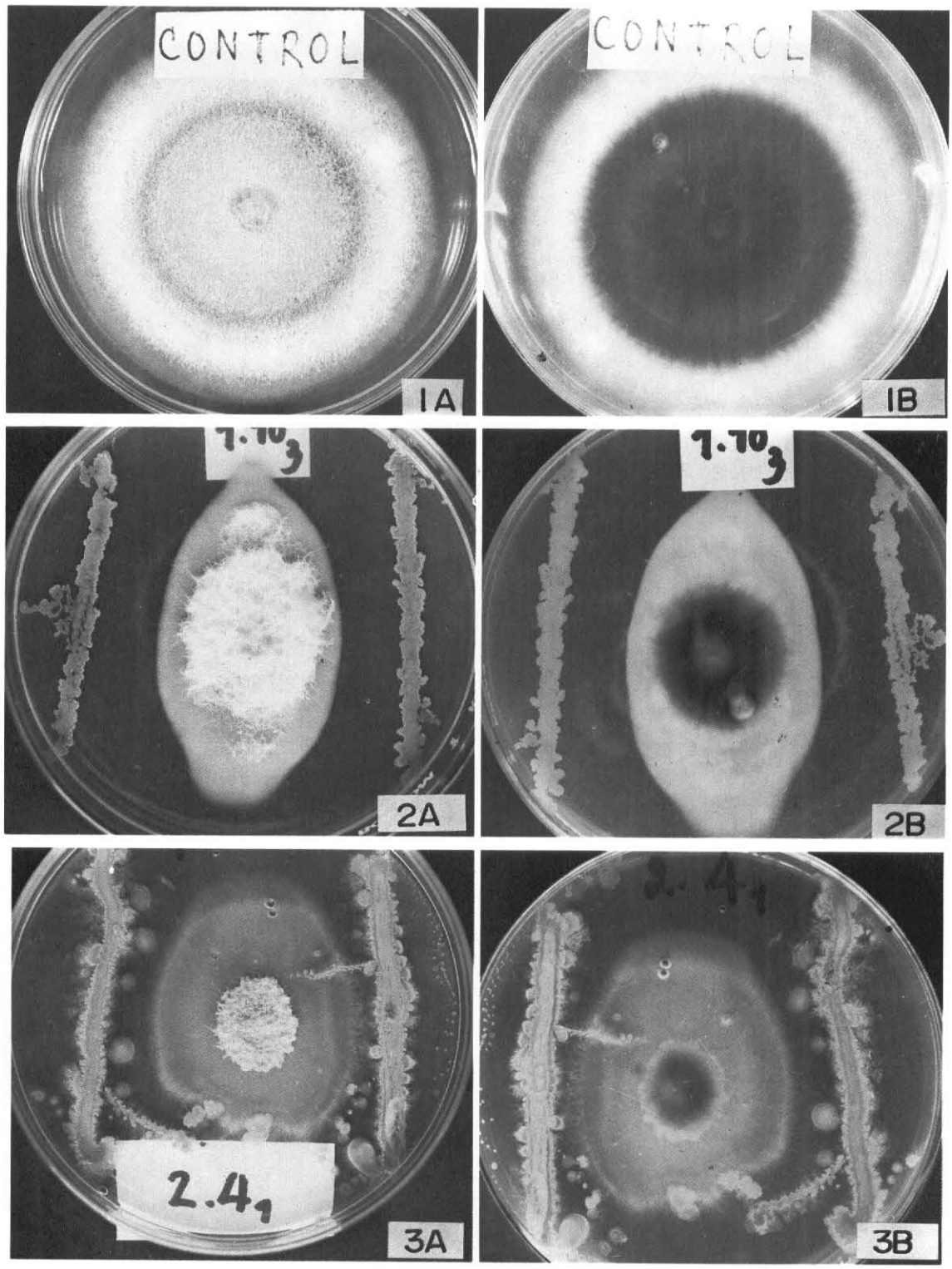

Fig. 1-3 *In vitro* inhibitory effect on vegetative growth of *Fusarium moniliforme* by 13 isolates of *Bacillus spp.*
(A) photograph taken from the surface of the colonies
(B) photograph taken from the reverse of colonies.

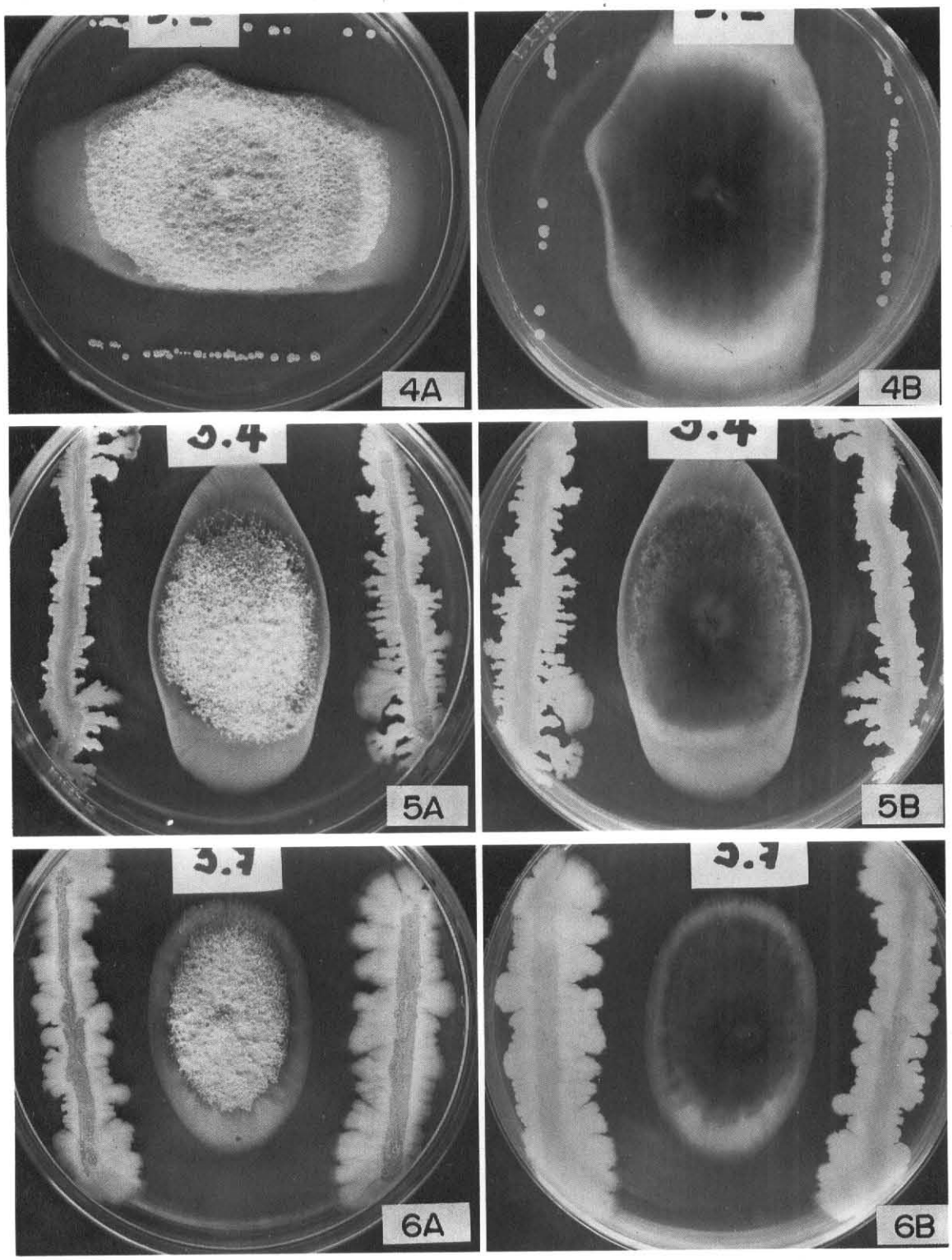

Fig. 4-6 *In vitro* inhibitory effect on vegetative growth of *Fusarium moniliforme* by 13 isolates of *Bacillus spp.*
(A) photograph taken from the surface of the colonies
(B) photograph taken from the reverse of colonies.

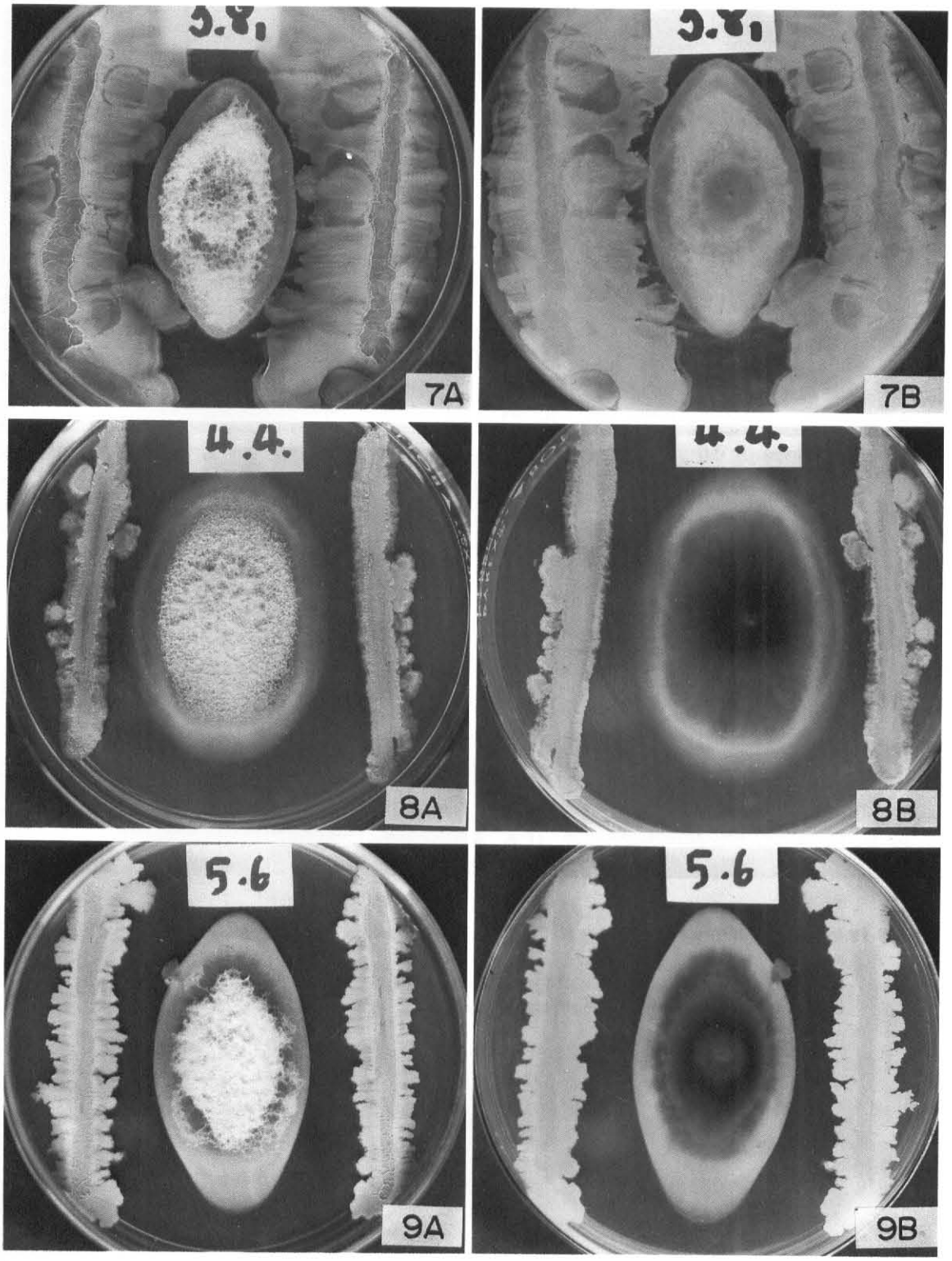

Fig. 7-9 *In vitro* inhibitory effect on vegetative growth of *Fusarium moniliforme* by 13 isolates of *Bacillus spp.*
(A) photograph taken from the surface of the colonies
(B) photograph taken from the reverse of colonies.

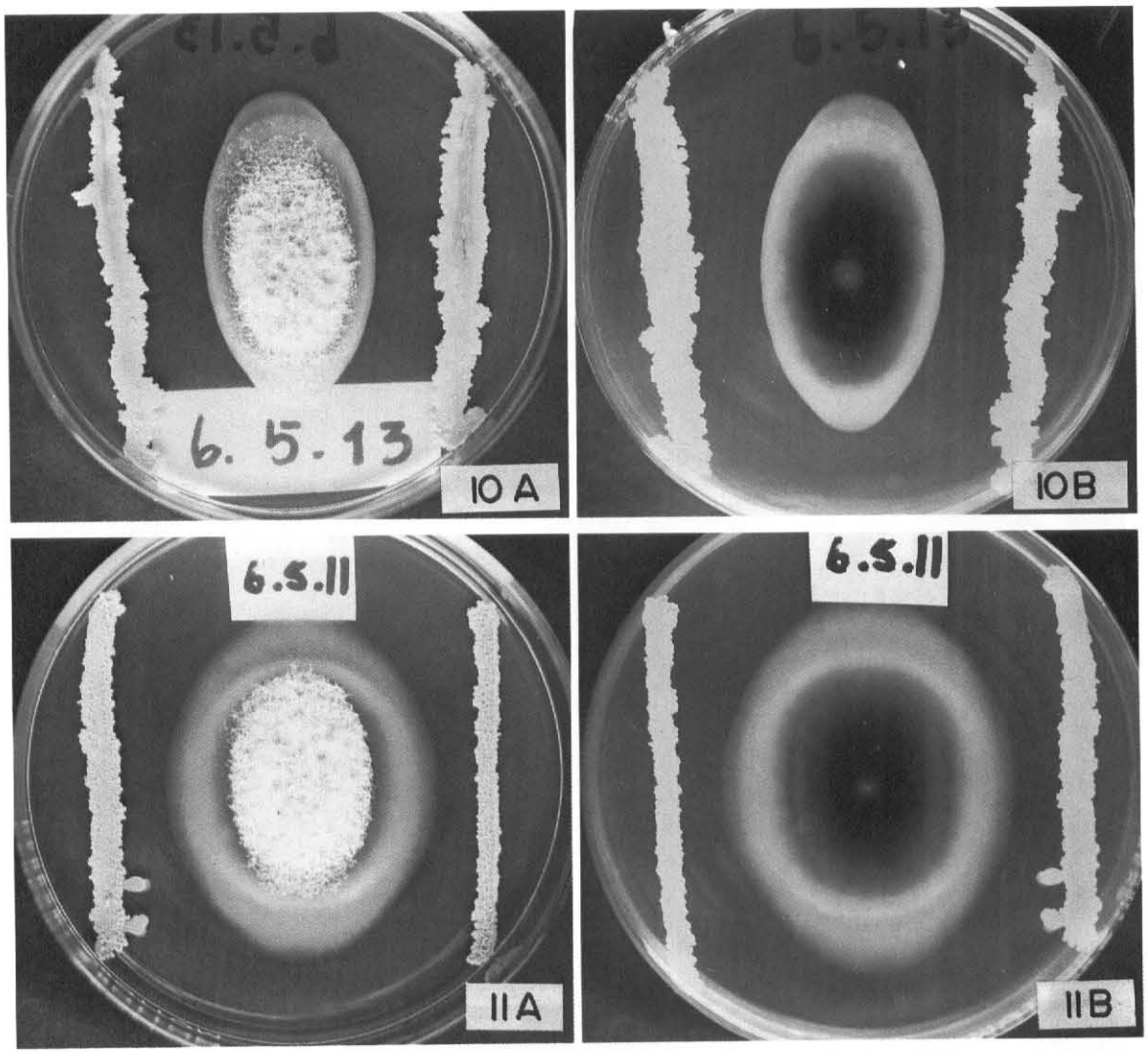

Fig. 10-11 *In vitro* inhibitory effect on vegetative growth of *Fusarium moniliforme* by 13 isolates of *Bacillus spp.*
(A) photograph taken from the surface of the colonies
(B) photograph taken from the reverse of colonies.

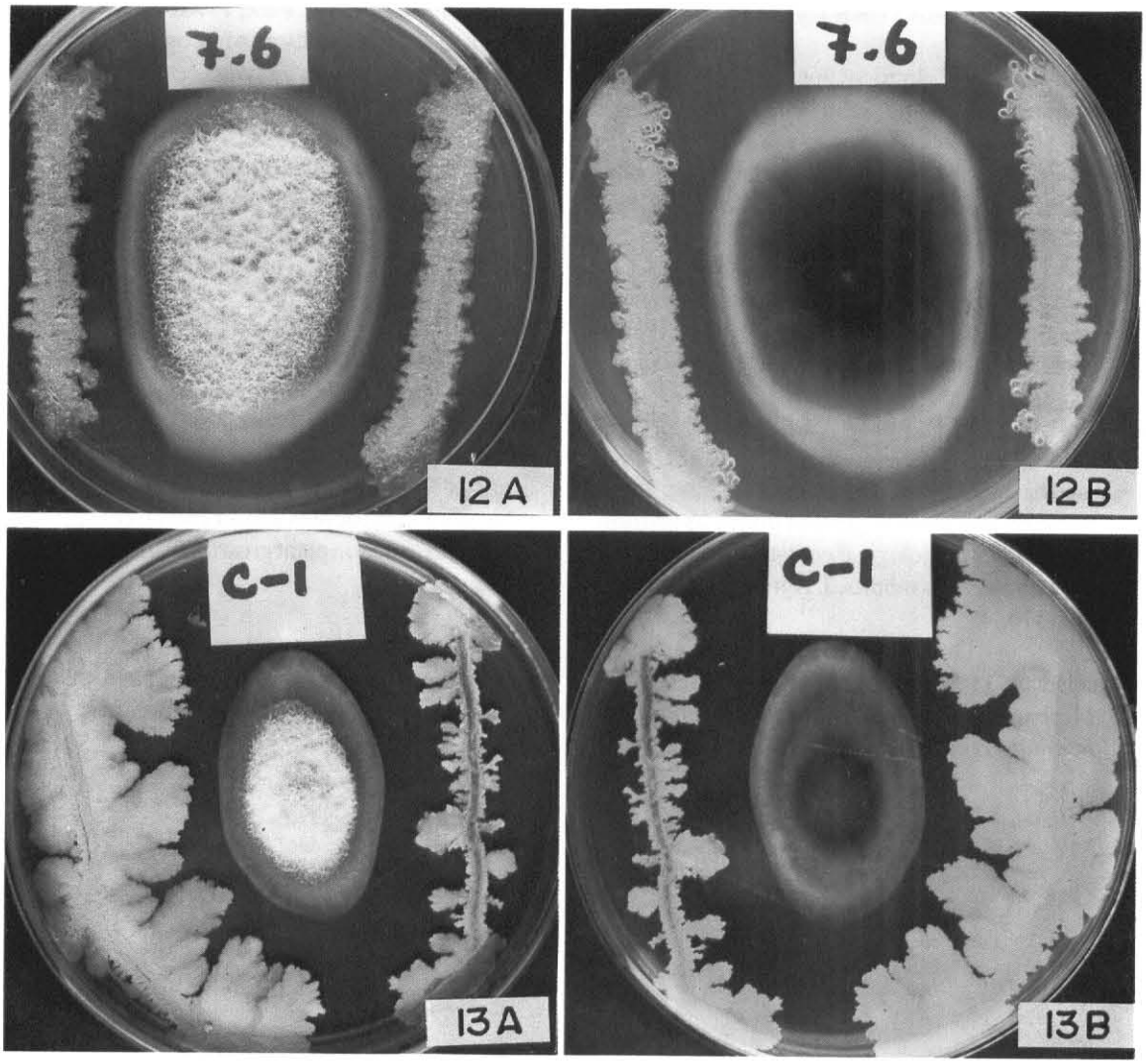

Fig. 12-13 *In vitro* inhibitory effect on vegetative growth of *Fusarium moniliforme* by 13 isolates of *Bacillus spp.*
(A) photograph taken from the surface of the colonies
(B) photograph taken from the reverse of colonies.

LITERATURE CITED

Alexander, M. 1977. Introduction to Soil Microbiology, 2nd, ed, 467 pp. John Wiley & Sons.

Buchanan, R.E. and N.E. Gibbons, 1974. The Bergey's Manual of Determinative Bacteriology, 8th. ed. The Williams and Wilkins Co.

Cook, R.J. and K.F. Baker, 1983. The Nature and Practice of Biological Control of Plant Pathogens. The American Phytopathological Society.

Gueldner, R.C.; C.C. Reilly; P.L. Pusey; C.E. Costello; R.F. Arrendale; R.H. Cox; D.S. Himmelsbach; F.G. Crumley and H.G. Cutler. 1988. Isolation and identification of iturins as antifungal peptides in biological control of peach brown rot with *Bacillus subtilis*. J. Agric. Fd. Chem. 36. 366-370.

Handelsman, J.; E.H. Mester, and S. Raffel, 1988. Mechanism of Biocontrol of *Phytophthora* by *Bacillus cereus* UW 85. Cited in S.G. Gilbert, J. Handelsman and J.L. Park, 1990 Role of Ammonia and Calcium in Lysis of Zoopores of *Phytophthora cactorum* by *Bacillus cereus* Strain UW 85. Experimental Mycology 14, 1-8/

Roveratti, D.S. 1989. *Bacillus thuringiensis*-a new perspective for an induced protection of coffee leaf rust. Journal of Phytopathology 126, 149-159.

Ryter, J.L.; F.L. Lukezic; R.Craig and G.W. Moorman, 1989. Biological control of Geranium rust by *Bacillus subtilis*. Phytopathology 79,367-370.

Signh, P.J. and R.S.Mehrotra. 1980. Biological control of *Rhizoctonia bataticola* on grain by coating seed with bacillus and *Streptomyces* spp. and their influence on plant growth. Plant and Soil 56, 475-483.

Utkhede, R.S. 1989. Evaluation of *Bacillus subtilis* for potential control of apple replant disease, Journal of Phytopathology 126, 305-312.