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BIOLOGY AND SURVIVAL OF PSEUDOMONAS SOLANACEARUM IN MALAYSIA

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BIOLOGY AND SURVIVAL OF *PSEUDOMONAS SOLANACEARUM* IN MALAYSIA

by

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TABLE OF CONTENTS

		Page
ACKNOWLEDGEMENTS		ii
LIST OF TABLES		ix
LIST OF FIGURES		xii
LIST OF PLATES		xiv
ABSTRACT		xvi
ABSTRAK		xxi
CHAPTERS		
I	INTRODUCTION	1
	Pseudomonas solanacearum	1
	Survival and Control	4
	Objectives	6
II	LITERATURE REVIEW	7
	Historical Background	7
	Distribution and Economic Significance	9
	Etiology	14
	Strain Variation	16
	Biovar	21
	Races	22
	Bacteriophage	23
	Bacteriocin	26

CHAPTERS Page

Environmental Factors 28

Survival 30

Control 32

III DISTRIBUTION OF BACTERIAL WILT IN MALAYSIA 37

Introduction 37

Materials and Methods 42

Distribution of Bacterial Wilt in Malaysia 42

Isolation of Pseudomonas solanacearum and Preparation of Stock Culture 43

Pathogenicity Studies 45

Results 49

Distribution of Bacterial Wilt 49

Distribution of Bacterial Wilt According to Weather Conditions in Malaysia 54

Distribution of Bacterial Wilt According to Soil Types in Malaysia 66

Distribution of Bacterial Wilt According to Hosts 68

Isolation of Pseudomonas solanacearum 69

Pathogenicity 70

Discussion 70

CHAPTERS Page

IV BIOLOGICAL CHARACTERIZATION OF *PSEUDOMONAS SOLANACEARUM* 79

Introduction 79

Materials and Methods 80

Selection of Isolates 80

Cultural Characteristics 80

Host Range 83

Biovar Differentiation 85

Race Determination 86

Isolation and Purification of Bacteriophages 87

Determination of Routine Test Dilution and Phage Concentration 93

Typing of Bacteriophages 94

Results 94

Cultural Characteristics 94

Host Range 98

Biovar Differentiation 107

Race Determination 110

Isolation and Purification of Bacteriophages 110

Plaque Morphology 113

Bacteriophage Designation 113

Bacteriophage Typing 116

CHAPTERS Page

Discussion 120

Cultural Characteristics 120

Host Range 122

Biovar 124

Race 125

Bacteriophages 126

V SURVIVAL AND CONTROL OF PSEUDOMONAS SOLANACEARUM 127

Introduction 127

Materials and Methods 128

Bacteriocin Production 128

Culture Media 128

Bacterial Culture 128

Detection of Bacteriocin Production 129

Effect of Mitomycin C on the Concentration of Bacteriocin 132

Determination of Bacteriocin Concentration 133

Determination of Bacteriocin-Sensitivity Patterns 134

Dynamics of the Virulent and Avirulent Strains of Pseudomonas solanacearum 135

Production of Streptomycin Resistant Mutants 135

CHAPTERS Page

Preparation of Inoculum and Soil Inoculation 137

Determination of the Population of P. solanacearum in the Soil 138

Determination of the Influence of Host and Non-host on Population Density of P. solanacearum in the Soil 139

Results 144

Bacteriocin Production 144

Effect of Mitomycin C on Bacteriocin Production and Concentration 145

Bacteriocin Concentration 145

Bacteriocin Sensitivity Pattern 150

Survival of P. solanacearum in Artificially Infested Soil 160

Influence of Host and Non-host on Population Density of P. solanacearum in the Soil 167

Discussion 170

Bacteriocin Production 170

Survival of Pseudomonas solanacearum in the Soil 172

Survival of Pseudomonas solanacearum in the Presence of Host and Non-host 174

CHAPTERS Page

VI SUMMARY AND CONCLUSION 176

LITERATURE CITED 185

APPENDICES

A CULTURE MEDIA USED FOR THE ISOLATION AND PREPARATION OF STOCK CULTURE OF *PSEUDOMONAS SOLANACEARUM* 204

B CHARACTERISTICS OF RUDUA SERIES 205

C CHARACTERISTICS OF SELANGOR SERIES 206

D CHARACTERISTICS OF TOK YOUNG SERIES 207

E BASAL MEDIUM USED FOR THE DETERMINATION OF ACID PRODUCTION 208

F MEDIA USED FOR THE DETERMINATION OF NITRATE FROM NITRITE AND GAS PRODUCTION 209

G CULTURE MEDIA USED IN THE STUDY OF BACTERIOPHAGE 210

H CULTURE MEDIA USED IN THE STUDY OF BACTERIOCIN PRODUCTION 211

LIST OF TABLES

Table		Page
1.	Recorded hosts of *Pseudomonas solanacearum* in Malaysia	39
2.	Selected test plants inoculated with *Pseudomonas solanacearum* for pathogenicity studies	46
3.	Natural hosts of *Pseudomonas solanacearum* recorded in this study	50
4a.	Soil types from the Northern part of Peninsular Malaysia where *Pseudomonas solanacearum* was isolated	56
4b.	Soil types from the Central part of Peninsular Malaysia where *Pseudomonas solanacearum* was isolated	57
4c.	Soil types from the Southern part of Peninsular Malaysia where *Pseudomonas solanacearum* was isolated	59
5.	Locality, family and original hosts of selected isolates of *Pseudomonas solanacearum*	81
6.	Isolates of *Pseudomonas solanacearum* tested as indicator strain for the production of temperate bacteriophages	91
7.	Hosts and locations of *Pseudomonas solanacearum* isolates used for bacteriophage typing	95
8.	Colony characteristics of selected isolates of *Pseudomonas solanacearum* on a tetrazolium chloride medium and a medium containing 0.1% L-tyrosine	97
9.	Pathogenicity of selected *Pseudomonas solanacearum* isolates to ginger, groundnut, tobacco, tomato and Irish potato	103

Table		Page
10.	Biochemical reactions of selected isolates of *Pseudomonas solanacearum*	108
11.	Biotypes and races of selected isolates of *Pseudomonas solanacearum*	109
12.	Production of temperate bacteriophage from *Pseudomonas solanacearum* isolates	112
13.	Temperate bacteriophage designations and origin	117
14.	Reaction of 68 isolates of *Pseudomonas solanacearum* to 15 bacteriophages	118
15.	Lysotypes in *Pseudomonas solanacearum*	121
16.	Hosts and locations of *Pseudomonas solanacearum* isolates used to test for the production of bacteriocin	130
17.	The crops planted in each rotation for each treatment	141
18.	Bacteriocin production of Malaysian isolates of *Pseudomonas solanacearum* by Cuppels's broth culture technique	146
19.	Effect of various concentration of Mitomycin C on the concentration (AU^{-ml}) of bacteriocin produced by four bacteriocin producers	147
20.	Thirty selected bacteriocinogenic isolates of *Pseudomonas solanacearum* and their host origin	148
21.	Concentration (AU^{-ml}) of bacteriocin produced by 30 bacteriocinogenic strain against 5 virulent isolates	149
22.	Bacteriocin sensitivity patterns of 68 isolates of *Pseudomonas solanacearum* to bacteriocin produced by 30 avirulent bacteriocinogenic strains	151

Table | Page

23. Survival of virulent (21034) and avirulent (M29012) P. solanacearum individually in sterilized and non-sterilized soils at different times after artificial inoculation 163

24. Survival of virulent (21034) and avirulent (M29012) P. solanacearum in the ratio of 1:1 in sterilized and non-sterilized soil at different times after artificial inoculation 164

25. Survival of virulent (21034) and avirulent (M29012) P. solanacearum in the ratio of 1:10 in sterilized and non-sterilized soil at different times after artificial inoculation 165

26. Survival of virulent (21034) and avirulent (M29012) P. solanacearum in the ratio of 1:100 in sterilized and non-sterilized soil at different times after artificial inoculation 166

27. Population of Pseudomonas solanacearum (21034) after the first, second and third crop rotation 169

LIST OF FIGURES

Figure		Page
1.	Locations of the area sampled for bacterial wilt caused by *Pseudomonas solanacearum*	38
2.	Climatic conditions of different locations nearest the sampling sites for selected locations for bacterial wilt survey. (Anon, Meteorological data from 1975-1984)	
	(i) Mean monthly air temperature for 10 years (1975-1984)	60
	(ii) Mean monthly rainfall for 10 years (1975-1984)	62
3.	Mean (1976-1979) annual variation of the soil temperature - average of all depths (After Todorov, 1980)	65
4.	A flow chart of the phage isolation procedure	88
5.	Pathogenicity of *Pseudomonas solanacearum* isolates to ginger, groundnut, tobacco, tomato and Irish potato	104
6.	Diagram to illustrate the arrangement of pots in the glasshouse	143
7.	Dendrogram of the cluster analysis of bacteriocin sensitivity patterns of 68 isolates of *Pseudomonas solanacearum* to 30 producer strains by CLUSTAN procedure CENTROID	155
8.	Dendrogram of the cluster analysis of bacteriocin sensitivity patterns of 68 isolates of *Pseudomonas solanacearum* to 30 producer strains by CLUSTAN procedure DENSITY	157

Figure Page

9. Population density trends of avirulent, M29012 (AV) and virulent, 21034 (V) strains of Pseudomonas solanacearum alone and incombination in sterilized (S) and non-sterilized (NS) soils 161

10. Population of Pseudomonas solanacearum in the soil after each crop rotation 168

LISTS OF PLATES

Plate		Page
1.	Typical symptoms of bacterial wilt of *Capsicum annuum* L. in the field	51
2.	Typical symptoms (arrowed) of bacterial wilt of *Arachis hypogeae* L. in the field	51
3.	Typical symptoms of bacterial wilt of *Solanum melongena* L. in the field	52
4.	Typical symptoms of bacterial wilt of *Zingiber officinale* Rosc. in the field	52
5.	Typical symptoms of bacterial wilt of *Euphorbia prunifolia* Jacq. in the field	53
6.	Typical symptoms of bacterial wilt of *Croton hirtus* L. in the field	53
7.	Milky exudate from an infected stem of *Psophocarpus tetragonolobus* DC.	55
8.	*Lycopersicon esculentum* Mill. inoculated with *Pseudomonas solanacearum* in the greenhouse showing bacterial wilt symptoms	71
9.	Wilting of *Arachis hypogeae* L. inoculated with *Pseudomonas solanacearum* in the greenhouse	71
10.	*Solanum tuberosum* L. inoculated with *Pseudomonas solanacearum* showing bacterial wilt symptoms	72
11.	*Nicotiana tabacum* L. inoculated with *Pseudomonas solanacearum* showing symptoms of bacterial wilt	72
12.	*Physalis minima* L. inoculated with *Pseudomonas solanacearum* showing wilting symptoms in the greenhouse	73

Plate		Page
13.	Euphorbia prunifolia Jacq. inoculated with Pseudomonas solanacearum showing wilting symptoms	73
14.	Croton hirtus L' Herit. inoculated with Pseudomonas solanacearum showing bacterial wilt symptoms	74
15.	Inoculated Zingiber officinale Rosc. showing bacterial wilt symptoms in the greenhouse	74
16.	Pseudomonas solanacearum on TZC agar plates after an incubation for 48 hours at 32 °C, showing the difference in amount of formazan formed. (A), isolate 24024 (B), isolate 25001 and (C), isolates 27013 (x 0.45)	99
17.	Fourty-eight hour culture of Pseudomonas solanacearum on TZC medium: (A) 26042 showing fluidal elliptical colonies (x10) and (B) 29127 showing fluidal irregularly round colonies (x10)	100
18.	Fourty-eight hour culture of Pseudomonas solanacearum isolate (29035) on TZC showing less fluidal, round colonies and non-helical formazan pattern (x20)	101
19.	Plaques formed by virulent bacteriophage of P. solanacearum after an incubation for 24 hr. at 30°C. (A, x 0.9; B, x 5) . . .	114
20.	Plaques formed by temperate bacteriophage of P. solanacearum after an incubation for 24 hr. at 30°C. (A, x 0.9; B, x 5) . . .	115
21.	Pots in the greenhouse for the experiment on the determination of the influence of host and non-host on population density of P. solanacearum in the soil (A & B) . . .	142

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ABSTRACT

Bacterial wilt, caused by *Pseudomonas solanacearum* (Smith, 1896) Smith 1914, is an important disease of many crop plants. The lack of a truly effective method of control for the disease, especially in the tropics, has been attributed to the many strains of the pathogen and the lack of information on the ecology and the variation in the pathogen population. The present study was therefore conducted to elucidate some aspects of the ecology and variation of the pathogen in Malaysia which would be useful in understanding the pathogen for the formulation of more effective control measures.

A study of the distribution of bacterial wilt caused by P. solanacearum in Malaysia showed that the disease was widespread. It was observed in all areas (the Northern, Central and Southern parts of Peninsular Malaysia, and East Malaysia) sampled. The disease occurred on many crop plants, weeds and ornamentals. A total of 243 isolates of the pathogen was obtained from 25 different plant species belonging to 9 families. The pathogenicity of all isolates was confirmed on its respective host and/or tomato. The study indicated the need for strict quarantine measures to be taken to exclude the introduction of exotic strains into the country and the need to reconfirm the status of some of the known host of the pathogen.

The environmental conditions in Malaysia were conducive for the growth of the pathogen and the development of the disease, being observed throughout the year regardless of dry or wet weather conditions. The disease also occurred in many types of mineral (clayey, loamy and sandy) and organic (peat and muck) soils. The type of hosts and its varietal susceptibility also played important roles in determining the occurrence of the disease in nature.

Cultural characteristics of isolates of P. solanacearum on a tetrazolium medium and a medium containing 0.1% L-tyrosine showed that isolates differed in colony size, fluidity and

production of formazan and melanin. However, these could not be used to differentiate strains within race 1.

Greenhouse pathogenicity studies revealed the presence of pathovars of race 1 which differed in their pathogenicity to ginger, groundnut and tobacco. Tomato and Irish potato were the universal hosts of all Malaysian isolates but all isolates were non-pathogenic to Heliconia sp. Biovar determination study indicated that isolates were biovar 2, 3, and 4. The majority of the isolates were biovar 3 followed by biovar 4 and 2. Determination of race based on host range, origin and biovar showed that isolates were in race 1 and 3. Race 1 was the predominant race and was widespread, while race 3 was confined to the highlands.

Attempts were made at isolating virulent and temperate bacteriophages of P. solanacearum. Only one virulent bacteriophage was obtained from diseased brinjal (Solanum melongena L.) plants and their rhizosphere soils. However, temperate bacteriophages were obtained from lysogenic strains of the pathogen. Forty-three percent of the P. solanacearum isolates were lysogenic and from these 14 temperate bacteriophages were isolated. Reaction of 68 P. solanacearum isolates to all the phages showed that none of the phages were specific to race or biovar. However, 13 lysotypes were distingushed and all isolates could be typed.

Thirty-eight percent of the isolates tested for bacteriocin production were bacteriocinogenic. Studies of the concentration of bacteriocin produced and the sensitivity of P. solanacearum isolates to the bacteriocin, showed that a few avirulent bacteriocinogenic strains produced relatively high concentrations of bacteriocin and were able to inhibit the growth of a large number of the virulent P. solanacearum isolates tested. This bacteriocin and its avirulent producer could therefore be useful in the development of biological control measures for the pathogen. However, a few isolates were not inhibited by any of the bacteriocin produced by the 30 avirulent bacteriocinogenic strains tested.

A study on the population of the virulent strain (race 1, biovar 3) in sterilized and non-sterilized soil showed that the pathogen was not capable of long term survival in the soil. Population density trends of the virulent strain in the presence of the avirulent bacteriocinogenic strain at ratios of 1:0, 1:1, 1:10 and 1:100 showed similar trends. This indicated that the avirulent strain was not effective in controlling the population of the virulent strain in the soil at the ratios tested or that the method of soil application was not effective.

Greenhouse study on the population dynamics of the virulent strain (race 1, biovar 3) of P. solanacearum showed

that the bacterial population in the soil decreased significantly after planting with a non-host crop. This indicates that the non-host crops viz. Ipomoea aquatica Forsk., Brassica rapa L. and Raphanus sativus L. var. hortensis Baker could be used in crop rotation programs.

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BIOLOGI DAN KEMANDIRAN UNTUK *PSEUDOMONAS SOLANACEARUM* DI MALAYSIA

oleh

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ABSTRAK

Penyakit layu bakteria yang disebabkan oleh *Pseudomonas solanacearum* (Smith, 1896) Smith 1914, adalah satu penyakit yang penting bagi banyak tanam-tanaman. Kekurangan cara kawalan yang benar-benar berkesan untuk mengawal penyakit ini telah disebabkan oleh adanya banyak strain-strain bagi patogen ini dan kekurangan pengetahuan mengenai ekologi dan variasi di dalam populasi patogen ini. Dari itu kajian ini telah dijalankan untuk memberi penjelasan mengenai beberapa aspek ekologi dan variasi patogen ini di Malaysia untuk memahami patogen ini supaya dapat perumusan cara kawalan yang lebih berkesan.

Kajian taburan penyakit layu bakteria yang disebabkan oleh Pseudomonas solanacearum di Malaysia, menunjukkan penyakit ini berlaku dengan berluasan. Penyakit ini dijumpai di semua kawasan (di utara, pertengahan dan selatan Semenanjung Malaysia dan Malaysia timur) yang dikaji. Penyakit ini juga terdapat pada banyak pokok tanaman, rumput-rumpai dan pokok perhaisan. Sejumlah 243 asingan patogen ini telah diperolehi daripada 25 spesies tumbuh-tumbuhan daripada 9 famili. Patogenisiti bagi semua asingan dipastikan kepada perumah asalnya dan/atau tomato.

Keadaan alam sekitar di Malaysia adalah sesuai untuk pertumbuhan patogen ini dan pengembangan penyakit layu bakteria. Penyakit ini berlaku sepanjang tahun dengan tidak mengira sama ada musim panas atau hujan. Penyakit ini juga berlaku pada banyak jenis tanah, daripada jenis bertanah liat kepada jenis berpasir dan tanah organik. Jenis perumah dan kepekaan varietinya juga memainkan peranan yang penting di dalam menentukan kejadian penyakit ini.

Ciri-ciri kultura asingan-asingan P. solanacearum di atas media tetrazolium dan media yang mengandungi 0.1% L-tyrosine telah menunjukkan bahawa asingan-asingan berbeza dari segi ukuran garis pusat dan kecairan koloni; dan pengeluaran formazan dan melanin. Walau bagaimanapun variasi yang terdapat

tidak boleh digunakan untuk membuat perbezaan antara strain-strain di dalam ras 1.

Kajian patogenisiti di rumahkaca menunjukkan adanya patovar-patovar ras 1 yang berbeza dari segi patogenisitinya terhadap halia, kacang tanah dan tembakau. Tomato dan kentang adalah perumah umum bagi semua asingan-asingan dari Malaysia, tetapi semua asingan-asingan tidak patogenik kepada Heliconia sp. Kajian penentuan biovar menunjukkan bahawa asingan-asingan adalah biovar 2, 3, dan 4. Biovar 3 adalah biovar yang terbanyak di Malaysia, diikuti oleh biovar 4 dan 2. Penentuan ras berdasarkan kepada bidang perumah, asal dan biovar menunjukkan bahawa asingan-asingan adalah ras 1 dan 3. Ras 1 adalah ras yang terbanyak dan luas sebarannya. Ras 3 pula terhad sebarannya kepada kawasan pergunungan.

Beberapa percubaan telah dibuat untuk mengasingkan virulen dan temperat bakteriofaj bagi P. solanacearum. Hanya satu bakteriofaj virulen telah diasingkan daripada pokok-pokok terong (Solanum melongena) yang berpenyakit dan tanah-tanah rizosferanya. Walaupun demikian temperat bakteriofaj telah diasingkan daripada strain-strain lisogenik patogen itu. Empat puluh tiga peratus daripada asingan P. solanacearum adalah lisogenik dan daripadanya 14 bakteriofaj temperat telah diasingkan. Tindakbalas 68 asingan P. solanacearum kepada semua faj menunjukkan bahawa tiada faj yang terhad kepada

sesuatu ras atau biovar. Walau bagaimanapun, 13 lisotip-lisotip telah dibezakan dan semua asingan boleh ditipkan.

Tiga puluh lapan peratus daripada asingan-asingan yang diuji untuk pengeluaran bakteriosin didapati bakterisinogenik. Satu kajian kepekatan bakteriosin yang diperolehi menunjukkan bahawa ada sebilangan kecil strain-strain avirulen yang bakteriosinogenik berupaya mengeluarkan bakteriosin yang agak pekat kalau dibandingkan dengan yang lain-lain. Strain-strain itu juga boleh menghalangi pertumbuhan sebilangan besar asingan-asingan P. solanacearum yang virulen. Dari itu, bakteriosin dan pengeluarnya yang avirulen mungkin berguna di dalam langkah-langkah kawalan biologi bagi patogen ini. Walau bagaimanapun, ada sebilangan kecil asingan-asingan yang kehidupannya tidak terhalang oleh kesemua bakteriosin yang dikeluarkan oleh 30 strain bakteriosinogenik yang diuji.

Kajian populasi strain virulen (ras 1, biotip 3) di dalam tanah steril dan tidak steril menunjukkan patogen ini tidak boleh hidup lama di dalam tanah. Trend ketumpatan populasi bagi strain virulen, dengan strain avirulen yang bakteriosinogenik pada nisbah 1:0, 1:1, 1:10 dan 1:100 menunjukkan trend yang serupa. Ini menunjukkan bahawa strain avirulen tidak berkesan di dalam pengawalan populasi virulen didalam tanah pada nisbah yang diuji atau kaedah penggunaannya tidak berkesan.