UNIVERSITI PUTRA MALAYSIA

ANTAGONISTIC ACTIVITIES OF SELECTED BACTERIAL ISOLATES AGAINST SOILBORNE DISEASE PATHOGENS OF TURFGRASS

NENI KARTINI CHE MOHD. RAMLI

FP 1999 24

ANTAGONISTIC ACTIVITIES OF SELECTED BACTERIAL ISOLATES AGAINST SOILBORNE DISEASE PATHOGENS OF TURFGRASS

By

NENI KARTINI CHE MOHD. RAMLI

Thesis submitted in fulfillment of the Requirement for the Degree of Master of Agricultural Science in the Faculty of Agriculture, Universiti Putra Malaysia

February 1999

DEDICATION

I find great delight in being able to dedicate this thesis, firstly to my beloved husband, Samsuri Surif for all his sacrifices and understanding.

My dear mother Pn. Azizah for her supportive and encouraging.

My very much- adored daughter, Nurul Shuhada, the little person who was an inspiration for me to finish my thesis and to date keeps on lightning up my life.

Last but not least, to all my relatives and friends.

ACKNOWLEDGEMENTS

I am extremely grateful and greatly indebted to my supervisor Assoc. Prof. Dr. Kamaruzaman Sijam of the Department of Plant Protection, Faculty of Agriculture, for his supervision, friendly support, dedicated efforts, stimulating discussions, active participation and constant encouragement throughout the planning and execution of this research.

My sincere appreciation and gratitude to members of my Supervisory Committee, Assoc. Prof. Dr. Mohd. Fauzi Ramlan of the Department of Crop Science, Faculty of Agriculture, and Dr. Suhaimi Napis of the Department of Biotechnology, Faculty of Food Science and Biotechnology, University Putra Malaysia for their critical comments and suggestions during the course of this project.

My sincere thanks go to Junaina Jaafar, Yusoff Yasin from Microbiology Laboratory and also to Johari Sarikat, Khir Abdul Rani and Nazri Abdul Rahman from Pathology Laboratory of Department of Plant Protection for their kind assistance and cooperation in conducting the research.

Thanks are also extended to Mazlan Bangi of the Department of Crop Science for his technical assistance in using the Spectrophotometer, other staff members of the Department of Plant Protection for their assistance.

Last but not least, I am greatly indebted and wish to thank my friends, Norida Mazlan and Puteri Edaroyati for their encouragement and support throughout the course of this study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Agricultural Science.

ANTAGONISTIC ACTIVITIES OF SELECTED BACTERIAL ISOLATES AGAINST SOILBORNE DISEASE PATHOGENS OF TURFGRASS

By

NENI KARTINI CHE MOHD. RAMLI

February 1999

Chairman: Associate Professor Kamaruzaman Sijam, Ph.D.

Faculty : Agriculture

Disease samples exhibiting symptoms of brown patch, yellow patch and *Pythium* blight were taken from several golf courses in Malaysia. Isolation and identification of the pathogens indicated that *Rhizoctonia solani* Kuhn, *Rhizoctonia cerealis* Van der Hoeven and *Pythium aphanidermatum* (Edson) Fitzpatrick were the causal agents respectively. Identification of the pathogens was based on their morphological characteristics.

Isolation of bacteria from thatch resulted in the isolation of ten bacterial isolates of which five were found to be antagonistic against the pathogens. The bacterial isolates were identified as *Burkholderia cepacia* (syn. *Pseudomonas cepacia*), *Serratia marcescens*, *Chromobacterium violaceum*, *Pseudomonas aeruginosa* and *Bacillus megaterium*.

B. cepacia, *P. aeruginosa* and *B. megaterium* were found to be antagonistic to *R. solani* with radial growth of 2.1 cm, 2.1 cm and 1.8 cm respectively; to *R. cerealis* with radial growth of 2.1 cm, 2.1 cm and 1.7 cm respectively; and to *P. aphanidermatum* with radial growth of 2.1 cm, 2.5 cm and 1.5 cm respectively; while *S. marcescens* and *C. violaceum* were only antagonistic to *P. aphanidermatum* with radial growth of 2.6 cm and 2.0 cm respectively. All the above measurements were taken 3 days after inoculation.

Microscopic observation of antagonistic mechanism displayed by the bacteria against the pathogens revealed that *B. cepacia*, *B. megaterium*, *C. violaceum* and *S. marcescens* degraded the cell wall, while *P. aeruginosa* suppressed or retarded the fungal growth. Cell wall degradation was due to the action of enzymes such as β-1.3 glucanase and chitinase, whereas growth suppression was due to the action of siderophores. *R. solani* and *R. cerealis* were inhibited by the bacteria via the action of β-1.3 glucanase, siderophores and antibiotics, while *P. aphanidermatum* was inhibited by the bacteria via the action of the above chemicals including chitinase.

Abstrak tesis ini diserahkan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk penganugerahan ijazah Master Sains Pertanian.

AKTIVITI-AKTIVITI ANTAGONISTIK ASINGAN BAKTERIA TERPILIH TERHADAP PATOGEN-PATOGEN PENYAKIT BAWAAN TANAH RUMPUT TURF

Oleh

NENI KARTINI CHE MOHD. RAMLI

Februari 1999

Pengerusi : Profesor Madya Kamaruzaman Sijam, Ph.D.

Fakulti : Pertanian

Sampel-sampel yang menunjukkan simptom penyakit 'brown patch', 'yellow patch' dan '*Pythium* blight' telah diambil dari beberapa padang golf di Malaysia. Pengasingan dan pengenalpastian patogen mendapati *Rhizoctonia solani* Kuhn, *R. cerealis* Van der Hoeven dan *Pythium aphanidermatum* (Edson) Fitzpatrick, masing-masing adalah penyebab penyakit-penyakit tersebut. Pengenalpastian patogen ini adalah berdasarkan kepada ciri-ciri morfologinya.

Pengasingan bakteria daripada 'thatch' telah menghasilkan 10 asingan bacteria yang mana 5 daripadanya memberikan kesan antagonistik kepada patogen. Asingan-asingan bacteria tersebut telah dikenalpasti sebagai *Burkholderia cepacia* (syn. *Pseudomonas cepacia*), *Serratia marcescens*, *Chromobacterium violaceum*, *Pseudomonas aeruginosa*. dan *Bacillus megaterium*.

B. cepacia, *P. aeruginosa* dan *B. megaterium* didapati antagonistik kepada *R. solani* dengan jejari pertumbuhan masing-masing bernilai 2.1 cm, 2.1 cm dan 1.8 cm; kepada *R. cerealis* dengan jejari pertumbuhan masing-masing bernilai 2.1 cm, 2.1 cm dan 1.7 cm; dan kepada *P. aphanidermatum* dengan jejari pertumbuhan masing-masing bernilai 2.1 cm, 2.5 cm dan 1.5 cm, manakala *S. marcescens* dan *C. violaceum* hanya antagonistik kepada *P. aphanidermatum* dengan jejari pertumbuhan masing-masing bernilai 2.6 cm dan 2.0 cm. Kesemua nilai di atas diambil 3 hari selepas inokulasi.

Cerapan mikroskop terhadap mekanisma antagonistik oleh bakteria menunjukkan bahawa *B. cepacia*, *B. megaterium*, *C. violaceum* dan *S. marcescens* mengurai dinding sel kulat, manakala *P. aeruginosa* merencat atau membantutkan pertumbuhan kulat. Penguraian dinding sel adalah disebabkan tindakan enzim seperti β-1,3 glukanase dan kitinase, manakala perencatan pertumbuhan adalah akibat tindakan siderofor. *R. solani* dan *R. cerealis* didapati direncat oleh bakteria melalui tindakan β-1,3 glucanase, siderofor dan antibiotik, manakala *P. aphanidermatum* direncat oleh bakteria melalui tindakan kesemua bahan kimia di atas termasuk kitinase.

CHAPTER I

INTRODUCTION

Turfgrass industry has developed very rapidly. This can be seen through the demand for turfgrasses to be used for golf courses and landscaping. Turfs are important in human activities from the functional, recreational and ornamental standpoint (Beard, 1973). In functional, turf is able to control wind and water erosion of soil and is essential in eliminating dust and mud problems on areas surrounding homes, factories, schools and businesses. Many outdoor sports and recreational activities utilise turf for cushioning effect that reduces injuries to participants, particularly in the more active sports such as rugby, American football and soccer. Turf also provides beauty and attractiveness for human activities. The clean, cool and natural greeness of turf provides a pleasant environment in which to live and work.

There are six basic components of turfgrass quality. They include uniformity, density, texture, growth habit, smoothness and colour (Beard, 1973). The high quality turfgrass should have the uniformity in visual. Any negative changes to the uniformity would decrease the quality of the turfgrass. In Malaysia the common turfgrasses grown are bermudagrass (*Cynodon dactylon*), zoysiagrass (*Zoysia* sp.), St. Augustinegrass (*Stenotaphrom secundatum*), centipedegrass (*Eremochloa ophioroides*), carpetgrass (*Axonopus compressus*) and bahiagrass (*Paspalum notatum*) (Nor Ain, 1993).

Diseases play major roles in determining the success or failure of a turfgrass stand. According to Vargas (1981), fungi were the most important cause of turfgrass diseases followed by nematodes and viruses. Turfgrass diseases caused by fungi are of great economic importance where in 1989, more than 48 % of the fungicides were used on turfgrass than on any other single crop in the United States (Couch & Smith, 1991). Some of the comman turfgrass diseases are brown patch, dollar spot, anthracnose, *Curvularia* leaf spot, *Helminthosporium* leaf spot, *Pythium* blight and *Fusarium* blight caused by *Rhizoctonia solani, Sclerotinia homoecarpa, Colletotrichum graminicola, Curvularia lunata, Helminthosporium cynodontis, Pythium* sp. and *Fusarium roseum* f. sp. *cerealis*, respectively.

Cultural practices and chemical control using fungicides are the most effective control methods for turfgrass diseases. Many problems have arisen from the repeated and prolonged use of chemicals in disease control. Chemical control can also caused deleterious effect on non-target organisms, particularly those involved in carbon and nitrogen cycling (Vyas, 1988). Dekker & Georgopoulus, (1982) reported that the development of resistant of pathogens to fungicides had become a major problem. Fungicides applied to control one disease may enhance the severity of other diseases, and may also cause thatch accumulation (Smiley et al., 1992).

Biological control is an alternative method of disease control to reduce fungicide dependency. It can also prevent many undesirable biological and environmental effects of excessive fungicide use. Baker and Cook (1974) defined biological control as a reduction of the amount of inoculum or disease producing activity of a pathogen accomplished by or through one or more organisms. Biological control has no deleterious effect on nontarget organisms and the environment. It may has some beneficial effects on the environment and usually last longer in the environment (Cook and Baker, 1983). Lo et al., (1996) demonstrated that *Trichoderma harzianum* was an effective biocontrol agent against brown patch, dollar spot and *Pythium* root rot and blight caused by *Rhizoctonia solani*, *Sclerotinia homoeocarpa* and *Pythium graminicola* respectively. Meanwhile, Nelson and Craft (1991) showed that *Enterobacter cloacae* significantly reduced the incidence of dollar spot caused by *Slerotinia homoeocarpa* when this bacterium was applied with sand as top dressing. Nelson and Craft (1992) reported that *Pseudomonas sp* and *Enterobacter cloacae* were able to suppress *Pythium* blight of turfgrasses caused by *Pythium aphanidermatum*. Although the practice of biological control in turfgrass diseases is still in the early developmental stage, the future of microbial inoculants for turfgrass disease control is extremely bright. The future use of antagonists as microbial inoculants will only come from a better understanding of how antagonists function and how they involve and interact with other turfgrass management inputs. Recent development in molecular biology has tremendously increased our ability to answer some of these questions.

The objectives of this study were to: a) determine and identify the major soil borne turfgrass disease of Bermudagrass in Malaysia, b) isolate, screen and identify the potential antagonistic bacteria to be used as bio-control agents for turfgrass diseases and c) study the mechanisms of antagonistic activity of the bacteria.

CHAPTER II

REVIEW OF LITERATURE

Turfgrass Industry

The extent and value of the turfgr

world. In the seventeenth and eighteenth centuries, turfs were cultured for use in lawn gardens, flower gardens, pleasure gardens and greens, and was most widely used in North America, England, New Zealand, Japan and Australia (Beard, 1973). However today, the demands for the turfgrasses were high in golf course industries. In Malaysia, this could be seen through the rapid development of golf courses where, in 1997 the number had reached close to 200 (Anonymous, 1997).

Soilborne Turfgrass Diseases

One of the most important components of turfgrass quality is uniformity. Injury caused by diseases, insects, nematodes and any other small animals had disrupted the uniformity of the turf and had been shown to reduce shoot density substantially (Beard, 1973). As any other crop, turfgrasses are vulnerable to diseases, depending on turfgrass Turfgrass cultivars varied in their resistance to diseases, determined by various heritable internal and external plant characteristics (Vargas, 1981). The favourable combination of environmental factors, such as high temperature, high moisture, weak host and high humidity will determine the severity of disease development (Beard, 1973).

Soilborne turfgrass, diseases such as *Rhizoctonia* diseases, *Pythium* diseases, *Fusarium* blight, and dollar spot diseases are the major diseases of warm season turfgrasses caused by *Rhizoctonia* sp., *Pythium* sp., *Fusarium* sp., and *Sclerotinia homeocarpa*, respectively (Smiley et al., 1992; Fermanian et al., 1997).

Diseases Caused by *Rhizoctonia* Species

The common soilborne fungi, *Rhizoctonia solani, R. cerealis, R. oryzae* and *R. zeae* are the causal agents of *Rhizoctonia* diseases of turfgrass, practically in all soil types throughout the world. The fungi feed equally well on living plant tissues or on organic matter present in the thatch and soil. All species of *Rhizoctonia* live in the soil as saprophytes, but not all attack living turfgrass plant. Some strains had been found to cause severe brown patch disease while others caused little damage (Nor Ain, 1993 ; Fermanian et al., 1997).

Smiley et al., (1992) reported that symptoms of diseases caused by these fungi vary greatly depending on the specific combination of turfgrass cultivars or species, soil and air environmental conditions, cutting height and the specific species or strains (races) of the fungi. It could be easily confused with symptoms of other diseases (Smith et al., 1989). Symptoms were found to differ on cool and warm season turfgrasses and were strongly affected by prevailing environmental conditions during the infection period (Smiley et al., 1992). *Rhizoctonia* sp. affects all known turfgrass species causing foliar blight, seed rot and seedling blight.

Species of *Rhizoctonia* produced several forms of hyphae frequently used for diagnostic purposes to the genus level. These include branching near the distal septum of cells in young vegetative hyphae, formation of septa near the origin of hyphal branches, constriction of hyphal branches near their origin, absence of conidia clamp connections and development of bulbils
(1970) reported that the mature hyphae of *R. solani*, *R. zeae* and *R. cerealis* usually branched at right angle whereas the hyphae of *R. oryzae* branched at an acute angle. The hyphal branches of all *Rhizoctonia* species were constricted at the point where they originate and septa separated the hyphal branches from their parent hyphae close to their point of origin. The mycelial cells were mostly multinucleated containing two (*R. cerealis*) to four (*R. oryzae*) nuclei per cell.
shade of brown (*R. solani*), buff-coloured to white (*R. cerealis*) or white to salmon or pink (*R. zeae* and *R. oryzae*) in colour (Smiley et al., 1992).

Rhizoctonia Seedling Diseases

Rhizoctonia sp. had been reported to cause seed rot, pre- and post-emergence blight on many different species of turfgrasses (Andrew, 1943; Couch, 1995). These pathogens commonly occur as complexes with other seed- and soil-borne fungi such as species of *Bipolaris, Curvularia, Drechslera, Fusarium* and *Pythium* causing turfgrass seed and root rots. *R. solani* was found to be the dominant species of *Rhizoctonia* to cause seedling diseases and was often associated with *R. zea, R. oryzae* and *R. cerealis* (Fermanian et al., 1997).

The symptoms normally occur as seed rotting, pre-emergent blight and damping-off when the soils were infested with *Rhizoctonia*, and environmental conditions favours the growth of the pathogen (Smith et al., 1989). The sclerotia of *R. solani* could be mixed with grass seeds and when the bulbils (sclerotia) germinate, they served as a source of inoculum (Smiley et al., 1992). The optimum temperature for seedling infection was found to be between 15.6°C to 23.9°C (60°F to 75°F) and invasion of turfgrass tissue was by direct penetration of epidermal cells and root hairs (Fermanian et al., 1997). Infected emerged seedlings showed necrosis at the soil level followed by withering and 'pinching' of the shoots (wire stem) causing seedling plants to collapse and turning light brown (post-emergence damping off) in colour. Less severely diseased seedlings developed eyespot lesions at or close to the soil line.

Rhizoctonia Foliar Diseases

Rhizoctonia foliar diseases are commonly known as brown patch or *Rhizoctonia* blight, and yellow patch. Brown patch, which is caused by *R. solani*, attacked all warm season turfgrasses causing severe damage to St. Augustinegrass, centipedegrass, zoysiagrass and hybrid bermudagrass (Fermanian et al., 1997; Smith et al., 1989). *Rhizoctonia* sp. also attack cool season turfgrass such as creeping bentgrass, tall fescue and perennial ryegrass particularly in locations where extended periods of high temperature, high relative humidity and moisture (Fermanian et al., 1997). This disease occurs at any time during the growing season and the severity could be extreme during hot and humid weather. The first symptom of brown patch could be seen as light green patches ranging from 5 cm to 0.9 meters wide (Smith et al., 1989; Fermanian et al., 1997). When condition is favourable for the development of the disease, the colour of the area changes to bright yellow and then to brown. On closely mown turf, such as greens, the leaves of infected grass plants become water soaked, blacken, then wither to a light brown colour in irregular or roughly circular patches from a few centimetre to a meter in diameter (Smiley et al., 1992).

Yellow patch was reported to be caused by *R. cerealis* by Boerema and Van der Hoeven in 1977. The disease normally occur during prolonged wet conditions when air temperature is between 10°C to 20°C (Burpee, 1980). The disease is also known as cool weather brown patch. Smith et al., (1989) reported that the symptom of yellow patch was commonly seen as light yellow, tan or straw coloured patches ranging from 2.5 cm to 0.9 meters in diameter. The grass in the centre of the larger patches normally recover, leading to the formation of frog-eye pattern of areas of green plants with 2.5 cm to 5.0 cm light yellow to tan outer rings (Fermanian et al., 1997).

Diseases Caused by *Pythium* Species

Pythium species, which are closely related to algae than fungi, had been reported to be cosmopolitan in soils and in water worldwide (Vargas, 1981; Smiley et al., 1992; Fermanian et al., 1997). Many species of *Pythium* have a very broad host range including turfgrasses. All turfgrasses were found to be susceptible to attack by species of *Pythium* under a variety of environmental conditions such as wet and high humidity condition (Smith et al., 1989; Smiley et al., 1992) causing seed rot, seedling blight, crown and root rot and foliage blight (Fermanian et al., 1997).

The symptom expression is normally related to the site of infection. Soil inhibiting species of *Pythium* incited the greatest damage in saturated or overlay wet soils, where their germination and growth were encouraged by stimulatory seed and root exudate (Smiley et al., 1992).

Seed Rot and Seedling Diseases

Species of *Pythium* involved in seed rot and seedling diseases include *P. aphanidermatum, P. aristosporum, P. arrhenomanes, P. dissotcum, P. graminicola, P. irregulare, P. multisporum* and *P. myriotylum* (Smiley et al., 1992). These diseases were reported to be most common when seed germination and the growth of seedling was unhealthy due to sub-optimal temperature, moisture, oxygen and lights conditions that favoured the growth and invasion of pathogens (Smith et al., 1989). Infected