General papers

The study on plant disease biocontrol by using antagonistic bacteria

Li Debao, Zhu Weiguang, Chen Xiongfeng, Chen Weiliang, Zhong Jingping, Xu Ping

(Institute of Biotechnology, Zhejiang Agricultural University, Hangzhou 310029, P. R. China)

Abstract

One hundred and thirty antagonistic bacteria were screened out, most of them belonged to *Bacillus* spp., showing very strong inhibitive effect to various plant pathogens. Eight antagonistic proteins(peptides) were purified (P11—I, P11—II, B8, B826—I, B826—II, A30—I, A30—II, G35). Two antagonistic protein related DNA fragments (B826—I, A30—II) were cloned and sequenced. B826—I DNA fragment composed by 906bp, it contains two ORF encoding 95, 53 amino acids respectively. By using Rif^r, Kam^r as the selective markers, we found the bacteria could colonize on rice leaf for at least 40 days. In greenhouse the antagonistic bacteria show certain degree control efficacy.

Key words: Biological control; Plant pathogen; Antagonistic bacteria; Antagonistic proteins

A brief review on the using of antagonistic bacteria

Biological control of plant diseases is to control plant diseases by directly introduction of the microbic antagonists to inhibit the pathogens. It can overcome the disadvantage of the using of chemical pesticides. People aware its potential prospect, more and more attentions are paid on this field. There are many reports on successful control of plant diseases by *Trichoderma* spp., *Pseudomonas* spp., *Streptomyces* spp., *Agrobacterium* spp., *Bacillus* spp. and other antagonists or their antagonistic substances. For examples, Peng, Y. F. et al.[17] applied a mutant strain of *Pseudomonas fluorescens* to control wheat take—all in the field successfully.

Kerr, A [9] first reported the successful application of *Agrobacterium radiobacter* K84 to control peach canker in Australia, following succeeded in United States and Greece. He purified a bacteriocin, Agricin K84 ,from strain K84. Later, Imler, J. K. also isolated a bacteriocin, Syringacin 4—A, from *Pseudomonas syringae* pv. *syringae* 4—A. Syringacin 4—A could protect soybean from the infection of *Pseudomonas phaseolicola* and increase the germination of soybean seeds[3].

Some mechanisms of antagonism have been studied. Konisky[11] found that bacteriocin combined with the receptors on the surface of sensitive bacteria and stimulated the specific transportation system on the membrane of protoplasm, as a result, it inhibited various physiological activity , the cell became unstable. Hardy[6], Holland[7] reported that colicin increased the permeability of cell membrane, made the K^+ move outside of the cell, decreased the ATP concentration, inhibited the movement of amino acids, saccharides and killed the cells. Obviously, different mechanisms are involved in the bacteriocin activities.

Many bacteriocins, which produced by the genus of *Escherichia*, *Bacillus*, *Agrobacterium*, *Streptococcus*, *Clostridium*, *Rhizobium*, *Klebsiella*, *Erwinia* and etc. , have been known as plasmid— encoded. Pugsley, A. P. [18] identified the bacteriocin—related region in plasmid colN and cloned it into pBR322. Michael, A. et al. [13] have transformed the genes of megacin A—216 and A—192134 into *Bacillus megaterium* VT—1660. San Millen, J. L. et al. [22] reported that four regions on the plasmid pMCC B17 were related to the production of microcin 87.

Bacillus spp. are not important plant pathogens but potential biocontrol agents. People become interested in the study on biocontrol by *Bacillus* spp. In many reports, *Bacillus* spp. were directly applied to control various diseases of different crops in different areas, especially to control the plant diseases caused by soil— born fungi. Some works involved in the study on antagonistic substances.

Goodman found *B. cereus* could produce bacteriocin which inhibited plant pathogen. Guldner, R. C. [5] purified the antagonistic substance "Iturins" from *B. subtilis* which could inhibited *Monilinia fructicola*, the pathogen of peach fruit brown rot. Pusley, P. L. et al. [19,20] reported *B. subtilis* could control the above disease in post— harvest storage stage, and inhibit the root rot of soybean and *Rhizoctonia solani* as well.

Turner, J. T. and Backman, P. A. [28] studied the antagonistic mechanism of *Bacillus* spp. to fungi. They found that when the peanut seeds were treated with *B. subtilis*, the bacteria could colonize on the roots of peanut, increased resistance of peanut to stress, improved the germination of seeds and the peanut's nutrition, finally decrease the root rot caused by *R. solani*. Choe[2] reported that *Phytophthora* blight of green pepper was suppressed by *Bacillus* spp. , and which could retained their activity at least 1 month after application.

Ferreira et al. [4] reported that the extract of *Bacillus* spp. could inhibit the mycelia growth and ascospore germination of *Eutypa lata*. Tschen[27] reported *B. subtilis* F—29—3

could inhibited the growth of *R. solani*. The extract of F—29—3 culture contained bacilycin and fengycin, and could limit the expansion of local lesion, moreover it is stable in soil.

B. subtilis NCIMB could inhibit *Xanthomonas campestris* pv. *phaseoli*. An active substance was purified from the bacterial culture after precipitated by acid, butanol, the extract could also inhibit *Botrytis cinerea*. Lux plasmid are going to transform *B. subtilis* for further study[8].

Most reported works on plant disease biocontrol were taken in plate test or pot test, and some in field, all of which showed different degrees of control efficacy (Table 1).

Table 1 Incomplete plant pathogens which were inhibited by *Bacillus* spp.

Alternaria radicina (carrot)	Hentschel, K. D. (1992)
Alternaria solani (tomato)	Wu, Wei—shi et al. (1993)
Botrytis cinerea (apple)	Ferreira, J. H. S. (1993) Holliday, G. et al. (1993)
Claviceps fusiformis	Mahadevamurthy, S. et al. (1988)
Colletotrichum gloeosporioides	Yang, Z. Z. (1990)
C. corchrium (jute)	Purkastha, R. P. (1989)
Dothiorella gregaria (to cankers)	Jin, J. R. et al. (1989)
Erwinia carotovora	Wang, J. S. et al. (1989),
Eutypa lata (grapevine)	Ferreira, J. H. S. et al. (1991)
Fusarium oxysporium f. sp. *ciceris* (cicer wilt)	Dhedhi, B. M. et al. (1990)
Fusarium oxysporium f. sp. lycopersici (tomato wilt)	Kaporr, I. J. et al. (1989) Purkastha, R. P. (1989)
F. solani (sunflower, soybean, *Vinga*, abelmoschus, carnation)	Obleglo, U. et al. (1990) Sarhan A. R. T. (1989)
Macrophomina phaseolina (soybean, *Vinga*.)	Ehteshamul—Hague, S. et al. (1990)
Monilinia fructicola (peach)	Guldner (1988)
Phytophthora capsici (pepper)	Kim, Y. K. et al. (1988) Choe, J. S. (1991)
Penicillium digitatum (citrus)	Ni, H—F et al. (1993)
Pseudomonas solanaceanum (tomato)	Phao, C. G. et al. (1990)
P. parasitica var. *nicotianae* (tobacco)	Handelsman, J. et al. (1991)
P. phaseolicola	Imber, J. K. (1976)
Pyricularia oryzae (rice)	Mizubuti, E. S. G. et al. (1993)
Pyrenophora tritici (wheat)	Mehdizadegan, F. et al. (1987)
Rhizoctonia solani (peanut, chrysanthemum potato, bean, pea, rice, rape—seed)	Wu, W. S. et al. (1990) Fiddaman, R. J. et al. (1993) Tschen, J. S. M. (1987) Turner, J. T. et al. (1991) Yang, Z. Z. (1990) Tong, W. H. et al. (1993)
R. betaticola (sugar—beet)	Byadgi, A. S. et al. (1988)
Sclerotium rolfsii	Singh, R. K. et al. (1987)
Streptomyces scabies (potato)	Schmiedeknecht, G. et al. (1993)
Uromyces appendiculatus (bean rust)	Mizubuti, E. S. G. et al. (1993)
Xanthomonas campestris pv. *phaseoli* (bean)	Holliday, G. et al. (1993)
Nematodes—*Melidogyne incognita*	Chahal, V. P. S. et al. (1993)

The study of antagonistic proteins of bacteria to *Xanthomonas oryzae* pv. *oryzae*

Rice bacterial leaf blight is a severe disease in the rice planting area. The bacteria invades plants through rice leaf water pore or wound especially after flood and heavy raining.

There is still no effective method to control it. The study on plant disease biocontrol by using antagonistic bacteria or antagonistic substances of bacteria has been made greatly progress since 1972. As mentioned above, the development of molecular biology and the success in transgenic plant bring a new approach for crop improvement, the combination of traditional biocontrol with genetic engineering may provide a new way for diseases management.

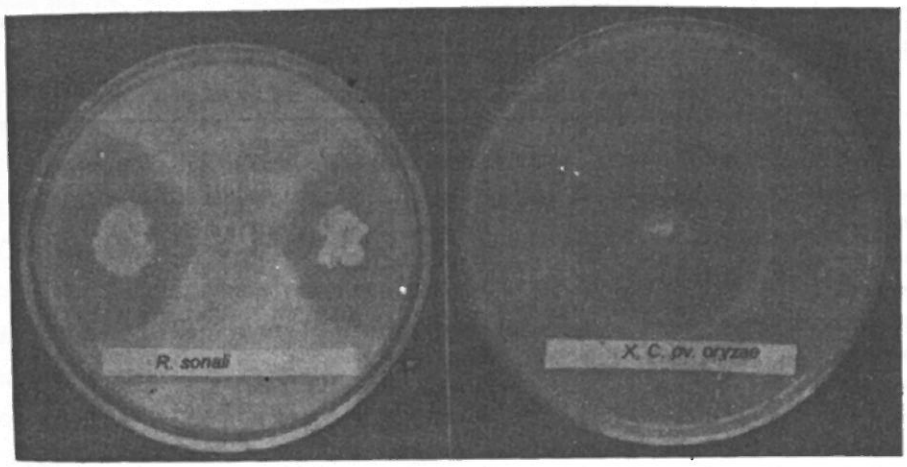

Fig. 1 The inhibiting zone of *Bacillus subtilis* to plant pathogens in plate tests

The program of "the study of antagonistic proteins of bacteria to *X. oryzae* pv. *oryzae*" has been studied in our laboratory since 1986. Attempts were focused as following:

(1) Screening antagonistic bacterial strains from rhizosphere, phyllosphere, atmosphere and etc., and studying their potential use in control of crop diseases.

(2)Purification and analysis of some antagonistic proteins from those antagonistic microbe,especially to chromosome coding or plasmid coding ones. Here, we prefer the term of "antagonistic substances(proteins)" rather than "bactericin".

(3)Characterization and cloning of antagonistic protein genes.

(4)Preparation for the directly use of antagonistic bacteria in the field. Study on the colonization of these antagonistic bacteria.

(4)Transforming the antagonistic protein genes into rice crops plant to get disease— resistant one, or into the dominant bacteria colonizing on the rice leaf to obtain genetic engineered bacteria and use them in the practical control.

1. Screening the antagonistic bacterial strains

More than 800 bacterial isolates were collected and tested for their antagonistic activity to *Xanthomonas oryzae* pv. *oryzae* and other plant bacteria as well as fungi pathogens.

One hundred and thirty antagonistic bacterial strains were screened out, most of them belonged to *Bacillus* spp. They could inhibit the growth of *Xanthomonas oryzae* pv. *oryzae* and other pathogens (Table 2,Fig. 1). Table 3 showed the antagonistic effects of strain B8,

B826 , P11, G35 on ten tested indicator strains of *X. oryzae* pv. *oryzae* in plate test. It shows the antagonistic specificity even to different strains.

Table 2 The inhibiting spectrum of some antagonistic bacteria to following plant pathogens

	B8	B826	A30	B034	P11	G35	31
Xanthomonas oryzae pv. *oryzae*	++++	++++	++++	++++	++	++	+
Xanthomonas oryzae pv. *oryzicola*	++	+++	+++	/	++	/	+
Pseudomonas solancearum	++	++	++	/	++	++	+
Pseudomonas fluorescent	−	+++	+++	/	/	/	/
Pseudomonas syringas	/	/	/	/	++	+	+
Erwinia carotovora	−	/	+++	/	++	+	+
Corynebacterium spp.	−	+++	/	/	/	+	+
Bacillus subtilis	++	/	/	/	−	/	+
Agrobacterium tumefaciens	++	++	/	/	/	/	/
Rhizoctonia salani	−	−	++	−	+	/	+
Fusarium spp.	−	−	++	−	/	/	/
Sclerotium rolfsii	−	−	++	/	/	/	/
Pyricularia oryzae	−	−	++	/	/	/	/

Table 3 The antagonistic effect of bacteria to different strains of *X. oryzae* pv. *oryzae**

	COX1	COX2	COX3	COX4	COX5	COX6	COX26	COX61	COX17	COX18
B8	3.4	4.0	3.0	4.0	3.2	3.7	3.5	3.4	3.5	3.4
B826	3.0	3.5	3.0	3.5	2.9	3.0	3.0	2.8	2.5	3.0
P11	2.8	2.5	3.0	3.0	2.8	2.8	3.0	2.7	2.4	2.7
G35	1.3	1.5	1.5	2.2	1.9	1.5	2.2	1.4	1.0	1.2

* Numbers in the table were the radius zone(cm) of inhibition

Most of the isolated antagonistic bacteria could also inhibit the growth of several other pathogens, such as *X. campestris* pv. *campestris*, *X. oryzae* pv. *oryzicola*, *Pseudomonas solanacearum*, *Erwinia carotovora* subsp. *carotovora*, *Rhizoctonia solani*, *Pyricularia oryzae* and etc..

Six of them, identified as *Bacillus subtilis* B826, P11, *B. cereus* G35, *Bacillus* sp. A30, B034 and *Enterobacter cloacae* B8, were selected for the further study because of their strong inhibiting activities and wide antagonistic spectrum. For example, strain A30 could inhibit *X. oryzae* pv. *oryzae*, *X. oryzae* pv. *oryzicola*, *X. campestris* pv. *campestris*, *Pseudomonas solanacearum*, *Erwinia carotovora* pv. *cartovora*, *Rhizoctonia solani* (rice and cotton) , *Fusarium oxysporium*, *F. vasinfectum*, *Pyricularia oryzae* and etc..

2. Analysis of antagonistic protein

Several antagonistic proteins (peptides) were purified with ion—exchange column chromatography, gel filtration chromatography and HPLC with a fractional collector (Fig. 2, 3, 4). The purified antagonistic proteins showed very strong activities, 1—2 μg purified proteins could inhibited *X. oryzae* pv. *oryzae* very strongly in plate tests. All these purified proteins showed a single band on SDS—PAGE, which suggested that each protein was composed of

one homogeneous subunit. It was found that there were different kinds of antagonistic sub-

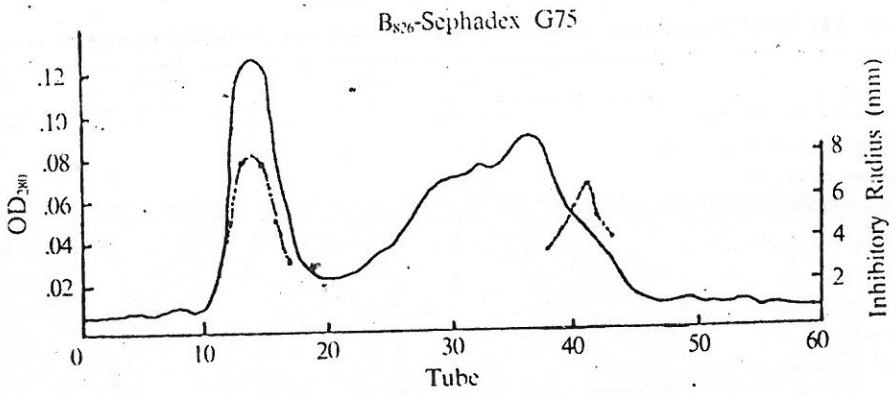

Fig. 2 Elution profile of B826 extraction on Sephadex G75——OD280……Antagonistic activity peak to *X. oryzae* pv. *oryzae*

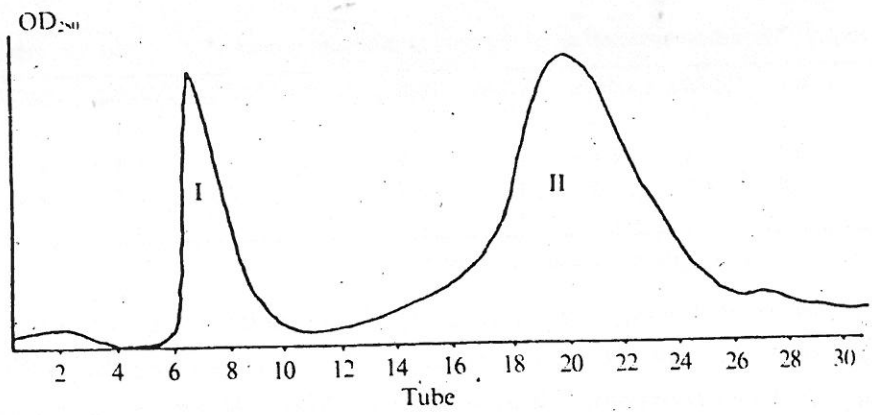

Fig. 3 Profile of the crude antagonistic proteins extract (after precipitated with ammonium sulfate) produced by B826 strain on Sephadex G75 (Mobile phase: 0. 02mol/L phosphate buffer, pH7. 2; Flow rate: 10 ml/30min. tube; Detector: 280nm)

stances in the antagonistic bacteria. Some bacterial strains could even produce different antagonistic substances which could inhibit the growth of *Xanthomonas* spp., *Pseudomonas solanacearum* (bacteria) , *Pyricularia oryzae*, *Rhizoctonia solani* (fungi) and etc., respectively. For example, B826 could produce two kinds of antagonistic proteins, both of them inhibited *X. oryzae* pv. *oryzae* . Whereas A30, P11 and other strains could produce more than two kinds

Table 4 List of the purified proteins (peptides)

Protein	Strain	MW
P11-I	*Bacillus subtilis* P11	14. 0 KD cloned
P11-II	*Bacillus subtilis* P11	10. 0 KD
B8	*Enterobacter cloacae* B8	5. 0 KD
B826-I	*Bacillus subtilis* B826	37. 0 KD cloned
B826-II	*Bacillus subtilis* B826	8. 0 KD
A30-I	*Bacillus* sp. A30	3. 0 KD
A30-II	*Bacillus* sp. A30	1. 3KD sequenced, cloned
G35	*Bacillus cereus* G35	3. 5 KD sequenced

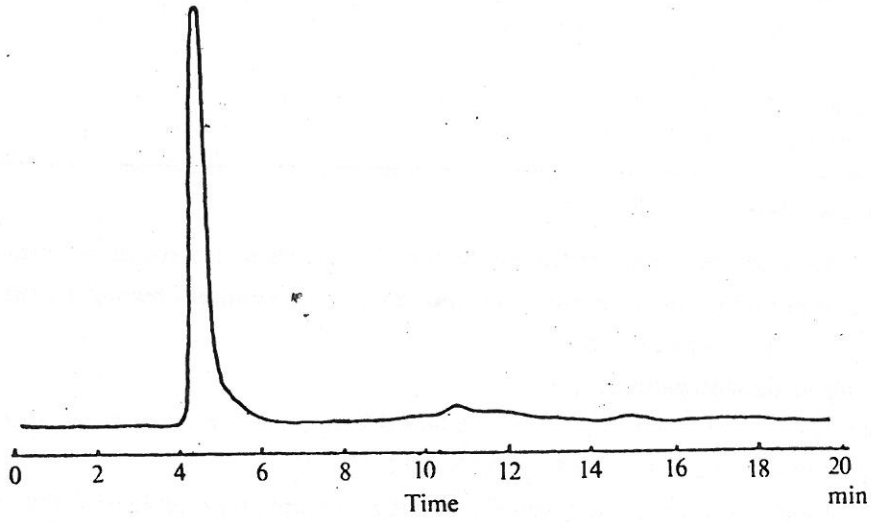

Fig. 4 Purified B826—I by HPLC(Pak—300s.w)

of proteins against *R. solani*, respectively(Table 4).

It was proved that these antagonistic proteins didn't have the properties of proteinase and amylase themselves.

Table 5 shows these protein properties of the antagonistic substances:

Table 5 Stability of antagonistic protein to the different treatments

	ProteinaseK	PronaseE	Trypsin	60 ℃ 30min	80 ℃ 30min	100 ℃ 15min	Phenol
P11—I	+	+	+	—	—	—	+
P11—II	+	+	+	—	—	—	+
B826—II	+	+	+	—	—	—	+
B034	+	+	+	+++	+++	+*	+

+:sensitive; —:stable; +++:partially sensitive; *:20min

The amino acid compositions and partial amino acid sequences of some antagonistic proteins (peptides) were shown in table 6 and 7.

Table 6 Amino acid composition of P11—I and P11—II

	Asp	Thr	Ser	Glu	Pro	Gly	Val	Ile
P11—I	11.9	4.76	7.14	9.54	4.76	11.9	7.14	4.76
P11—II	8.82	2.94	4.14	4.41	8.82	22.05	2.94	7.35
	Leu	Trp	Arg	Tyr	Phe	Ala	Cye	Lys
P11—I	2.38	9.52	7.14	11.9	7.14	—	—	—
P11—II	8.82	4.41	1.74	—	—	16.18	2.94	4.41

Table 7 Partial amino acid sequences of A30—I and G35

A30:	Met—Tyr—Met—Ile —Lys—Trp—Met—Arg—Thr
G35 *:	Fragment 1. Tyr—Trp—Ala—Asn—Lys
	Fragment 2. Ile—Leu—Gly—Trp—Ile—Ser—Tyr—Ser—Asn—Lys
	Fragment 3. Ser—Ile—Val—His—Pro—Arg

* Cooperated with Dr. Chen Z. L., Beijing Univ.

Besides the proteins, other antagonistic compounds were also found in many strains. For example, strain H31 could produce four antagonistic substances, two of them were non—protein substances, and dialysable.

3. Cloning of the antagonistic genes

(1)DNA library construction: Using lambda ZAPII/pBS cloning system, four DNA libraries have been constructed, i.e. B826, A30, P11, and B8.

(2)Antisera preparation: Rabbit polyclonal antisera were prepared against the antagonistic proteins of P11 and A30. The monoclonal antibody (McAb) to B826—I protein had been prepared.

(3)Immunological screening of specific recombinant clones: Using these antisera and McAb, we screened out the positive clones from the DNA library of antagonistic bacteria by IPTG inducing. 12 positive clones expressing B826—I protein, clone 9 which contained a 2.1 kb insertion fragment showed higher antagonistic activity against *X. oryzae* pv. *oryzae*, and the antagonistic protein expressed by clone 9 was identified by Western blot. 3 positive clones expressing A30 protein. All 3 positive recombinants from strain A30 had antagonistic activity when induced by IPTG. But 2 positive clones of P11 showed no antagonistic activity to *X. oryzae*.

(4)Analysis of the inserted fragments: The DNA insert of B826 in clone 9 is about 2.1 kb. The insert DNA of A30 is about 0.5 kb.

(5)Sequencing of the genes: By subcloning, an about 1 kb inserted subclone from 2.1 kb positive clone, which also showed antagonistic activity, was obtained and sequenced. As showed following , the inserted fragment is 906 bp, in which there were two open reading frames which could code two proteins with 95 and 53 amino acids, respectively. Further work is to be carried out on the functions of the proteins and genes.

From the partial amino acid sequence of the antagonistic protein of A30—II, we synthesized a 25—mer degenerate primer, and got a DNA fragment about 0.5 kb by polymerase chain reaction of A30. The fragment was sequenced and showed that it coded only 16 amino acids. The 16AA protein is conformed to the M. W. of the purified active protein (A30—II) (Fig. 5).

```
...TGCAGGAATTCGAT  ATG  TAT  ATG  ATT  GAT  TGG  TGG  ATG  AGG  GAT
                   Met  Tyr  Met  Ile  Asp  Trp  Trp  Met  Arg  Asp
ACG  CAG  AGC  CTT  GCA  GGA  TAAGATGATCCTTGTACACTAA
Thr  Gln  Ser  Leu  Ala  Gly
AACGTGATGTGATTTTTTTCGTTTTTTAAAATTAAGATGTAACATAGAGGA
```

Fig. 5 Sequence and encoded amino acid of a cloned fragment from A 30

(6)Study on the plasmid coding antagonistic protein: We also studied the bacteria with antagonistic protein coded by plasmid, and found that 18 from 86 antagonistic bacteria strains harbored plasmids. Among them, *Bacillus* sp. B034, isolated from rice leaves, had a 8kb plasmid and had antagonistic activity very strongly to *X. oryzae* pv. *oryzae*. After cured its plasmid with the treatment of acridine orange, it lost antagonistic activity. It indicated that there is a possible relationship between the plasmid and the antagonistic activity.

The restriction map of the B034 plasmid showed it had 4 EcoR I sites. All four EcoR I fragments were cloned in the shuttle vector pMK4 respectively. The recombinant DNAs were expressed in *E. coli* XL1—Blue and *B. subtilis* DB104. One recombinant harboring fragment—II (—3kb) show some antagonistic activity to *X. oryzae* pv. *oryzae*.

4. Study on the colonization of antagonistic bacteria and there effect on rice bacterial blight in greenhouse

In order to study whether the antagonistic bacteria could be used directly in field, we studied the colonization of strain B8 on rice. Firstly, we obtained a drug resistant mutant B×8 of strain B8 (Rif^r and Km^r) by stress selection and Tn5 mutagenesis, with the same strong antagonistic activity as B8. The marked bacterial suspension of B×8 (about 10^8 cells/ml) was sprayed on rice leave, the drug resistant bacteria were recovered and count from the rice leave at different time. It was shown that the bacterium could colonize on rice leave and its population become stable even the 40th day after spraying (Fig. 6). But it was affected strongly by the weather. Same results were recorded when we studied the colonization of other antagonistic bacteria on rice leave.

We inoculated rice leave at the end of tillering stage by clipping methods with spraying suspension of *X. oryzae* pv. *oryzae* (10^5— 10^6 cells/ml). The antagonistic bacteria suspension (about 10^8 cells/ml) of strains B8, P11, B826, and A30 or their filtrated cultural media were sprayed on rice leave before, while, and after inoculation of *X. oryzae* pv. *oryzae*, then scored the lesion length at different time. All tested strains reduced the lesion by 9%—39% (the highest one was 50%) of rice bacterial leaf blight compared with control. Best control efficacy of antagonistic bacteria were recorded when sprayed at the same inoculation time with *X. oryzae* pv. *oryzae* and that sprayed before *X. oryzae* pv. *oryzae* inoculation was better than that sprayed after *X. oryzae* pv. *oryzae* inoculation.

Discussion

From the works mentioned above, more than 130 strains of antagonistic bacteria were

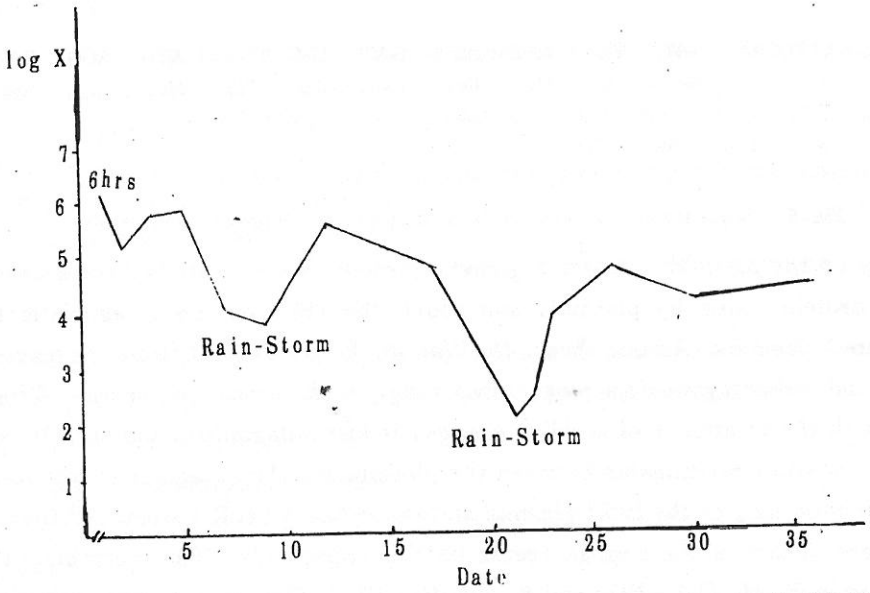

Fig. 6 Change of the population of strain B8 on rice leaves(X: CFU/cm² leaf)

screened out from rhizosphere, phyllosphere and other sources. Among them, *Bacillus* spp. are always easy to isolate and have strong inhibiting effects with wide antagonistic spectrum to various plant pathogens. It indicated that *Bacillus* spp. could serve as a important biocontrol agent with promising prospect, more screening work are going on. Although most work were plate tests, some showed good results on pot test in green—house. Further study is going to undertake in our laboratory.

The study of bacterial colonization on rice leaves provide the evidence for the direct use of those antagonistic *Bacillus* spp. . We recovered the marked bacterium even after 40 days with high colony density, this results indicated that bacteria could colonize on rice leaf surface and it is possible to prevent the disease before the invasion of pathogen. Some antagonistic bacteria such as B8, A30, B034 etc. , were isolated from rice leaf surface.

Eight antagonistic proteins (peptides) were purified and analyzed from the antagonistic *Bacillus* spp.. As mentioned above, more than one kinds of antagonistic components with different degree of antagonistic activity to different pathogen are occurred and there are antibiotics occurred also. Evidence showed the complexity of antagonistic mechanism. In a plate test, *Bacillus subtilis* A30 showed strong inhibition to *Rhizoctonia solani*. The hyphae of *R. solani* was dissolved. The study on the antagonistic proteins and their relation will be very important to understand the antagonistic mechanism. There are some difficulty to purify and identify the antagonistic substance.

After studying antagonistic genes, two DNA fragments which involved the encoding of antagonistic proteins were cloned and sequenced. Works are being undertaken to transform these two fragments into plants or bacteria which could colonize on plants to prevent the infection of related plant pathogens with strong antagonistic activity. We studied only a few genes, more genes should be studied in detail, especially the relationship and the interaction

between the antagonistic genes before we put the genes transformation work in practice.

References

[1]Chen, W. Y. et al. (1984), *Pl. Pathol.* 33:245—254.

[2]Choe, J. S. (1991), Horticultural Abstracts 061—09082.

[3]Dhedhi, B. M. et al. (1990), *Indian J. of Mycol. & Pl. Pathol.* 20:70—77.

[4]Ferreira, J. H. S. et al. (1991), *Phytopathol.* 81:283—287.

[5]Guldner,R. C. (1988), *J. Agri. Food Chem.* 36:366—370.

[6]Hardy, K. G. (1975), *Bacteriol. Rev.* 39:464—515.

[7]Holland, I. B. (1975), *Advances in Microbiol. Physiol.* 12:56— 139.

[8]Holliday, G. et al. (1993), The 6th International Congress of Plant Pathology (3. 3. 6).

[9]Kerr, A. (1972), *J. Appl. Bacteriol.* 35:493—497.

[10]Kerr, A. et al. (1974), *Physiol. Pl. Pathol.* 4:37—44.

[11]Konisky, J (1982), *Ann. Rev. Microbiol.* 36:125—144.

[12]Mayr—Harting, A. et al. (1972), Method in Microbiology Vol. 7A:351—322.

[13]Michael,A. et al. (1984), *J. of Bacteriol.* 160(3):854— 859.

[14]Mizubuti, E. S. G. (1993), The 6th International Congress of Plant Pathology (3. 2. 36).

[15]Ni, H. F. et al. (1993), The 6th International Congress of Plant Pathology (3. 3. 15).

[16]Nomura, M. (1967), *Ann. Rev. Microbiol* 21:257—284.

[17]Peng, Y. F. and Chen, S. M. (1990), *Acta Phytopathologica Sinica* 20(3):229—233.

[18]Pugsley, A. P. (1984), *J. Bacteriol.* 158:523—529.

[19]Pusley, P. L. and Wilson, C. L. (1984), *Pl. Diseases* 68:753— 756.

[20]Pusley, P. L. et al. (1988), *Pl. Diseases* 72:622—626.

[21]Reeve, P (1972), The Bacteriocins Molecular——biology, biochemistry, and boiphysics. vol. II Springer —Verlag, New York.

[22]San Millen,J. L. et al. (1985), *J. Bateriology* 163:1016— 1020.

[23]Sarhan, A. R. T. (1989), *Acta Phytopathologica et Entomologica, Hungarica* 24:271—275.

[24]Schmiedknecht, G. et al. (1993), The 6th International Congress of Plant pathology (16. 1. 10).

[25]Smidt, M. L. et al. (1982), *Can. J. Microbiol* 28:600—604.

[26]Smidt, M. L. et al. (1986), *Can. J. Microbiol* 32:231—236.

[27]Tschen, J. S. M. (1987), *Transaction of the Mycological Society of Japan* 28(4):483—493.

[28]Turner,J. T. ,Backman, P. A. (1991), *Pl. Diseases.* 75:347— 351.

[29]Utkhede R. S. and Sholberg, P. L. (1986), *Can. J. Microbol.* 32:963—967.

[30]Vidaver, A. K. (1976), *Ann. Rev. Phytopathology* 14:451—465.

[31]Vidaver, A. K. (1983), *Pl. Diseases* 67:471—475.

[32]Wang, J. S. et al. (1989), *J. Nanjing Agri. Univ.* 12(4) 59—62.