Recent Advances in Biofertilizers and Biofungicides (PGPR) for Sustainable Agriculture

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CHAPTER ONE

PRIVATE SECTOR PERSPECTIVE: GLOBAL SUCCESS OF PRATHISTA INDUSTRIES ORGANIC AGRI-INPUTS FOR SUSTAINABLE AGRICULTURE

K. V. S. S. Sairam[1] and N. Sree Rama Reddy[2]
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Abstract

The adoption of organic farming alone will bring about sustainability in agriculture. The indiscriminate use of chemical fertilizers over the years has resulted in the accumulation of toxic chemical substances in the soil, depletion of organic carbon content, micro flora and fauna, and damage to soil fertility with deleterious effects on crop productivity. The application of certified organic agri-inputs revives soil health and brings about ecological balances, reducing pollution and increasing the quality and quantity of crop yield. The availability of such organic inputs is a limiting factor for the farming community.

In an effort to overcome this limitation, Prathista Industries of Hyderabad, India has developed microbial-derived organic agri-inputs for all plant macro and micro nutrients. National and international experiments have proved the efficacy of these products in increasing the yield and quality of different crops. The present chapter reviews the results of experiments on yield improvement attained in various crops under diverse agro-climatic zones. Organic agriculture is a holistic production management system which promotes and enhances agro ecosystem health, including biodiversity, biological cycles and soil biological activity. Organic farming is necessary in view of economic, environmental and social concerns. The green revolution increased crop yields to attain self-sufficiency in food production by using high input external techniques.

The continuous use of inorganic fertilizers has had a negative impact on soil health resulting in a reduction in organic carbon content, nutrient uptake and expression of micro nutrient deficiencies, in addition to soil erosion and environmental pollution. The adoption of organic farming combats the ill effects of soil, water and the environment. In this direction, Prathista Industries manufactures microbial-derived organic agri inputs for all plant macro and micro nutrients developed through continuous research and development. The products are eco-friendly, bioavailable with a shelf life of 3–5 years, and do not contaminate the groundwater. These products are certified as organic by the Indian Organic Certification Agency (INDOCERT) in India, and the Organic Materials Review Institute (OMRI) in the USA. Field-level demonstrations of these products on various food crops (rice, corn, soyabean etc.), commercial crops (sugarcane and cotton), vegetables (cabbage, tomato, onion, cauliflower, aubergine and onion), horticultural crops (banana, mango, citrus, rose etc.), and spices (cardamom and cloves) were conducted in various parts of India and abroad (the USA, the Philippines, Uganda, Turkey and Kenya). The present chapter reviews the bio-efficacy results of Prathista organic inputs on different crops conducted at various locations.

Rice

Large-scale demonstrations on farmers' fields were conducted in different states of India, adopting the protocol of Prathista products (125 kgs of Aishwarya + 75 kgs each of Bio Potash and Bio Phos as basal, 12.5 kgs of bio zinc twenty days after planting [DAP], and foliar application of 625 mL each of Organic NPK +Suryamin and Bio Potash at 40 and 60 DAP, respectively/ha), which has convincingly indicated 20%–30% increased yield over the recommended dose of chemical fertilizers (80 N + 40 P_2O_5 + 40 K_2O/ha). The increase in the yield was attributed to the greater number of tillers, more grains per panicle, a greater number of panicles per plant, and the test weight of the grain. Trials conducted at Agricultural Research Institutions, Andhra Pradesh, on the rice-pulse cropping system utilizing Prathista products have indicated a greater uptake of plant nutrients from the soil, an increase in organic carbon content, and the enhancement of the enzymatic activity of Ureases, Phosphotases and Dehydrogenases, which ultimately resulted in a significant increase in the yield of rice as well as the succeeding pulse crop (*Phaseolus aures*).

Field trials on rice as per food and product agreement FPA protocols using a randomized complete block design (RCBD) with six treatments were conducted at Tiaong Quezyn in the Philippines to evaluate the

effectiveness of Bio Phos on the growth and yield of lowland rice (RC 18). Treatments include control, recommended rate of conventional fertilizer (90–60–60 Kg/ha), recommended rate of Bio Phos (2 mL/L), and different combinations of chemical fertilizer and Bio Phos. Straw and grain yields were significantly affected by the different treatments. The recommended rate of conventional fertilizer and recommended rate of Bio Phos increased the straw yield significantly over the control, but improvement due to Bio Phos was nevertheless comparable only with the improvement due to half the recommended rate of chemical fertilizer. The grain yield performance of Bio Phos was significantly better than the performance of control, but comparable with that of half the recommended rate of chemical fertilizer. This indicates a positive interaction between recommended rate of Bio Phos and half the recommended rate of chemical fertilizer. Based on the experiment results, Bio Phos has been recommended for FPA registration in the Philippines.

A similar study was conducted in various parts of Uganda by the Department of Crop Science, Makerere University, using the following products: New Suryamin, Megacal, Bio Zinc, Bio Phos, Bio Potash and Push (biopesticide). These products were also subjected to pathological and quality analyses, together with their field efficacy on various crops including rice. The results indicated that the products were organic and free from disease-causing plant pathogens. Field efficacy results emphasized the increased rice yields by 20%–22% compared to conventional fertilizers.

Studies conducted at Kansas State University on the effect of Suryamin, Bio Phos, Bio Potash and Bio Zinc on wheat, soyabean, and sorghum indicated the positive impulse of these products in increasing grain quality and yield. Application of New Suryamin increased the total nitrogen and protein content by 11% compared to control. Application of Suryamin, Bio Phos, Bio Potash and their combination on Soyabean improved the photosynthesis rate, transpiration rate leaf chlorophyll content, and photochemical efficiency. Due to the improved physiological traits, the grain yield was increased by 12%–20% and the protein content increased to 5% more than control.

Research studies conducted by the Department of Plant and Soil Sciences at Mississippi State University, USA, revealed an increase in corn yield and quality with the application of Bio Zinc and Bio Potash (8%), Suryamin (5%) and Bio Phos (15%) over control (1100 Kg/ha). The highest percentage of grain protein (9.5%) was observed with the application of Suryamin and Bio Phos compared to control (8.2%).

Sugarcane

The on-farm semi-commercial and commercial demonstrations conducted in sugarcane by Kakira Sugars, Uganda, using Prathista products indicated significantly higher cane and sugar yields compared to the recommended dose of chemical fertilizers. Further, this study has concluded that an integrated approach of adopting 50% of inorganic fertilizer recommendation (50 Kg N + 80 Kg P_2O_5 + 50 Kg K_2O) along with the recommended Prathista products (125 Kg Bio Phos + 50 Kg Bio Potash + 50 Kg New Suryamin) is the better strategy to realize higher yields with a low production cost. Field demonstrations conducted in collaboration with the World Health Organization (WHO) utilizing Prathista products at the Regional Agricultural Research Station, Anakapalle, India, indicated that the Prathista protocol received (Suryamin + Bio Phos + Bio Potash each 115 Kg/ha as basal and sprayings of Organic NPK @ 650 mL/ha at 45, 60, and 90 DAP) recorded the highest cane and sugar yields. Increases of 23% of cane yield and 33% sugar yield were recorded over farming practice. The same treatment, when compared with RDF (112 Kg N + 100 Kg P_2O_5 + 120 Kg K_2O), also recorded 7.6% and 11.7% increases in cane and sugar yields, respectively. Fertigation demonstrations with liquid organic formulations of Prathista indicated that 100% Prathista organic soluble nutrients contribute to higher cane yields, a greater number of millable canes, high quality juice and jaggery, and a higher B:C ratio compared to the recommended dose of chemical fertilizers. Higher populations of beneficial microbes like *Trichoderma* spp. (8.8×10^4 cfu/g) and *Pseudomonas flourescens* (6.3×10^6 cfu/g) were recorded in plots that received 100% Prathista organic fertilizers compared to control (RDF- 3.2×10^4 cfu/g, and 1.9×10^6 cfu/g, respectively). Field demonstrations of over thirty locations conducted by the Prudential Sugars Corporation (India) indicated an average additional yield of 25–30 t/ha of sugarcane with Prathista organic products compared to chemical fertilizers.

Vegetables

The Prathista Organic fertilizers New Suryamin, Aishwarya, Wonder, Miracle, Bio Phos, Bio Zinc and Jado were studied for their efficacy by the University of the Philippines both during the wet season (July to November) at the Central Experiment station, and at Barangay Sambat, Laguna during the dry season (March to May). The results show that organic formulations are effective in increasing Pechay crop yields over the control. A further improvement in yields was obtained by combining

Prathista Organic formulations with half the recommended rate of inorganic fertilizer. New Suryamin in combination with half the recommended rate of inorganic fertilizers resulted in the highest crop yield (11 t/ha) followed by Wonder (10.2 t/ha), Bio Zinc (10.2 t/ha), Safe (10.2 t/ha), Miracle (9.3 t/ha), Jado (8.4 t/ha), and Bio Phos (8.3 t/ha) in comparison to the recommended dose of conventional fertilizer (6–7 t/ha). Based on these results, the products were recommended for FPA registration in the Philippines.

Demonstrations of Prathista organic products conducted on various vegetable crops by officials of the Department of Agriculture, Sikkim (declared as an organic state in India) showed a significant increase in yield over control (cow dung 10 t/ha). An average additional yield of 8 q/ha in tomato was recorded followed by 5 q/ha and 4 q/ha in cabbage and cauliflower, respectively, using Prathista protocol (Aishwarya 75 Kg + Bio Phos 50 Kg + Bio Potash 50 Kg as basal and sprayings of Pushkal / New Suryamin + Megacal 4 mL/L at 35 DAP).

PART I.

PGPR AND OTHER MICROBIALS: GROWTH PROMOTION AND BIOLOGICAL CONTROL IN CROPS

CHAPTER TWO

CONTROL OF PLANT DISEASES BY THE ENDOPHYTIC RHIZOBACTERIAL STRAIN *PSEUDOMONAS AERUGINOSA* 23_{1-1}

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Abstract

The term endophyte refers to microorganisms colonising the interior of plants which do not have pathogenic effects on their hosts. Various endophytes have been found to play important roles in plant vitality and suppression of plant diseases as the action of endophytic microorganisms has been demonstrated in several pathosystems. Endophytic bacteria are able to enter the host plant and become systemically disseminated, actively colonising the xylem and occasionally the intracellular spaces. This colonisation represents an ecological niche, similar to that occupied by plant pathogens. Thus, endophytic bacteria can act as biological control agents against several plant pathogens. In our previous studies, the endophytic and antagonistic rhizobacterial strain *Pseudomonas aeruginosa* 23_{1-1}, isolated from the rhizosphere of a watermelon plant grown in the Mekong Delta of Vietnam, showed significant control of two important fungal diseases of watermelon, i.e. gummy stem blight caused by *Didymella bryoniae*, and vascular wilt caused by *Fusarium oxysporum* f.sp. *niveum* under greenhouse and field conditions. In this study, *Ps.*

aeruginosa 23_{1-1} was tested against a range of pathogens under *in vitro* and greenhouse conditions. All pathogens were significantly inhibited under *in vitro* conditions. Furthermore, *Ps. aeruginosa* 23_{1-1} also significantly reduced sheath blight disease caused by *Rhizoctonia solani* and bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* in rice, fruit rot caused by *Phytophthora capsici* and anthracnose caused by *Colletotrichum lagenarium* in watermelon, as well as soft rot caused by *Erwinia carotovora* and damping off caused by *R. solani* in cabbage under greenhouse conditions. These results suggest that *Ps. aeruginosa* 23_{1-1} is a promising biological control agent for plant disease management.

Introduction

Rhizobacteria are important microorganisms which play many roles in promoting plant growth. They can directly promote plant growth by producing phytohormones, solubilise phosphate, or fix nitrogen. Indirectly, they also improve plant growth through inhibition of pathogen development (Lugtenberg & Kamilova 2009). Many rhizobacteria isolated from different plant species were able to reduce a range of diseases, including soilborne and airborne diseases. *Pseudomonas aeruginosa* has been found to be antagonistic to different plant pathogens and pests such as *Pythium* sp. and the root-knot nematode *Meloidogyne javanica* (Ali et al. 2002; Wahla et al. 2012). The endophytic bacterium *Pseudomonas aeruginosa* 23_{1-1} was isolated from the rhizosphere of a watermelon plant in the Mekong Delta of Vietnam, and this bacterium is able to systemically protect watermelon from gummy stem blight caused by *Didymella bryoniae* both under greenhouse and field conditions (Nga et al. 2010). The protection was found to involve antibiosis and to induce resistance. In addition, the bacteria were found living inside the watermelon plant where they can grow to a high population density when the pathogen is present (Nga et al. 2010). *Ps. aeruginosa* 23_{1-1} also shows antagonistic ability against *Fusarium oxysporum* f.sp. *niveum* in dual culture tests on potato dextrose agar (PDA) plates. When drenching bacterial suspensions into the soil, it was possible to reduce Fusarium wilt on watermelon under greenhouse and field conditions (Pham & Nguyen 2010; Nguyen 2010). In the present study we investigated whether the bacterium could inhibit other bacterial and fungal pathogens on rice, watermelon and cabbage, including *Xanthomonas oryzae* pv *oryzae* causing bacterial leaf blight and *Rhizoctonia solani* causing sheath blight in rice, *Phytophthora capsici* causing fruit rot, and *Colletotrichum lagenarium* causing anthracnose on watermelon and cucurbits plants, as well as *Erwinia carotovora* causing

soft rot, and *Rhizoctonia solani* causing damping off on cabbage *in vitro* and under greenhouse conditions.

Materials and Methods

Microorganisms and culture conditions

Pseudomonas aeruginosa $23_{1\text{-}1}$ was cultured on agar plates containing King's B medium for 48 h. Then, the bacterium was harvested by adding sterile distilled water and gently releasing the cells. The bacterial density was determined by measuring OD at 600 nm and then, based on a standard curve, the bacterial density in the suspension was determined. Bacterial suspensions of 10^7, 10^8 and 10^9 cfu/ml were obtained by dilution.

Xanthomonas oryzae pv *oryzae* and *Erwinia carotovora* were cultured agar plates containing King's B medium for 48 h. Harvest, determination of cell density and dilution to 10^9 cfu/ml took place as for *Pseudomonas aeruginosa* $23_{1\text{-}1}$.

Rhizoctonia solani was cultured on PDA plates for two d. *Colletotrichum lagenarium* was cultured on modified PDA plates (PDA to which was added 5 g peptone per litre) for two weeks for sporulation. *Phytophthora capsici* was cultured on potato carrot agar medium for 7 d. Sterile distilled water was added to the plates, just submerging the colonies in water for 7–10 d to produce sporangia. Harvesting of zoospores took place by adding water to the plates covered with colonies of sporangia and then incubating the culture plates in a refrigerator at 4°C for 30 min. Subsequently, the suspension was filtered through cheese cloth to remove debris and this suspension with zoospores was used.

The conidial density of *C. lagenarium* and a zoospore density of *Ph. Capsici* were determined by a haemocytometer, and the inoculum concentrations were diluted to 10^5 spores/ml of *C. lagenarium* and 5×10^4 zoospores/ml for *Ph. capsici*.

In vitro experiments

Dual culture tests for the antagonistic ability of *Ps. aeruginosa* $23_{1\text{-}1}$ were performed with *Xanthomonas oryzae* pv *oryzae* and *Erwinia carotovora.* All bacteria were cultured as before. A volume of 200 µl *Ps. aeruginosa* suspension was added to a test tube containing 10 mL melted King's B agar at 50°C. After thorough mixing, the medium was poured onto a plate and sterile dishes of blotter paper were dipped into bacterial suspensions and then placed on the surface of the plate (five dishes across the plate).

The plates were incubated for 2 d and inhibition zones were induced by *Ps. aeruginosa* $23_{1\text{-}1}$ against *X. oryzae* pv. *Oryzae*, and *E. carotovora* were recorded.

For dual culture tests for the antagonistic ability of *Ps. aeruginosa* $23_{1\text{-}1}$ against *R. solani*, *Ph. Capsici,* and *C. lagenarium*, *Ps. aeruginosa* $23_{1\text{-}1}$ was cultured as described above. *R. solani*, *Ph. Capsici* and *C. lagenarium* were cultured on PDA for 2–4 d. Subsequently, fungal culture plugs (5 mm in diameter) were placed in the middle of agar plates. Two dishes of sterile blotter paper (5 mm in diameter) dipped in a *Ps. aeruginosa* $23_{1\text{-}1}$ suspension were placed on opposite sides of the fungal agar plug. The plates were incubated at room temperature for several days and the inhibition zones between the bacterium and the three pathogens were recorded each day after inoculation.

Greenhouse experiments

All experiments were conducted with cv. Jasmine.

Control of sheath blight and bacterial leaf blight in rice under greenhouse conditions

***Sheath blight* (*Rhizoctonia solani*).** The experiment followed a completely randomised block design with 7 treatments and 4 replications. Three treatments with sprayings of *Ps. aeruginosa* $23_{1\text{-}1}$ at 10^7, 10^8, and 10^9 cfu/ml on entire plants at 2 d before inoculation with the pathogen; three treatments with sprayings of *Ps. aeruginosa* $23_{1\text{-}1}$ at 10^7, 10^8, and 10^9 cfu/ml on entire plants at 2 d after inoculation; and one control treatment with a spraying of distilled water. Soil from a rice field was well mixed and weighed at 1 Kg/pot, then rinsed and washed with water several times for one week. One pot (17.5 × 12 cm, containing 10 plants) served as one replication. Pathogen inoculation was conducted at 40 d after sowing following the method of Khoa et al. (2011) by placing a plug from a 3 d old *R. solani* PDA culture (5 mm in diameter) inside the sheath at 2 cm above the soil line. The plants were incubated in a growth chamber at 25°C and high humidity for 2 d. Subsequently, they were transferred to a greenhouse at ambient environmental conditions. At 2, 4 and 6 d after inoculation, the relative lesion height (Sharma et al. 1990) [RLH (%): (lesion length/plant height) × 100] and the percentage of infected plants = (the number of infected plants/total number of plants×100) were measured.

Bacterial leaf blight in rice (*Xanthomonas oryzae* pv oryzae)
The experimental design and bacterial treatments were as described above, except that applications of *Ps. aeruginosa* $23_{1\text{-}1}$ took place 1 d before and 1 d after inoculation. Inoculation with *X. oryzae* pv *oryzae* was conducted at 40 d after sowing following the method of Kauffman et al. (1973) by using a pair of scissors dipped in a *X. oryzae* pv *oryzae* suspension (10^9 cfu/ml) to cut the top of two fully mature leaves per plant in each pot. Then, the plants were incubated in the growth chamber at 25°C and high humidity for 24 h. Subsequently, plants were transferred to greenhouse conditions. Disease recordings were performed according to the method described by Kauffman et al. (1973) using a scale with 5 levels: (1): no or only a trace of disease; (3): lesions covering less than 25% of the leaf; (5): lesions covering 25%–50% of the leaf; (7): lesions covering more than 50% of the leaf; (9): lesion reaching down to the sheath or "kresek."

Control of anthracnose and fruit rot in watermelon under greenhouse conditions

Anthracnose disease on watermelon (*Colletotrichum lagenarium*)
The experimental design was as described above, but with 7 treatments and 12 replications. Three treatments with soil drenched with *Ps. aeruginosa* $23_{1\text{-}1}$ at 10^7, 10^8, and 10^9 cfu/ml with 5 m of bacterial suspension for each 7-day-old-seedling; three treatments with a spraying of *Ps. aeruginosa* $23_{1\text{-}1}$ at 10^7, 10^8, and 10^9 cfu/ml on the surface of the leaves at 1 d before and 2 d after inoculation with the pathogen, and one control treatment inoculated with the pathogen and sprayed with distilled water instead of *Ps. aeruginosa* $23_{1\text{-}1}$ at 1 d before and after pathogen inoculation. Each replication comprised one pot containing 4 plants. Inoculation with the pathogen was conducted at 15 d after sowing by spraying a *C. lagenarium* suspension (0^6 spores/ml) onto the surface of the first true leaf until run-off. Subsequently, the plants were incubated in the growth chamber at 25°C and high humidity for 1 d, after which time they were transferred to the greenhouse. Disease was recorded as a percentage of infected leaf area per plant each day when symptoms appeared at 5 d after inoculation until the plants died.

Fruit rot in watermelon (Phytophthora capsici)
The experimental design was as described above with 10 treatments and 6 replications. Three treatments with a spraying of *Ps. aeruginosa* $23_{1\text{-}1}$ at 10^7, 10^8, or 10^9 cfu/ml on the surface of watermelon fruits at 1 d before pathogen inoculation; three treatments with a spraying of *Ps. aeruginosa*

23_{1-1} at 10^7, 10^8, or 10^9 cfu/ml on the surface of watermelon fruits at 1 d before and 1 d after pathogen inoculation, and three treatments sprayed with *Ps. aeruginosa* 23_{1-1} at 10^7, 10^8, or 10^9 cfu/ml on the surface of watermelon fruits at 1 d after pathogen inoculation. Spraying with distilled water followed by inoculation with the pathogen served as a control. Each replication was with one young watermelon fruit. Pathogen inoculation was conducted by pipetting 15 µl of zoospore suspension (6×10^4 zoospores/ml) onto the middle of the fruit. Inoculated fruits were placed in nylon bags supplemented with a plug of moist cotton with the bags being placed at room temperature (25°C). The diameters of the fruit rot lesions were recorded when symptoms appeared (at 3 d after pathogen inoculation and until the entire fruit was rotten [5 d]).

Control of anthracnose and fruit rot in cabbage under greenhouse conditions

Damping off on cabbage (*Rhizoctonia solani*)
The experimental design was as described above with 7 treatments and 7 replications. Three treatments with soil drenching with *Ps. aeruginosa* 23_{1-1} at 10^7, 10^8, or 10^9 cfu/ml before sowing, three treatments with soil drenching with *Ps. aeruginosa* 23_{1-1} at 10^7, 10^8, or 10^9 cfu/ml at 5 d after sowing, and a control treatment with application of distilled water instead of *Ps. aeruginosa* 23_{1-1} followed by pathogen inoculation. A pot with 40 plants was a replication. Pathogen inoculation took place by adding a mixture of 50 g sterilised rice straw with a fully grown PDA plate of *R. solani* (3 d-old) into a pot containing 950 g sterile soil and mixing well. Cabbage seeds were treated with 50°C warm water for 30 min and then incubated at 30°C for 2 d for seed germination. A total of 40 germinated seeds were sown in each pot. The pots were placed in the greenhouse. Disease was recorded as a percentage of infected plants at 5, 9, and 13 d after pathogen inoculation.

Soft rot disease on cabbage *(Erwinia carotovora)*
The experimental design was as described above with 7 treatments and 10 replications. This comprised three treatments with a spraying of *Ps. aeruginosa* 23_{1-1} at 10^7, 10^8, or 10^9 cfu/ml on the surface of cabbage leaves at 1 d before pathogen inoculation, three treatments with a spraying of *Ps. aeruginosa* 23_{1-1} at 10^7, 10^8, or 10^9 cfu/ml on the surface of cabbage leaves at 1 d after pathogen inoculation, and a control treatment inoculated with the pathogen and sprayed with distilled water 1 d before and 1 d after

inoculation. Each replication was one pot with 5 plants. Inoculation took place by using a pipette to inject 5 µl of bacterial suspension (10^9 cfu/ml) in the middle of the fourth true leaf of each plant. Inoculated plants were incubated in the growth chamber at 25°C in darkness for 24 h. After inoculation, the plants were placed in the growth chamber at 25°C with cycles of 12 h light and 12 h darkness. Disease recordings were performed using a scale modified from Ren et al. (2001), with nine levels: (1) No symptoms; (2) Lesions smaller than 5 mm; (3) Lesions 5–10 mm; (4) Lesions bigger than 10 mm but not reaching the petiole; (5) Lesions spreading on leaf and petiole; (6) The stem infected, uninoculated leaves not infected; (7) Stem and uninoculated leaves infected; (8) Plant going to die soon; (9) Plant dead.

Data analysis

The data were subjected to analysis of variance (ANOVA) using the MstatC software, and the means were compared using Duncan's multiple range test at a significance level of 5%.

Results and Discussion

Disease control by *Pseudomonas aeruginosa* 23_{1-1} in rice, watermelon and cabbage

Pseudomonas aeruginosa 23_{1-1} showed a high antagonistic ability against both fungal and bacterial pathogens on rice, watermelon and cabbage (see Table 2.1 below). *Ps. aeruginosa* 23_{1-1} expressed antagonistic ability against the two rice pathogens *Rhizoctonia solani* and *Xanthomonas oryzae* pv *oryzae* with inhibition zones of 5.6 and 9.0 mm at 2 DAI, respectively (see Table 2.1 and Fig. 2.1 below). *Ps. aeruginosa* 23_{1-1} also had an inhibiting effect against the watermelon pathogens *Ph. capsici* (inhibition zone 11.0 mm), and against *C. lagenarium* (inhibition zone 6.0 mm) (see Table 2.1 and Fig. 2.2 below).

For cabbage pathogens, *Ps. aeruginosa* 23_{1-1} likewise showed a high antagonistic ability to *R. solani* and *Erwinia carotovora* with inhibition zones of 11.0 and 3.0 mm (see Table 2.1 and Fig. 2.3 below).

These results indicate that *Ps. aeruginosa* 23_{1-1} possesses a wide spectrum of antagonistic activity with both bacterial and fungal pathogens from different plants.

In a previous study, *Ps. aeruginosa* 23_{1-1} also inhibited *Didymella bryoniae* causing gummy stem blight and *Fusarium oxysporum* f.sp.

niveum causing vascular wilt in watermelon under *in vitro*, greenhouse, and field conditions (Nga et al. 2010; Pham & Nguyen 2010; Nguyen 2010).

Table 2.1. **Inhibition zones induced by *Pseudomonas aeruginosa* 23_{1-1} against six pathogens of rice, watermelon and cabbage**

Pathogens	Inhibition zones (mm)
From rice	
Xanthomonas oryzae pv *oryzae* (2 dai)	5.6
Rhizoctonia solani (2 dai)	9.0
From watermelon	
Colletotrichum lagenarium (6 dai)	6.0
Phytophthora capsici (5 dai)	11.0
From cabbage	
Rhizoctonia solani (2 dai)	11.0
Erwinia carotovora (2 dai)	3.0

The dual culture tests were conducted with four to five replications.
Dai: days after inoculation.

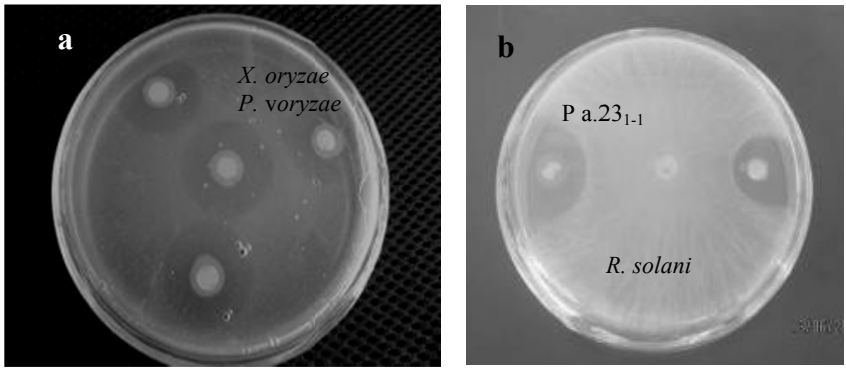

Fig. 2.1. Effect of *Ps*. *aeruginosa* 23_{1-1} against (a) *X. anthomonas oryzae* pv *oryzae* and (b) *R. hizoctonia solani* in dual culture tests at 2 d after inoculation

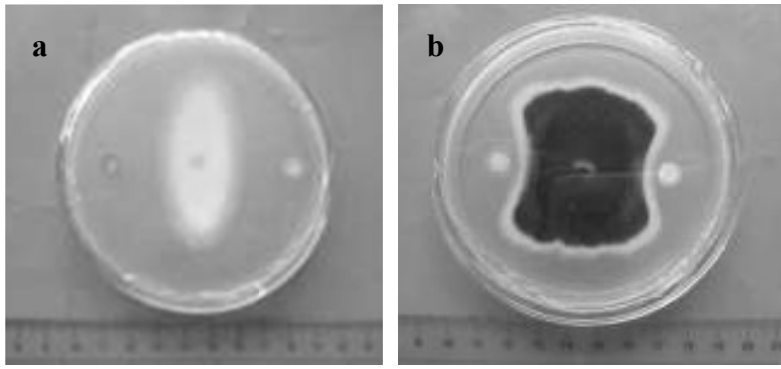

Fig. 2.2. Effect of *Ps. aeruginosa* $23_{1\text{-}1}$ against (a) *Ph. capsici* and (b) *C. lagenarium* after 5 and 6 d of inoculation

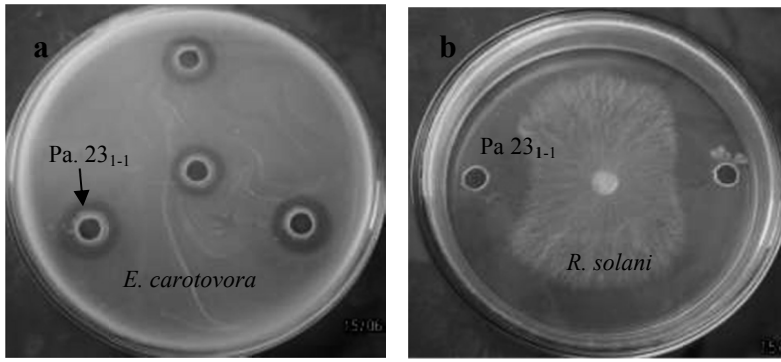

Fig. 2.3. Effect of *Ps. aeruginosa* $23_{1\text{-}1}$ against (a) *E. carotovora* and (b) *R. solani* in dual culture tests after 2 d of inoculation

Control of sheath blight and bacterial blight in rice under greenhouse conditions

Sheath blight caused by R. solani. Among six spray treatments with *Ps. aeruginosa* $23_{1\text{-}1}$ on rice for control of sheath blight, a concentration of 10^7 cfu/ml before pathogen inoculation resulted in a significant reduction in the percentage of diseased plants and relative lesion height at all-time points (see Table 2.2 and Fig. 2.4 below). The only other treatment which gave a significant reduction in relative lesion height was spraying with 10^8 cfu/ml before pathogen inoculation at 2 d. Thus, spraying with *Ps. aeruginosa* $23_{1\text{-}1}$ at a concentration of 10^7 or 10^8 cfu/ml before pathogen

inoculation could reduce infection by *Rhizoctonia solani* in rice.

Table 2.2. **Effects of bacterial treatments on the percentage of diseased plants and relative lesion height of sheath blight lesions caused by *R. solani* in rice under greenhouse conditions**

Treatment	% of diseased plants			Relative lesion height		
	Days after inoculation					
	2	4	6	2	4	6
Spraying with 10^7 cfu/ml *Ps. aeruginosa* 23_{1-1} before pathogen inoculation	52.5 b	83.2 a	83.2 a	1.1 c	3.6 b	4.5 b
Spraying with 10^8 cfu/ml *Ps. aeruginosa* 23_{1-1} before pathogen inoculation	75.7 a	95.8 a	95.8 a	1.8 bc	4.9 ab	6.7 a
Spraying with 10^9 cfu/ml *Ps. aeruginosa* 23_{1-1} before pathogen inoculation	72.7 a	92.7 a	97.2 a	2.3 abc	5.1 ab	6.0 ab
Spraying with 10^7 cfu/ml *Ps. aeruginosa* 23_{1-1} after pathogen inoculation	62.0 ab	87.5 a	92.5 a	2.1 abc	4.6 ab	5.7 ab
Spraying with 10^8 cfu/ml *Ps. aeruginosa* 23_{1-1} after pathogen inoculation	80.5 a	90.2 a	95.2 a	2.5 ab	5.5 a	7.1 a
Spraying with 10^9 cfu/ml *Ps. aeruginosa* 23_{1-1} after pathogen inoculation	73.1 a	93.8 a	93.8 a	2.2 abc	4.5 ab	6.2 ab
Control	81.4 a	94.7 a	94.7 a	3.3 a	6.1 a	7.1 a
Level of significance	*	NS	NS	*	*	*

*Note: Means within a column followed by the same letter are not significantly different based on Duncan's multiple range test least significant difference (α= 0.05). NS: non-significant difference; *: significant at P < 0.05.*

Fig. 2.4. Sheath blight symptoms on cv. Jasmine after treatment with *Ps. aeruginosa* 23_{1-1} and water (control) under greenhouse conditions. (a) Spraying with *Ps. aeruginosa* 23_{1-1} at a concentration of 10^7 cfu/ml before pathogen inoculation; (b) Control sprayed with water

Bacterial blight caused by Xanthomonas oryzae *pv* oryzae. Six treatments with *Ps. aeruginosa* 23_{1-1} were tested for the control of bacterial leaf blight caused by *Xanthomonas oryzae* pv *oryzae* (see Table 2.3 and Fig. 2.5 below). Only two treatments reduced lesion length and disease level, i.e. the spray treatments with 10^9 cfu/ml before pathogen inoculation and the spray treatment with 10^8 cfu/ml after pathogen inoculation. Thus, the results indicate that spraying with *Ps. aeruginosa* 23_{1-1} can reduce bacterial leaf blight.