General Distribution **OCDE/GD(97)22**

Series on Harmonization of Regulatory Oversight in Biotechnology No. 6

CONSENSUS DOCUMENT ON INFORMATION USED IN THE ASSESSMENT OF ENVIRONMENTAL APPLICATIONS INVOLVING PSEUDOMONAS

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

Paris

49010

Document complet disponible sur OLIS dans son format d'origine

Complete document available on OLIS in its original format

Also published in the Series on Harmonization of Regulatory Oversight in Biotechnology:

No. 1, *Commercialisation of Agricultural Products Derived through Modern Biotechnology: Survey Results* (1995)

No. 2, *Analysis of Information Elements Used in the Assessment of Certain Products of Modern Biotechnology* (1995)

No. 3, *Report of the OECD Workshop on the Commercialisation of Agricultural Products Derived through Modern Biotechnology* (1995)

No. 4, *Industrial Products of Modern Biotechnology Intended for Release to the Environment: The Proceedings of the Fribourg Workshop* (1996)

No. 5, Consensus Document on General Information concerning the Biosafety of Crop Plants Made Virus Resistant through Coat Protein Gene-Mediated Protection (1996)

Consensus Document on the Biology of Brassica Napus L *(Oilseed Rape)* (in preparation)

Consensus Document on the Biology of Solanum tuberosum *(Potato)* (in preparation)

Consensus Document on Information Used in the Assessment of Environmental Applications Involving Rhizobiacea (in preparation)

Consensus Document on Information Used in the Assessment of Environmental Applications Involving Bacillus (in preparation)

© OECD 1997

Applications for permission to reproduce or translate all or part of this material should be made to: Head of Publications Service, OECD, 2 rue André-Pascal, 75775 Paris Cedex 16, France.

OECD Environmental Health and Safety Publications

Series on Harmonization of Regulatory Oversight in Biotechnology

No. 6

Consensus Document on Information Used in the Assessment of Environmental Applications Involving *Pseudomonas*

Environment Directorate

Organisation for Economic Co-operation and Development

Paris 1997

About the OECD

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 29 industrialised countries in North America, Europe and the Pacific, as well as the European Commission, meet to co-ordinate and harmonize policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialized Committees and subsidiary groups composed of Member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD's Workshops and other meetings. Committees and subsidiary groups are served by the OECD Secretariat, located in Paris, France, which is organised into Directorates and Divisions.

The Environmental Health and Safety Division publishes complimentary documents in six different series: **Testing and Assessment**; **Good Laboratory Practice and Compliance Monitoring**; **Pesticides**; **Risk Management**; **Harmonization of Regulatory Oversight in Biotechnology**; **and Chemical Accidents.** More information about the Environmental Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (see below).

This publication is available electronically, at no charge.

For the complete text of this and many other Environmental Health and Safety publications, consult the OECD's World Wide Web site (http://www.oecd.org/ehs/)

or contact:

OECD Environment Directorate,
Environmental Health and Safety Division

2 rue André-Pascal
75775 Paris Cedex 16
France

Fax: (33-1) 45 24 16 75

E-mail: ehscont@oecd.org

FOREWORD

The OECD'S Expert Group on Harmonization of Regulatory Oversight in Biotechnology decided at its first session, in June 1995, to focus its work on the development of ***consensus documents*** which are mutually acceptable among Member countries. These consensus documents contain information for use during the regulatory assessment of a particular product.

This document contains information for use during regulatory assessments of environmental applications involving organisms which are fluorescent members of the genus *Pseudomonas* (rRNA group I pseudomonads). The first draft (initially prepared by the United Kingdom) was completed in August 1995. It was then sent to national co-ordinators, nominated by the Expert Group, for technical comments. Following receipt of their comments, and a meeting of a task group established by the Expert Group, it was decided that the document should be revised further. This work was undertaken by Canada, as the lead country.

At its second session, in March 1996, the Expert Group agreed in principle that this document should be recommended for derestriction after the incorporation of certain changes. A revised version was forwarded for consideration to the national co-ordinators who had commented on the previous draft.

The Joint Meeting of the Chemicals Group and Management Committee of the Special Programme on the Control of Chemicals subsequently recommended that this document be made available to the public. It is published on the authority of the Secretary-General of the OECD.

TABLE OF CONTENTS

Preamble 13

Section I **General Introduction** 15

Section II **Introduction to the Genus *Pseudomonas*** 16

Section III **Format of the Information in this Consensus Document** 18

Section IV **Information Used in the Assessment of Environmental Applications Involving *Pseudomonas*** 21

A. General Considerations 21

1. Subject of the document: species included and taxonomic considerations 21

1.1 Species included 21
1.2 Taxonomic considerations 21

1.2.1 The genus Pseudomonas 21
1.2.2 The "fluorescent" subgroup 22

Fluorescence 22
Plasmid-encoded characteristics 26

2. Characteristics of the organism which permit identification and the methods used to identify the organism 26

2.0 General considerations 26

2.1 Methods used for identification and classification 26

2.1.1 Numerical taxonomy 26
2.1.2 Genotypic approaches 27

Polymerase chain reaction (PCR) 29

2.1.3 Other biomarkers 29

3. Information on the reproductive cycle (sexual/asexual) 30

(continued on next page)

4. Biological features and environmental conditions which affect survival, reproduction, growth, multiplication or dissemination 30

4.0 General considerations 30
4.1 *P. aeruginosa* 30
4.2 *P. chlororaphis* 30
4.3 *P. fluorescens* 31
4.4 *P. fragi* 32
4.5 *P. putida* 32
4.6 *P. syringae* 32
4.7 *P. tolaasii* 33

5. Behaviour in simulated natural environments such as microcosms, growth rooms, greenhouses, insectaries, etc 34

5.1 *P. aeruginosa* 34
5.2 *P. chlororaphis* 34
5.3 *P. fluorescens* 34
5.4 *P. fragi* 34
5.5 *P. putida* 34
5.6 *P. syringae* 35
5.7 *P. tolaasii* 36

6. History of use (examples of environmental applications of the organism and information derived from these examples) 36

6.0 General considerations 36
6.1 *P. aeruginosa* 36
6.2 *P. chlororaphis* 36
6.3 *P. fluorescens* 38
6.4 *P. fragi* 38
6.5 *P. putida* 38
6.6 *P. syringae* 39
6.7 *P. tolaasii* 39

7. Characterisation of the genome (e.g. presence of large plasmids, insertion sequences) and stability of these characteristics 39

8. Genetic transfer capability 40

Conjugation 41
Transduction/bacteriophage mediated gene transfer 42
Transformation 43

B. Human Health Considerations 43

9. Diseases caused and mechanism of pathogenicity including invasiveness and virulence 43

9.0 General considerations 43
9.1 *P. aeruginosa* 44
9.2 *P. fluorescens* 46
9.3 *P. fragi* 46
9.4 *P. putida* 46
9.5 *P. chlororaphis, P. syringae, P. tolaasii* 46

10. Communicability 46

11. Infective dose 47

12. Host range, possibility of alteration 47

13. Capacity for colonisation 47

14. Possibility of survival outside the human host 47

15. Means of dissemination 48

16. Biological stability 48

17. Antibiotic-resistance patterns 48

17.1 *P. aeruginosa* 48
17.2 *P. fluorescens* and *P. putida* 48
17.3 *P. chlororaphis*, *P. fragi*, *P. syringae, P. tolaasii* 48

18. Toxigenicity 48

19. Allergenicity 49

20. Availability of appropriate prophylaxis and therapies 51

20.1 *P. aeruginosa* 51
20.2 *P. fluorescens* and *P. putida* 51
20.3 *P. chlororaphis*, *P. fragi*, *P. syringae, P. tolaasii* 51

(continued on next page)

C. Environmental and Agricultural Considerations...51

21. Natural habitat and geographic distribution. Climatic characteristics of original habitats..51

21.0 General considerations..51
21.1 *P. aeruginosa*...52
21.2 *P. chlororaphis*..52
21.3 *P. fluorescens*...52
21.4 *P. fragi*...52
21.5 *P. putida*..53
21.6 *P. syringae*...53
21.7 *P. tolaasii*...53

22. Significant involvement in environmental processes, including biogeochemical cycles and potential for production of toxic metabolites...53

Toxic metabolites of hazardous wastes 53

23. Pathogenicity - host range, infectivity, toxigenicity, virulence, vectors 54

23.0 General considerations..54
23.1 *P. aeruginosa*...54

Pathogenicity to animals..54
Pathogenicity to plants 55

23.2 *P. chlororaphis*..56

Pathogenicity to animals..56
Pathogenicity to plants 56

23.3 *P. fluorescens*...56

Pathogenicity to animals 56
Pathogenicity to plants 57

23.4 *P. fragi*...58

Pathogenicity to animals..58
Pathogenicity to plants 58

23.5 *P. putida*..58

Pathogenicity to animals 58
Pathogenicity to plants 58

23.6 *P. syringae* ... 58

Pathogenicity to animals ... 58
Pathogenicity to plants ... 58

23.7 *P. tolaasii* ... 63

Pathogenicity to animals ... 63
Pathogenicity to plants ... 63

24. Interactions with and effects on other organisms in the environment ... 63

24.1 *P. aeruginosa* ... 63
24.2 *P. chlororaphis* ... 64
24.3 *P. fluorescens* ... 65
24.4 *P. fragi* ... 65
24.5 *P. putida* ... 65
24.6 *P. syringae* ... 66
24.7 *P. tolaasii* ... 68

25. Ability to form survival structures (e.g. spores, sclerotia) ... 68

26. Routes of dissemination, physical or biological ... 68

Physical ... 68
Biological ... 69

27. Containment and decontamination ... 69

28. Description of detection and monitoring techniques, including specificity, sensitivity and reliability ... 70

28.1 Techniques employed in the laboratory and/ or environment for detecting the presence of, and for monitoring, numbers of the organism ... 70

Selective plating ... 70
Most probable number ... 70
Simple chemotaxonomical approach ... 72
Immunological methods ... 72
Nucleic acid probes and primers ... 72
Polymerase chain reaction (PCR) based sequence amplification ... 73
Arbitrary PCR primers ... 73
Specific PCR primers ... 73

28.2 Specificity, sensitivity, reliability ... 73

(continued on next page)

Section V References 75

Appendix: Considerations from the OECD "Blue Book" 105

Questionnaire to Return to the OECD 109

PREAMBLE

OECD Member countries are moving rapidly towards the commercialisation and marketing of agricultural and industrial products of modern biotechnology. They have therefore identified the need for harmonization of regulatory approaches to the assessment of these products, in order to avoid unnecessary trade barriers.

At the end of 1992, a project on ***Environmental Applications of Modern Biotechnology*** (formerly called ***Industrial Products of Modern Biotechnology***) was initiated under the auspices of the OECD's Environment Policy Committee. The scope of the project includes microorganisms for use in applications such as bioremediation, bioprevention, biomining and bioleaching. Its objective is to assist countries in their regulatory assessment of such applications and to facilitate international harmonization.

The first step in this project was to organize a Workshop, held in Brussels in 1993, to identify the information used by regulatory authorities in OECD countries when assessing these applications. The results, which show considerable commonality among countries, are described in OECD Environment Monograph No. 100, *Analysis of Information Elements Used in the Assessment of Certain Products of Modern Biotechnology* (OECD, 1995).

Building on the work of the Brussels Workshop, a second Workshop held in Fribourg, Switzerland, in 1994 identified the ***types*** of information used to address the information elements which had been identified [see Environment Monograph No. 117, *Industrial Products of Modern Biotechnology Intended for Release to the Environment: The Proceedings of the Fribourg Workshop* (OECD, 1996)]. As a result of the Fribourg Workshop, it was shown that much of the information used in regulatory assessments is not case-specific but would be equally applicable to many assessments involving the same or similar host organisms. It was further found that much of this information, such as that related to the biological properties of the host organism, is available in the scientific literature.

In June 1995, at its first session, the Expert Group on the Harmonization of Regulatory Oversight in Biotechnology instituted the development of ***consensus documents*** which are ***mutually acceptable*** among Member countries, as an initial step in efforts to facilitate harmonization. The purpose of these consensus documents is to identify common elements in the safety assessment of environmental applications of modern biotechnology, to encourage information sharing, and to prevent duplication of effort among OECD countries.

The focus of this consensus document is on information which is not case-specific, and which is readily available from the scientific literature, related to fluorescent members of the genus **Pseudomonas** ***(rRNA group I pseudomonads).***

In order to ensure that scientific and technical developments are taken into account, it was agreed that these documents will be updated regularly. Additional areas relevant to the subject of each consensus document will be considered at the time of updating.

Users of this document are invited to provide the OECD with new scientific and technical information, and to make proposals for additional areas to be considered. ***There is a short, pre-addressed questionnaire for this purpose on page 109. The completed questionnaire (or a photocopy) should be returned to the Environmental Health and Safety Division.***

SECTION I – GENERAL INTRODUCTION

This document presents information that is accepted in the scientific literature concerning the known characteristics of fluorescent members of the genus *Pseudomonas* (rRNA group I pseudomonads). Regulatory officials may find this information useful in evaluating and establishing the properties of environmental applications of biotechnology which involve those microorganisms which are the focus of this document. Consequently, a wide range of information is provided without prescribing when the information would or would not be relevant to a specific risk assessment. This document represents a "snapshot" of current information that may potentially be relevant to such assessments. However, Member countries have not yet attempted to put together an exhaustive literature review on all aspects of these organisms.

The genus *Pseudomonas* may potentially be utilised in a number of different engineering applications. These include *in situ* applications such as groundwater reinjection, air sparging, and bioventing. They also include *ex situ* applications such as landfarming, slurry phase remediation, and biopiles. Many of the potential uses under development or envisioned for the genus *Pseudomonas* involve improvement of air, soil or water quality, or cleanup of otherwise intractable environmental contaminants.

In considering information that should be presented on this taxonomic grouping, the Task Group for Environmental Applications of Modern Biotechnology discussed the list of topics developed in the "Blue Book", *Recombinant DNA Safety Considerations* (OECD, 1986), and attempted to pare down that list to eliminate duplications, as well as those topics whose meaning is unclear, and to rearrange the presentation of the topics covered so they would be easier to understand and use (see Section III).

This effort at refining the exposition of safety considerations in the "Blue Book" for application to the genus *Pseudomonas* has also recognized the importance of a thorough understanding of the characteristics of the particular application for which these organisms will be used. Worker and other human exposures, and environmental exposures, will differ depending on the method of application. This knowledge is likely to affect the types of information on particular taxa that regulatory officials deem relevant in specific risk assessments. Group I pseudomonads are known to display a range of pathogenic and toxicological characteristics in regard to humans, animals and plants. However, even though some of the rRNA group I pseudomonads are known to exhibit pathogenic properties, exposures of and potential impacts on humans, animals and plants may be relatively limited in some circumstances, e.g. when the microorganisms are used in bioreactors of various sorts that have suitable controls on liquid and gaseous emissions, or when other specific mitigation or containment measures are in place. The factors discussed in this document may, therefore, have varying levels of impact on individual risk assessments, depending upon how and where the particular microorganisms are used, i.e. depending on the likely exposures presented by the application.

Given the breadth of information contained in this document, it is hoped that it will be useful not only to regulatory officials as a general guide and reference source, but also to industry and to scientists involved in research.

This document is a consensus document for environmental applications involving fluorescent members of the genus *Pseudomonas* (rRNA group I). Section II is an introduction to the genus *Pseudomonas* and to the species which are the subject of the document. The format of the information is described in Section III, and the information is presented in Section IV. Section V contains the References.

SECTION II – INTRODUCTION TO THE GENUS *PSEUDOMONAS*

Taxonomy

Pseudomonas is part of a large, heterogeneous and ubiquitous group of microorganisms generally referred to as pseudomonads. The pseudomonads are characterised as being highly metabolically versatile, bioactive, and prolific colonisers of surfaces. Pseudomonads are gram-negative, straight or slightly curved rods with polar flagella; they are chemo-organotrophs with a respiratory, non-fermentative type of metabolism and are usually catalase and oxidase-positive. The taxonomy of the group has been clarified using 16S ribosomal RNA sequence analysis (Table 1, Section IV).

The genus *Pseudomonas* corresponds to rRNA group I (Table 1). The type species for the genus is *Pseudomonas aeruginosa*. Strains are metabolically diverse, as well as having the capacity for denitrification and arginine degradation under anaerobic conditions. *P. aeruginosa* has been studied in more detail than any other pseudomonad using genetic techniques. Physical and genetic chromosome maps have been described (Romling et al., 1989; Ratnaningsih et al., 1990).

Applications

Pseudomonads have been identified to be of importance in bioremediation as a result of their tremendous capacity for biodegradation. They also offer considerable promise in agronomic applications, since many strains are bioactive, fast-growing, prolific colonisers of plant surfaces and are able to suppress or out-compete pathogenic and other deleterious microorganisms.

Pseudomonads as candidates for bioremediation

Nutritional versatility is exhibited widely amongst the pseudomonads. Combined with the presence or acquisition of catabolic plasmids by large numbers of strains, pseudomonads have the potential to mineralise a wide range of natural organic compounds, including aromatic hydrocarbons. This versatility allows for the rapid evolution of new metabolic pathways for the degradation of synthetic compounds (xenobiotics), leading to their complete oxidation and mineralisation. The complexity of the catabolic routes indicates sophisticated systems of regulation to control the expression and achieve the co-ordination of catabolic activities. Although the degradative pathways of pseudomonads vary considerably, the metabolic routes are convergent and lead to a limited number of common intermediates such as catechols. These represent key intermediates for aromatic compound degradation.

It is also anticipated that the nutritional versatility of pseudomonads and the application of molecular genetic techniques will be harnessed in the design of catabolic pathways for environmental purposes (Ensley, 1994; Timmis, 1994). For example, a *Pseudomonas* strain was recently isolated that can utilise TNT (2,4,6-trinitrotoluene) as a sole nitrogen source, producing toluene, aminotoluene and nitrotoluenes as end products. This organism was, however, unable to utilise toluene as a carbon source for growth. By introducing the entire toluene degradation pathway carried on the TOL plasmid pWWO-Km, an organism was produced that could potentially completely mineralise TNT (Ensley, 1994). Despite some of the TNT being completely mineralised, the formation of some dead-end metabolites by reduction of the nitrotoluenes to aminotoluenes remains a problem.

Agronomic applications

Pseudomonads also have great potential in agronomic applications, since they are prolific colonisers of plant surfaces and represent a significant component of plant microflora. Furthermore, they have been identified to possess traits that make them suitable as agents for biological pest control (O'Sullivan and O'Gara, 1992). These traits include an ability to produce antimicrobial molecules (antibiotics, antifungals and siderophores) and a capacity to compete aggressively with other microorganisms for niches and to exclude phytopathogens.

SECTION III – FORMAT OF THE INFORMATION IN THIS CONSENSUS DOCUMENT

The information format presented in this section is based on the ***General Scientific Considerations***, ***Human Health Considerations*** and ***Environmental and Agricultural Considerations*** from the OECD "Blue Book" (OECD, 1986) (see the Appendix to this consensus document). These Considerations were also used as the reference point in Environment Monograph No. 100, *Analysis of Information Elements Used in the Assessment of Certain Products of Modern Biotechnology* (OECD, 1995), which identified commonalities among OECD countries with respect to data elements used during regulatory assessments.

It was decided at a meeting of the OECD Task Group on Environmental Applications of Modern Biotechnology in October 1995 that, for the purpose of producing consensus documents containing information for use during regulatory assessments, a subset of the considerations addressed in the OECD "Blue Book" would be appropriate. The subset presented here reflects the removal of considerations in the "Blue Book" that were duplicative or were possibly ambiguous in meaning:

Information Elements

A. General Considerations

Taxonomy, identification, source, culture

1 Subject of document: species included and taxonomic considerations;

2 Characteristics of the organism which permit identification, and the methods used to identify the organism;

3 Information on the recipient organism's reproductive cycle (sexual/asexual);

4 Biological features and environmental conditions which affect survival, reproduction, growth, multiplication or dissemination;

5 Behaviour in simulated natural environments such as microcosms, growth rooms, greenhouses, insectaries, etc.;

6 History of use (examples of environmental applications of the organism and information derived from these examples);

Genetic characteristics of the organism

7 Characterisation of the genomes (e.g. presence of large plasmids, insertion sequences), and stability of these characteristics;

8 Genetic transfer capability;

B. Human Health Considerations

Characteristics of the organism

9 Diseases caused and mechanism of pathogenicity, including invasiveness and virulence;

10 Communicability;

11 Infective dose;

12 Host range, possibility of alteration;

13 Capacity for colonisation;

14 Possibility of survival outside of human host;

15 Means of dissemination;

16 Biological stability;

17 Antibiotic-resistance patterns;

18 Toxigenicity;

19 Allergenicity;

20 Availability of appropriate prophylaxis and therapies;

C. Environmental and Agricultural Considerations

Ecological Traits of the Organism

21 Natural habitat and geographic distribution. Climatic characteristics of original habitats;

22 Significant involvement in environmental processes, including biogeochemical cycles, and potential for production of toxic metabolites;

23 Pathogenicity - host range, infectivity, toxigenicity, virulence, vectors;

24 Interactions with and effects on other organisms in the environment;

25 Ability to form survival structures (e.g. spores, sclerotia);

26 Routes of dissemination, physical or biological;

Application of the Organism in the Environment

27 Containment and decontamination;

28 Description of detection and monitoring techniques, including specificity, sensitivity and reliability.

The information elements numbered 1-28 above were adopted as the framework for producing this* Pseudomonas *consensus document.

In Section IV, each of these information elements has been used as a prompt to collate the information in the scientific literature which is applicable to the assessment of the environmental application of the microorganisms. The information is that available in the literature as of 30 June 1995. The literature search covered a number of databases. The literature search for human health information elements (9-20) covered Medline, Biosis, C.A.B., Embase, and Food Science and Technology Abstracts, back to 1966 or when the particular electronic database was established. Other information elements (4-6, 21, 23-24) grouped information by species. The literature search for these elements covered the following databases: *P. aeruginosa* (C.A.B. Abstracts, 1972-June 1995; B.C.I., 1991-June 1995; Chapman and Hall CD-ROM, 1995); *P. chlororaphis* (and *P. aureofaciens*) (C.A.B. Abstracts, 1972-June 1995; B.C.I., 1991-June 1995; Agricola, 1970-1995; DNP CD-ROM, 1995); *P. fluorescens* (and *P. marginalis*) (C.A.B. Abstracts, 1979-June 1995); *P. fragi* (C.A.B. Abstracts, 1972-June 1995; B.C.I., 1991-June 1995); *P. putida* (C.A.B. Abstracts, 1979-June 1995); *P. syringae* (C.A.B. Abstracts, 1972-June 1995; B.C.I., 1991-June 1995); and *P. tolaasii* (C.A.B. Abstracts, 1972-June 1995; B.C.I., 1991-June 1995).

The information is restricted to that available for the following seven species of the genus *Pseudomonas*: *P. aeruginosa, P. chlororaphis* (including *P. aureofaciens*), *P. fluorescens* (including *P. marginalis*), *P. fragi*, *P. putida*, *P. syringae*, and *P. tolaasii*.

Further, the information is restricted to that available for the naturally occurring species; information on any genetically modified strains is excluded unless it bears directly on the properties of the naturally occurring organism.

SECTION IV – INFORMATION USED IN THE ASSESSMENT OF ENVIRONMENTAL APPLICATIONS INVOLVING *PSEUDOMONAS*

A. General Considerations

1. Subject of the document: species included and taxonomic considerations

1.1 Species included

The subject of this document is a subset of seven species within the genus *Pseudomonas*, most of which produce fluorescent pigments. Many members of this set have been, or are likely to be, employed in various biotechnological applications in the environment. The seven species are: *P. aeruginosa*, *P. chlororaphis* (including *P. aureofaciens*), *P. fluorescens* (including *P. marginalis*), *P. fragi, P. putida, P. syringae,* and *P. tolaasii.*

1.2 Taxonomic considerations

1.2.1 The genus Pseudomonas

Prior to 1973, *Pseudomonas* was seen as one large heterogenous genus with members sharing a few phenotypic features. Palleroni et al. (1973) concluded that five groups approximating genera, which were established on the basis of rRNA sequence homologies (Table 1), appeared phylogenetically distant from each other. Though these groupings were confirmed through DNA hybridisation experiments (Johnson and Palleroni, 1989), it nonetheless took a decade to transform them into discrete taxonomic units based on both phenotypic and genotypic associations. The groupings now comprise units of larger than genus rank. Species once called *Pseudomonas* are now classified as members of at least a dozen genera found within the original five homology groups (Table 1; Yabuuchi and De Vos, 1995a; Yabuuchi and De Vos, 1995b). The genus *Pseudomonas* is now strictly confined to members of the rRNA group I (Table 1).

The members of this genus still represent a somewhat heterogenous collection of bacteria, but they are far more closely allied to each other than they are to species formerly having the genus name *Pseudomonas*. The type species for the genus is *Pseudomonas aeruginosa*. Strains of *P. aeruginosa* can be isolated from many environmental substrates, and appear uniform in a number of diagnostic characters (Palleroni, 1992b). It can be argued that *P. fluorescens* is more "typical" of the genus than is *P. aeruginosa*, but due to the difficulty of establishing defining characteristics for *P. fluorescens, P. aeruginosa* remains the choice for the type species (Palleroni, 1992c).

Common characteristics of the genus* Pseudomonas*:

- gram-negative
- rod-shaped (straight, asporogenous, 0.5-1.0 X 1.5-4.0 µm)
- motile due to polar flagella
- oxidase-positive (except for *P. syringae*)
- oxidative metabolism (mostly saccharolytic, some non-saccharolytic species, no gas formation from sugars)

- chemo-organotrophs
- catalase-positive
- growth with acetate as sole carbon source, most non-fastidious, few require growth factors
- NO_3 reduced to NO_2 or molecular N_2
- accumulate longer-chained polyhydroxyalkanoates
- produce pigments
- indole-negative

1.2.2 The "fluorescent" subgroup

The seven species considered in this document [*P. aeruginosa, P. chlororaphis* (including *P. aureofaciens*), *P. fluorescens* (including *P. marginalis*), *P. fragi, P. putida, P. syringae,* and *P. tolaasii*] are considered to be closely related to each other except for *P. aeruginosa* and *P. syringae* (Molin and Ternström, 1986; Janse et al., 1992). These seven species are considered as the fluorescent subgroup of the rRNA group I, although *P. fragi* includes non-fluorescent strains.

P. fluorescens, P. putida and *P. chlororaphis* are seen as forming a complex, intertwined by a continuum of transitional strains (Molin and Ternström, 1986; Barrett et al., 1986). Complicating the classification scheme is the observation that both *P. fluorescens* and *P. putida* comprise several biovars, each of which may deserve species rank, but which are so interconnected that adequate methods have not been devised to clearly separate the member strains for each biovar. *P. chlororaphis*, which also encompasses the strains formerly called *P. aureofaciens* and *P. lundensis*, a recently described species, were once considered to belong to separate biovars of *P. fluorescens*.

Also closely associated with the *fluorescens-chlororaphis-putida* complex is *P. fragi*. This species has some fluorescent strains, but is primarily non-fluorescent. *P. fragi* is also a complex of different phenotypes, many of which are closely allied with some biovars of *P. fluorescens* and also could be misidentified as *P. putida* (Molin and Ternström, 1986).

P. aeruginosa, the type species and most clearly defined member of the genus, is seen as separate from the *fluorescens-chlororaphis-putida* complex.

P. syringae and *P. tolaasii* are pathogens in a group that also includes other pathogenic species (e.g. *P. cichorii* and *P. viridiflava*). However, *P. tolaasii* is an oxidase-positive mushroom pathogen related to, and potentially confused with, members of *the P. fluorescens* supercluster (Janse et al., 1992). *P. syringae* is an oxidase-negative plant pathogen comprising many pathovars derived from taxa that previously had species rank (Palleroni, 1984).

Fluorescence

Pigments often provide valuable diagnostic characters, since their production invariably correlates well with other group properties. Fluorescent pigments are produced abundantly in media with a low iron content; fluorescence varies from white to blue-green upon excitation with ultraviolet radiation. King's medium B is frequently used for the isolation of pseudomonads, especially by plant pathologists (King et al., 1954). Fluorescent species of *Pseudomonas* produce pyoverdin and/or phenazine pigments. Pyoverdin production is characteristic of most species. Palleroni (1984) indicates that *P. fluorescens* biovars II and V, along with *P. chlororaphis* and *P. putida* biovar B, have variable (11-89% positive) pyoveridin production. Although positive pyocyanin production is diagnostic for *P. aeruginosa*, the reverse is not necessarily true.

Table 1

Phylogeny and current classification of the pseudomonads

Proteobacteria subclass	rRNA group	Original name	Current classification	Characteristics
Gamma	I	* *P. aeruginosa*	*Pseudomonas*	type species; opportunistic pathogen
		* *P. fluorescens*		fluorescent supercluster; oxidase positive, mostly fluorescent, saprophytic or opportunistic pathogens
		* *P. chlororaphis*		
		P. lundensis	* *P. chlororaphis*	name reclassified
		* *P. putida*		
		* *P. tolaasii*		mushroom pathogen
		P. marginalis	* *P. fluorescens*	name reclassified
		P. aureofaciens	* *P. chlororaphis*	name reclassified
		* *P. fragi*		some strains non-fluorescent
		* *P. syringae*		fluorescent, plant or mushroom pathogen. *P. syringae* and *P. viridflava* are oxidase-negative. *P. syringae* comprises many pathovars
		P. viridiflava		
		P. cichorii		
		P. agarici		
		P. asplenii		
		P. flavescens		fluorescent
		P. alcaligenes		non-fluorescent
		P. citronella		
		P. mendocina		

(continued on next page)

Table 1

Phylogeny and current classification of the pseudomonads (cont.)

		P. oleovorans		
		P. pseudoalcaligenes		
		P. stutzeri		
Gamma	V	*P. maltophila*	*Stenotrophomonas maltophilia*	related to *Xanthomonas*
		P. marina	*Delaya marina*	
Beta	III	*P. acidovorans*	*Comamonas*	
		P. terrigena		
		P. testosteroni		
		P. avenae	*Acidovorax*	facultatively autotrophic species (hydrogen pseudomonad)
		P. delafieldii		
		P. facials		
		P. flava	*Hydrogenophaga*	facultatively autotrophic species (hydrogen pseudomonad)
		P. palleroni		
		P. pseudoflava		
		P. taenispiralis		
		P. saccharophila	*Pseudomonas*	
		P. ruhlandii	*Alcaligenes xylosoxidans*	

Table 1

Phylogeny and current classification of the pseudomonads (cont.)

Beta	II	*P. cepacia*	*Burkholderia*	heterogenous genus
		P. caryophylli		
		P. gladioli		
		P. mallei		
		P. pickettii		
		P. pseudomallei		
		P. solancearum		
Alpha	IV	*P. paucimobilis*	*Sphingomonas paucimobilis*	
		P. carboxidovorans	*Oligotropha carboxidovorans*	
		P. aminovorans	*Aminobacter aminovorans*	
		P. mesophilica	*Methylobacterium mesophilicum*	
		P. sp.	*Chelatobacter heintzii*	
		P. compransoris	*Zavarzinia compransoris*	
		P. diminuta	*Brevundimonas diminuta*	very distantly related to rRNA group I
		P. vesicularis	*Brevundimonas vesicularis*	

Compiled from Palleroni, 1992b and 1992c; Molin and Ternström, 1986; Yabuuchi et al., 1995a and 1995b, and Hildebrand et al., 1994. The species which are the focus of this document are indicated with an asterisk.

Plasmid-encoded characteristics

Plasmid-encoded characteristics such as antibiotic resistance, chemical resistance and metabolic capabilities are important components of the pseudomonad genome. Some examples, for the species under consideration, are given in Table 2. Many if not most of these characteristics are strain-specific and of little value in terms of taxonomy and identification. An exception to this rule, however, is phage susceptibility. Plasmid-encoded phage susceptibility can be important in differentiating *P. syringae* pathovars when combined with biochemical testing, and *P. aeruginosa* isolates have been typed to the subspecies level using phage sensitivity.

2. Characteristics of the organism which permit identification and the methods used to identify the organism

2.0 General considerations

P. aeruginosa is distinct and readily distinguished from other members of the genus, and the pathovars of *P. syringae* can be distinguished by determination of their host range.

Distinction of the other five species in the group *(P. fluorescens, P. chlororaphis, P. putida, P. tolaasii, P. fragi*) from each other is not straightforward, and the expression "continuum" is frequently used to describe their inter-relationship. Most authors agree that current methods are generally inadequate to ensure proper placement of new isolates within the related species *P. fluorescens*, and *P. putida* and their biovars (Palleroni, 1992b; Christensen et al., 1994; Barrett et al., 1986). Except for its pathogenicity, *P. tolaasii* is difficult to distinguish from *P. fluorescens* (Janse et al., 1992). *P. chlororaphis* is separable from *P. fluorescens* based on production of unique phenazine pigments (Palleroni, 1984), and has some distinctive substrate utilisation patterns (Barrett et al., 1986), but is otherwise well within the boundaries of the fluorescent supercluster (e.g. Janse et al., 1992). Finally, *P. fragi* shares many features with members of the fluorescent supercluster, but most strains are not fluorescent (Molin and Ternström, 1986).

2.1 Methods used for identification and classification

2.1.1 Numerical taxonomy

Numerical taxonomy has become the "traditional" method for classifying members of the genus *Pseudomonas*. This approach compares multiple features of the isolate, for which there is substantial discriminatory power, with a database of features of well-described members of the taxon. The accuracy of this type of approach will depend upon the quality and quantity of the data for strains comprising the reference database.

In order to achieve valid results, identical laboratory techniques need to be used for analysis of the isolate and the strains used to construct the reference database. The success of numerical taxonomy is also affected by the complexity of the relationships among the taxa being evaluated.

Use of a broad spectrum of substrates in numeric taxonomic evaluations has had some success for fluorescent species of *Pseudomonas* (e.g. Barrett et al., 1986). These techniques have permitted some assignment of strains to species and biovars within the fluorescent supercluster (a term applied to all of the species and biovars of *P. fluorescens, P. putida* and their allies, Table 1). However, Molin and Ternström

(1986) and Janse et al. (1992) both reported many unclassifiable strains among those they subjected to classical numeric taxonomic analyses.

Commercial suppliers have devised simplified, automated versions of this technique. Examples of commercial kits available for identification of *Pseudomonas* on the basis of carbon source utilisation patterns, and physiological and morphological characters, are the API20E (API, 200 Express Street, Plainview, New York 11803, USA; BioMerieux, F-69280 Marcy-L'Etoile, France) and the BIOLOG (BIOLOG Inc., Hayward, California, USA) systems. For these kits, the database for *Pseudomonas* is based on mainly clinical, not environmental, strains. As a result, the kits may fail to identify all environmental isolates.

Use of these kits requires experience. In addition, most of them are designed to determine the membership of the isolate within a taxon and not to distinguish strains within a species. That is, the test profile in most cases is not unique to a particular strain. So, in most cases, test profiles will not be sufficient to distinguish the isolate from other strains of the same species. If such a distinction is being made, it must be based on the detection of properties unique within the taxon.

Details of the test methodologies and profiles of the species can be found in Palleroni (1981; 1984; 1992c).

2.1.2 Genotypic approaches

The current classification of the pseudomonads is based on rRNA homologies. The variable and conserved regions of the RNA molecule are both important for identification purposes. The conserved regions serve as targets for polymerase chain reaction (PCR) primer binding sites and universal hybridization probes. The variable regions are the targets of the hybridization probes and primers that are taxon-specific. Probes and PCR primers directed at diagnostic rRNA sequences have facilitated the classification of pseudomonads into the five rRNA groups (Table 1).

Strong selection pressure for the conservation of 16S and 23S rRNA molecular structure and sequence has meant that rRNA molecules are powerful evolutionary clocks for describing phylogenetic relationships between rRNA groups of pseudomonads. At present, however, they are unable to position individual strains into species groups. This is particularly true for the fluorescent rRNA group I pseudomonads (Christensen et al., 1994). Using 23S rDNA methods, Christensen et al. (1994) found that "the method failed to provide a basis for distinguishing between *P. fluorescens, P. chlororaphis*, and *P. putida* Biovar B and to differentiate among the biovars of *P. fluorescens*." This study also showed that there did not seem to be a correspondence between taxonomies of this group based on 23S ribosomal sequences and from conventional numerical taxonomy. As pointed out by Janse et al. (1992), the large number of intermediate strains of all of these species shows "more variation than the present schemes (for classification) allow."

Schleifer et al. (1992) describe several probes for the rapid identification of members of the genus *Pseudomonas*. A 360 bp fragment of a 23S rRNA gene derived from *P. aeruginosa* (Festl et al., 1986) allowed differentiation of the eleven fluorescent and non-fluorescent group I species tested. A second probe was group-specific for *P. stutzeri, P. alcaligenes, P. pseudoalcaligenes* and *P. aeruginosa*. This probe comprises a 255 bp fragment of the 23S rDNA of *P. stutzeri* that is homologous to the bases 1366 to 1617 of *Escherichia coli* 23S rRNA. Both probes were tested by "dot-blot" hybridization against genomic DNA bound to filters.

Table 2

Examples of plasmids responsible for the metabolism of organic compounds or resistance to heavy metals in fluorescent *Pseudomonas* species

Organism	Plasmid	Substrate	Reference
P. putida	CAM TOL	camphor xylene, toluene	Jacoby, 1975 White & Dunn, 1978
	SAL	salicylate	Korfhagen et al., 1978
	NAH	napthalene	White & Dunn, 1978
	pRE4	isopropyl-benzene	Eaton & Timmis, 1986
	pEG	styrene	Bestetti et al., 1984
	pCINNP	cinnamic acid	Andreoni & Bestetti, 1986
	pAC25	3-chloro-benzoic acid	Chatterjee & Chakrabarty, 1983
P. fluorescens	pQM1	mercury	Bale et al., 1988
P. syringae pv. *tomato*	pPT23a; pPT23c	copper	Bender & Cooksey, 1986

Schleifer et al. (1992) used fluorescein or tetramethylrhodamine 5'end-labelled sequences as probes to identify *Pseudomonas* species. The first probe (P72; 5'-TTCAGTACAAGATACCTA) differentiated *P. aeruginosa, P. alcaligenes* and *P. pseudo-alcaligenes* from the other group I species. A second probe, Ps (5'-GAAGGCTAGGCCAGC), identified all species except *P. putida*. An oligonucleotide specific for the *P. putida* sequence, 5'-GAAGGUUAGGCCAGC, allowed differentiation of *P. putida,* and a mixed probe (i.e. both oligonucleotides) allowed detection of all species of *Pseudomonas*.

Polymerase chain reaction (PCR)

P. aeruginosa strains can be identified by PCR-based amplification of the 16S-23S rDNA internal transcribed spacer region with specific primers (Tyler et al., 1995).

2.1.3 Other biomarkers

Biomarkers such as fatty acid methyl esters (FAMES) have been used widely for the identification of bacterial species (Thompson et al., 1993b). A commercial identification system, the Microbial Identification System (MIDI, Newark, New Jersey, USA), offers an extensive database of strains, including many pseudomonad strains, to which the fatty acid profile of an unknown isolate may be compared. The libraries of strains contain well-described clinical, environmental and plant-pathogenic strains. Tahis system provides an identification at the species level and a diagnostic profile; however, it is unlikely to identify all environmental isolates, since many have not been described before. Whole cell fatty acid analysis was tested as a method to discriminate between members of the *Pseudomonas* fluorescent supercluster (Janse et al., 1992). This analysis resulted in recognition of a large supercluster that included most *P. fluorescens* and related strains (*P. chlororaphis, P. putida* and *P. tolaasii*). In the supercluster there were no separate clusters discriminating biovars of *P. fluorescens*, the other related species, or strains received as *P. marginalis* (a name formerly applied to plant-pathogenic members of the supercluster). Thus, the resolution of this technique appears to have limitations.

Diagnostic profiles for microorganisms may also be obtained using polyacrylamide electrophoresis of whole cell protein extracts, or DNA fingerprints produced via restriction endonuclease digestion of genomic DNA. Pseudomonads have genomes that are rich in GC DNA bases. Enzymes like SpeI that cut at sites with a high AT base composition will digest the DNA at only a few sites, producing large fragments which may be separated and analysed using pulse field gel electrophoresis.

The species of rRNA group I synthesize a ubiqionone with nine isoprene units (Q-9) in the side chain, whereas members of rRNA groups II, III, and V contain Q-8, and those of rRNA group IV a Q-10 (Oyaizu and Komgata, 1983).

Polyamine patterns are of similar utility: rRNA group I species have a high putrescine and spermidine content, rRNA group II and III species have 2-hydroxuputrescine and a high content of putrescine, rRNA group IV species only contain significant amounts of spermidine and sym-homospermidine, and rRNA group V species are characterised by high concentrations of cadaverine and spermidine (Busse and Auling, 1988; Auling, 1992; Yang et al., 1993).

In summary, the results of any of the methods of identification described above are only as good as the database of strains and isolates to which they are referenced. There are numerous techniques that, if applied at the optimum taxonomic level, may prove useful in identifying *Pseudomonas* and its species. Ribosomal RNA sequencing seems useful at the genus or higher level, and methods like fatty acid analysis can work at the strain and isolate level.

3. Information on the reproductive cycle (sexual/asexual)

Pseudomonas species reproduce by cell growth and binary cell division.

4. Biological features and environmental conditions which affect survival, reproduction, growth, multiplication or dissemination

4.0 General considerations

Pseudomonas species are efficient saprophytic chemo-organotrophs which grow at neutral pH and at temperatures in the mesophilic range (optimal growth between 20° and 45°C). Some species will grow at 4°C (*P. fluorescens, P. putida)* but not at the elevated temperature of 41°C. Optimal temperatures are *25*-30°C for *P. fluorescens, P. putida and P. syringae*, 30°C for *P. chlororaphis,* and 37°C for *P. aeruginosa.* Most, if not all, species fail to grow under acid conditions (< pH 4.5) (Palleroni, 1984).

Pseudomonas species thrive under moist conditions in soil (particularly in association with plants), and in sewage sediments and the aquatic environments. Environmental conditions which will affect their growth include nutrient availability, moisture, temperature, predation, competition, UV irradiation, oxygen availability, salinity, and the presence of inhibitory or toxic compounds. As nutritional demands are modest, pseudomonads can survive and multiply for months in moist environments such as tap water, sink drains, etc. (Palleroni, 1981; Bergen, 1981).

Competition and competitive niche exclusion are likely to limit the growth of introduced pseudomonad inoculants. Competitors are likely to include closely related pseudomonads and other bacteria able to compete for the same ecological niches with similar nutritional requirements (Lindow, 1992).

A number of environmental conditions may affect the dissemination of *Pseudomonas* species including surface water runoff, wind currents, and rain splash. It is likely that insects and other animals may also serve as vectors for dispersal. For example, *P. aeruginosa* can inhabit part of the normal gut or skin microflora of humans and animals. Thus dissemination would be associated with these vectors.

4.1 *P. aeruginosa*

P. aeruginosa is capable of growing in conditions of extremely low nutrient content (Palleroni 1984). The species was found to survive and proliferate in water for up to 100 days or longer (Warburton et al., 1994). Conditions of high humidity and temperature (80-90% humidity, 27°C) favoured the colonisation of lettuce and bean plants (Green et al., 1974).

4.2 *P. chlororaphis*

P. aureofaciens (*P. chlororaphis*) is an important coloniser of the rhizosphere and phyllosphere of plants (Thompson et al., 1993a; Legard et al., 1994). Kluepfel et al. (1991b) reported the colonisation of wheat roots in a field release of a recombinant *P. aureofaciens* (*lac*ZY). This population of *P. aureofaciens* reached a maximum of $2x10^6$ cfu/g root two weeks after inoculation and declined steadily to reach a level below detection (<100 cfu/g root) by 38 weeks post-inoculation. Angle et al. (1995) found that inoculations of recombinant *P. aureofaciens* (*lac*ZY) survived approximately twice as long in wheat rhizosphere as in non-rhizosphere bulk soil.

England et al. (1993) compared the survival and respiratory activity of *P. aureofaciens* in sterile and non-sterile loam and sandy loam soil microcosms. Recovery of *P. aureofaciens* was greater in sterile than non-sterile soils. Respiratory activity was higher in sandy loam soil than in loam soil, but soil type had no effect on survival.

The growth of *P. aureofaciens* in the spermosphere of seed-inoculated sugarbeets exhibited long lag phases (8-12 h) and their populations increased mainly between 12 and 24 hours (Fukui et al., 1994). The doubling time during the exponential growth phases was 2-3 h (Fukui et al., 1994).

4.3 *P. fluorescens*

P. fluorescens is commonly found inhabiting plant rhizosphere or phyllosphere environments. The plant rhizosphere provides an environment in which the species may show improved survival and growth. *P. fluorescens* distributed homogeneously in soil can result in significantly higher numbers in the rhizosphere of young wheat plants than in non-rhizosphere soil (Trevors et al., 1990).

The survival of *P. fluorescens* is affected by a variety of abiotic and biotic factors. Rattray et al. (1993) found that temperature and soil bulk density had a significant effect on lux-marked *P. fluorescens* colonisation of wheat rhizospheres. The greatest rates of colonisation occurred at the highest temperature (22°C) and lower bulk density (0.82 g/cm^3), and 100-fold higher numbers were found in the ectorhizosphere than in the endorhizosphere. Van Elsas et al. (1991) found *P. fluorescens* cells were able to withstand low temperatures, and could survive better at 4°C than at 15 or 27°C following introduction into natural soil, possibly due to an inhibition of the activity of the indigenous microflora. Van Elsas et al. (1986) found that *P. fluorescens* cell numbers declined slowly in both silt loam and loamy sand, but survival was better in the silt loam. Heijnen et al. (1993) found that *P. fluorescens* survived better in unplanted soils in the presence of bentonite clay. Stutz et al. (1989) demonstrated that survival of *P. fluorescens* in vermiculite clay was better than in montmorillonite, which was better than in illite.

Van Elsas et al. (1992) found *P. fluorescens* R2f survived above 10^7 cfu/g dry soil for up to 84 days in Ede loamy sand microcosms when encapsulated in alginate with skim milk and bentonite clay, while free cells declined below 10^5 cells/g dry soil after 21 days. Vandenhove et al. (1991) studied the survival of *P. fluorescens* inocula of different physiological stages in soil. Introduction of a late exponential phase inoculum into soil brought about a lower death rate compared to exponential or stationary phase inocula.

Handley and Webster (1993) studied the effect of relative humidity (RH at 20, 40, 60, and 80%) on airborne survival of *P. fluorescens* indoors. They found that *P. fluorescens,* suspended in distilled water, survived best at mid humidities and least at 80% relative humidity.

Boelens et al. (1994) and Bowers and Parke (1993) determined that motility of *P. fluorescens* did not affect its spread through soil. A non-motile mutant strain promoted plant growth and colonised roots as effectively as the motile strain. Water flow rates were more important than motility for dispersal through soil and rhizospheres. Knudsen (1989) developed a mathematical model for predicting aerial dispersal of bacteria during environmental release which predicted off-site dispersal patterns that were in qualitative agreement with results from a field release of a genetically engineered *P. fluorescens* in California.

Thompson et al. (1992) studied dissemination of *P. fluorescens* by placing bacterial populations on apple or pear pollen in the entrances of hives of honey bees. In a pear orchard, 72% of the flowers within 7.6 m of the hive were colonised with *P. fluorescens* eight days after the start of the study.

4.4 *P. fragi*

P. fragi is commonly found on refrigerated meat and dairy products (Jay, 1992). Psychrotrophs such as *P. fragi* generally have a lower metabolic rate than mesophiles (lower Q_{10} for the same substrate) and have membranes that transport solutes more efficiently (Jay, 1992). In addition, there is a correlation between the maximum growth temperature and the temperature at which respiratory enzymes are destroyed in psychrotrophs. Nashif and Nelson (1953) reported that extracellular lipase synthesis in *P. fragi* was inactivated at 30°C. The lipase of *P. fragi* is reported active at temperatures as low as -29°C (Alford and Pierce, 1961).

P. fragi has the ability to colonise stainless steel surfaces in food processing establishments to form "biofilms" (Hood and Zottola, 1995); attachment may involve a polysaccharide and protein matrix surrounding the cells (Herald and Zottola, 1989). Attachment of *P. fragi* to stainless steel surfaces occurred in 0.5 h at 25°C and in 2 h at 4°C through the development of attachment fibrils (Stone and Zottola, 1985).

4.5 *P. putida*

A variety of environmental factors can affect the survival of *P. putida.* For example, plant rhizospheres can provide an environment for improved survival. Gamliel and Katan (1992) studied the chemotaxis response of *P. putida* towards seed exudates and germinating tomato seeds and suggested this may contribute to its rapid establishment in plant rhizospheres. Temperature is also an important factor. Hartel et al. (1994) found that *P. putida* (*lac*ZY) declined from about 10^8 to 10^3 cfu/g of soil after 35 days at 35°C, while it did not survive after three days at 40°C.

Macnaughton et al. (1992), using pLV1013 as a marker plasmid in *P. putida* PaW8, investigated the effect of soil texture on survival and found that introduced bacteria survived better in soils with higher clay content. Compeau et al. (1988) studied survival of *P. fluorescens* and *P. putida* strains in sterile and non-sterile soil. Colonisation of sterile soil by one strain precluded normal colonisation of the second added strain and suggested there were limited sites for colonisation of *Pseudomonas* species in these soils.

Lynch (1990) found that *P. putida* WCS 358 survived in distilled water (without substrate input) for over a year. It was suggested that this could result from the utilisation of dead cells within the population, and from viable cells having a very low maintenance energy requirement in a state of arrested metabolism.

Madsen and Alexander (1982) found that cells of *P. putida* were not transported below 2.7 cm in moist soil in the absence of some transporting agent or in the presence of developing plant roots. Percolating water and a burrowing earthworm enhanced the vertical transport of *P. putida*.

4.6 *P. syringae*

Foliar plant pathogens such as *P. syringae* are generally not adapted to survival in the soil (Lindow et al., 1988). However, *P. syringae* has been isolated from plant debris in the soil and can overwinter in temperate climates (Hirano and Upper, 1983). *P. syringae* pv. *syringae* R32 have pili that function as adhesions anchoring the cell to the surface of plants, thereby enhancing epiphytic colonisation.

Wild-type bacteria became virtually resistant to displacement by rinsing within one day after inoculation, whereas non-pilated mutants were only partially resistant within three days (Suoniemi et al., 1995).

P. syringae metabolises a broad range of substrates, thus demonstrating flexibility in nutrient utilisation (Hirano and Upper, 1990). Criteria for viability of cells have been modified as the result of starvation experiments with *P. syringae*; it was determined that respiration of acetate and glycerol were more accurate determinants of viability than respiration of succinate (Cabral, 1995). The use of bactericides in agriculture (streptomycin and copper) has resulted in selection for strains resistant to these compounds; the resistance is often encoded on plasmids (Cooksey, 1990).

Plant-pathogenic strains grow to larger population sizes on susceptible plant hosts than on resistant ones (Stadt and Saettler, 1981), and therefore pathovars of *P. syringae* will grow to greater numbers on their respective hosts than on non-hosts. The presence of free water may be the most important factor contributing to the increase in population of *P. syringae* pv. *syringae* to infectious levels on bean leaves (Hirano and Upper, 1983; 1990; Beattie and Lindow, 1994). Immediately after rainfall, there is an initial decrease in population as bacteria are washed off the leaf surface, followed by a rapid increase in the population within 12 to 24 hours. Ambient temperature appears to have little effect on field populations of *P. syringae* pv. *syringae* on leaves but the age of annual crops does have an effect, with many more cells found on older leaves than on younger ones (Hirano and Upper, 1990; Jacques et al., 1995). *P. syringae* pv. *savastanoi* causes tumors on olive and oleander by producing the plant growth regulators indoleacetic acid (IAA) and cytokinins following infection; mutants deficient in IAA production grew as well as the wild type in culture and on plants, but the wild type reached a higher population density and maintained its maximum density at least nine weeks longer than the mutant populations.

Rainfall plays an important role in redistributing *P. syringae* within the plant canopy by washing bacteria from upper leaves onto lower ones, and by allowing individual bacterial cells to move using their flagella and find protected micro-sites on the surface of the leaf (Beattie and Lindow, 1994). Rainfall efficiently removed bacteria from foliar surfaces, but most of the cells were washed onto the soil; only a small portion were washed a relatively short distance from the source (Butterworth and McCartney, 1991).

. *P. syringae* is also dispersed on seeds (Hirano and Upper, 1983). When cells of *P. syringae* were applied as a spray to plots, an exponential decrease in numbers of cells was observed; some cells were detected 9.1 m downwind within 20 minutes of the spray application. When applied to oat plants (a non-host), viable cells could be detected for up to 16 days and were detected on plants up to 27 m downwind. In contrast to the plants, viable cells could not be detected in the upper layers of soil after two days (Lindow et al., 1988).

4.7 *P. tolaasii*

In the production of commercial mushrooms, *P. tolaasii* probably survives between crops on structural surfaces, in debris, and on equipment. It can be moved readily from one crop to another on the hands of pickers, on materials or equipment used in harvesting, and by insects, mites, water droplets and mushroom spores.

Conditions of high relative humidity and surface wetness encourage the expression of symptoms of brown blotch, an important mushroom disease, caused by *P. tolaasii.* Dispersal of the microorganisms occurs readily upon watering once the disease is established (Howard et al., 1994).

5. Behaviour in simulated natural environments such as microcosms, growth rooms, greenhouses, insectaries, etc.

5.1 *P. aeruginosa*

In a study by Sturman et al. (1994), the growth rate of *P. aeruginosa* appeared to be important in determining interspecies competition within packed-bed bioreactors filled with diatomaceous earth pellets.

5.2 *P. chlororaphis*

Angle et al. (1995) found that an intact soil core microcosm closely simulated survival results obtained from a field release of a recombinant *P. aureofaciens* (*lac*ZY). The strain of *P. aureofaciens* survived approximately 63 days in the bulk soil microcosm and 96 days in the rhizosphere microcosm.

5.3 *P. fluorescens*

Binnerup et al. (1993) found that kanamycin-resistant cells of *P. fluorescens* DF57-3 (Tn5 modified) inoculated in soil microcosms rapidly lost their culturability, as defined by visible colony formation on Kings B agar supplemented with kanamycin. After 40 days, only 0.02 to 0.35% of the initial inoculum was culturable. It was determined that about 20% of the initial inoculum represented viable, but non-culturable cells.

Compeau et al. (1988) studied survival of *P. fluorescens* and *P. putida* in sterile and non-sterile soil. Colonisation of sterile soil by one strain precluded normal colonisation of the second added strain and suggested there are limited sites for colonisation of *Pseudomonas* species in these soils. Similarly, Al-Achi et al. (1991) found that, when introduced as pairs into irradiated, sterile soils, a *P. fluorescens* strain prevented optimum colonisation by a *P. putida* strain. The addition of *P. putida* to sterile soil already populated by *P. fluorescens* impeded growth of *P. putida* in that soil. However, adding *P. fluorescens* to soil populated by *P. putida* did not prevent growth of *P. fluorescens* and caused a decrease in *P. putida*. These results suggested that there was competition for similar niches in soils, and that *P. fluorescens* was the more competitive species studied.

5.4 *P. fragi*

No information was found regarding the behaviour of this species in microcosms, greenhouses, insectaries, etc.

5.5 *P. putida*

Winstanley et al. (1993) studied the survival of *P. putida,* with an xy/E marker insert, in soil and lake water microcosms. When released into these microcosms, populations of the marked *P. putida* had a steady decline with little or no apparent division of cells. The rate of decline of *P. putida* in soil microcosms was significantly greater at 35% than at 50% field capacity water content, indicating that water content of the soil had an effect on survival. Similarly, Iwasaki et al. (1993; 1994) reported that the density of *P. putida* decreased rapidly to less than 10^2 to 10^3 level within five days in lake water and soil microcosms. The survival was influenced by protozoa density, light intensity, and soil water content. The addition of *P. putida* (10^7 cfu/ml) into natural water and soil had no effect on the density of indigenous microorganisms and enzyme activities.

Williams et al. (1992) studied the fate and effects of *P. putida* PPO200 genetically engineered for both nalidixic acid and kanamycin resistance in freshwater and marine microcosms. The freshwater microcosm contained fish (*Poecilia latipinna*), annelid worms (*Tubifex tubifex*), snails (*Gyraulus* sp.), freshwater mussels (*Margaritifera margaritifera*), freshwater shrimps (*Palaemonetes kadiakensis*), and plants (*Elodea canadensis*). The marine microcosm contained fish (*Cyprinodon variegatus*), sea anenomae (*Bunodosoma californica*), snails (*Turbo fluctosus*), oysters (*Crassostrea gigas*), estuarine shrimp (*Palaemonetes pugio*), shorefly larvae (*Ephydra* sp.), and plants (*Salicornia biglovii*). *P. putida* could be detected in the tissues of some non-target organisms, i.e. the bacterium survived. However, gross signs, survival, and the histological study of control and exposed non-target organisms indicated that there were no adverse effects.

Doyle et al. (1991) and Short et al. (1991) observed reductions in CO_2 evolution and the number of fungal propagules, as well as the enhancement of dehydrogenase activity in soil amended with 2,4-dichlorophenoxyacetate (2,4-D) and inoculated with *P. putida* PPO301(pRO103) genetically engineered to degrade 2,4-D. These unanticipated effects were not observed: (a) in uninoculated soil; (b) when the homologous, plasmidless parent *P. putida* PPO301 was inoculated; or (c) in the presence of the genetically engineered *P. putida* when 2,4-D was not added. Moreover, the effects were not predictable from the phenotype of this genetically engineered *P. putida*. While long-term, statistically significant differences were detected in some microbial populations and processes, the majority of the differences were transient.

The effects of *P. putida*, on nitrogen transformations and nitrogen-transforming microbial populations were studied in a soil perfusion system by Jones et al. (1991). Neither the genetically engineered strain nor its homologous plasmidless host had a significant effect on ammonification, nitrification or denitrification in the soil, or on the population dynamics of the microorganisms responsible for these processes.

5.6 *P. syringae*

Wendtpotthoff et al. (1994) monitored the fate of a genetically engineered strain of *P. syringae* applied to the leaves of bush beans in a planted soil microcosm. *P. syringae* established on the bean leaves at between 5 x 10^3 and 4 x 10^6 cfu/gm^{-1} fresh weight. During senescence of the bean plants, the strain was no longer detectable by selective cultivation and subsequent colony hybridization.

Significant differences within *P. syringae* strain MF714R were detected when the bacterium was cultured on agar or in broth or collected from colonised leaves and subsequently inoculated onto greenhouse-grown plants in growth chambers or in the field or onto field grown plants. Bacterial cells cultured in liquid medium survived the least well after inoculation under all conditions, whereas cells cultured on solid media exhibited the highest percent survival and desiccation tolerance in the growth chamber but survived less well in the field than did cells harvested from plants. Cells harvested from plants and inoculated onto plants in the field usually had the highest percent survival, started to increase in numbers earlier, and reached a higher number than did cells cultured *in vitro* (Wilson and Lindow, 1993a).

Wilson and Lindow (1993b) indicated that greenhouse-grown plants support larger epiphytic populations of an inoculated strain of *P. syringae* than do field-grown plants.

5.7 *P. tolaasii*

No information was found regarding the behaviour of this species in microcosms, greenhouses, insectaries, etc.

6. History of use (examples of environmental applications of the organism and information derived from these examples)

6.0 General considerations

Pseudomonads have been identified to be of importance in bioremediation as a result of their metabolic versatility. This metabolic versatility, and the ability to acquire additional versatility via plasmids, provides the potential for the rapid evolution of novel metabolic ability in *Pseudomonas* species. Examples of useful, or potentially useful, environmental applications of *Pseudomonas* isolates are given in Table 3. Some pseudomonad species have been introduced into the environment in bioremediation studies and have provided valuable information pertaining to characteristics such as survival. For example, Thiem et al. (1994) injected *Pseudomonas* sp. strain B13, a chlorobenzoate degrader, into a subsurface aquifer and found they could detect the strain 14.5 months after its environmental introduction.

Pseudomonads also have great potential in agronomic applications, since they are prolific colonisers of plant surfaces and represent a significant component of plant microflora. For example, they have been identified to possess traits that make them suitable as agents for biological pest control (O'Sullivan and O'Gara, 1992). These include an ability to produce antimicrobial molecules (antibiotics, antifungals and siderophores) and a capacity to aggressively compete with other microorganisms for niches and exclude phytopathogens. The possibility of the environmental application of strains to minimize frost damage on crop plants has also been investigated (Lindow and Panopoulos, 1988).

6.1 *P. aeruginosa*

Some strains of *P. aeruginosa* have been shown to produce biosurfactants which have potential uses in bioremediation for washing hydrocarbons from soil (Van Dyke et al., 1993). Jain et al. (1992) found that biosurfactants produced by *P. aeruginosa,* when added to soil, significantly enhanced the degradation of tetradecane, hexadecane and pristane.

Degradation of pentachlorophenol by *P. aeruginosa* has been investigated in shake-cultures. The bacteria were able to completely degrade pentachlorophenol up to 800 mg/l in six days with glucose as a co-substrate (Premalatha and Rajakumar, 1994). *P. aeruginosa* has also been found to degrade styrene in a continuous reactor at a rate of 293 mg $g^{-1}h^{-1}$. This could be applied to the industrial treatment of waste gas or polluted water (El Aalam et al., 1993).

6.2 *P. chlororaphis*

A strain of *P. chlororaphis,* genetically engineered to contain the *lacZY* genes, was introduced into the environment in a field trial in the United States in 1987, and its behaviour compared to the non-engineered strain (Kluepfel et al., 1991a, b, c). The non-engineered strain increased in number for two weeks, then declined to at or near the detection limit by 31 weeks.

Table 3

Examples of fluorescent species of *Pseudomonas* reported to have been used, or to have potential use, for bioremediation

Species	**Strain**	**Target chemical**	**Reference**
P. aeruginosa	JB2	halogenated benzoic acids	Hickey & Focht, 1990
	PaK1	polyaromatic hydrocarbons	Kiyohara et al., 1994
P. fluorescens	PHK	phthlate	Pujar & Ribbons, 1985
		dimethylphenol	Busse et al., 1989
		isopropylbenzene	Busse et al., 1989
P. putida		methyl-benzoates	Galli et al., 1992
		napthalene sulphonic acid	Zurrer et al., 1987
		dimethylphenol	Busse et al., 1989
	OUS82	polyaromatic hydrocarbons	Kiyohara et al., 1994
	G7	polyaromatic hydrocarbons	Kiyohara et al., 1994

6.3 *P. fluorescens*

P. fluorescens has been genetically engineered and used in a number of experimental field studies, including the environmental introduction of a Tn-5 marked strain in the Netherlands in 1986 and other studies in the United States with strains engineered by deletion of the ice gene, and by introduction of *lacZY* marker genes (Wilson and Lindow, 1993b). De Leij et al. (1995) found that field releases of a genetically engineered *P. fluorescens,* and the unmodified wild-type strain, resulted in significant but transient perturbations of some of the culturable components of the indigenous microbial communities that inhabited the rhizosphere and phylloplane of spring wheat, but no significant perturbations of the indigenous culturable microbial populations in non-rhizosphere soil were found. The release of both of these bacteria had no obvious effect on plant growth and plant health, and the observed microbial perturbations were considered minor.

P. fluorescens can produce large and unusual proteins that are a key component of bacterial ice nuclei (Warren, 1987). Warren (1987) and Lindow and Panopoulos (1988) reviewed the practical applications connected to ice nucleation, including snow-making and the use of ice nucleation gene-deletion strains to generate biological control agents for minimizing frost damage to plants. A naturally occurring *P. fluorescens* strain, A506, has been registered commercially for the control of frost injury of pear (Wilson and Lindow, 1993b).

P. fluorescens is also one of the more common bacterial species that has been used for the control of diseases in the phyllosphere of plants, and a naturally occurring strain of this species has been registered for the commercial control of fire blight on pear (Wilson and Lindow, 1993b). Hatai and Willoughby (1988) detected *P. fluorescens* and *Saprolegnia parasitica* in rainbow trout lesions and found that *P. fluorescens* could strongly inhibit the growth of the fungus. It was suggested that *P. fluorescens*, or an antibiotic derived from it, might be used in biological control of saprolegniasis.

Snyman et al. (1993) found that *P. fluorescens* genetically engineered to produce the insecticidal toxin from *Bacillus thuringiensis* was toxic to *Eldana saccharina*. An LC_{50} of 1.86 mg freeze-dried bacterial powder/ml of insect diet was calculated, and it successfully reduced sugarcane boring.

P. fluorescens has been shown to have the ability to degrade a wide variety of compounds, including: 3-chlorobenzoic acid (Fava et al., 1993); naphthalene, phenathrene, fluorene and fluoranthene (Weissenfels et al., 1990); chlorinated aliphatic hydrocarbons (Vandenbergh and Kunka, 1988); styrene (Baggi et al., 1983); and pure hydrocarbons and crude oil (Janiyani et al., 1993). *P. fluorescens* can also be used in biosensor applications. For example, the recombinant *P. fluorescens* strain HK9, which lights up in the presence of contaminants such as PAHs (due to the insertion of lux genes), allows easy detection of bioavailable fractions of pollutants in soils and sediments (King et al., 1990).

6.4 *P. fragi*

No information was found on the use of *P. fragi* in environmental applications.

6.5 *P. putida*

P. putida is capable of eliminating phytopathogenic microorganisms and stimulating plant growth (Vancura, 1988; Kloepper et al., 1988; Freitas and Germida, 1990). *P. putida* is also capable of degrading many unusual compounds by means of enzymatic systems encoded in plasmids. Chemicals degraded include polychlorinated biphenyls (PCBs) (Boyle et al., 1993; Lajoie et al., 1994); trichloroethylene (TCE) (Fujita et al., 1995); acetonitrile and sodium cyanide (Babu et al., 1994). *P. putida*

has also shown the ability to remediate non-ionic sewage (Turkovskaya et al., 1993), pulp mill waste (black liquor) (Jain et al., 1993), waste gases using a biofilter (Zilli et al., 1993), electroplating effluent with high concentrations of copper (Cu(II)) (Wong et al., 1993), and high-sulphur coal (Khalid and Aleem, 1991).

6.6 *P. syringae*

Lindow et al. (1988) monitored the fate of a strain of *P. syringae* in experimental field trials in the United States. They found an exponential decrease in numbers of viable cells deposited at increasing distances from sprayed field plots. The relative rate of survival of cells sprayed directly on plants was more than ten times higher than that of cells dispersed through the air to similar adjacent plants.

Use of *P. syringae* has been proposed to enhance snowmaking and to delay frost damage in plants (Lindow, 1983; Wilson and Lindow, 1993b). *P. syringae* has also been shown to incorporate aluminium, chromium and manganese, so the bioremediation of sites contaminated with these chemicals may be a potential use (Alaoukaty et al., 1992).

6.7 *P. tolaasii*

No information was found on the use of *P. tolaasii* in environmental applications.

7. Characterisation of the genome (e.g. presence of large plasmids, insertion sequences) and stability of these characteristics

Members of the genus *Pseudomonas* are known for their metabolic versatility. They are capable of degrading many recalcitrant xenobiotics due to their ability to recruit new genes and alter the expression of existing ones. An understanding of the relative chromosomal position of relevant genes, the diversity of mobile genetic elements found within this genus, and the role these mobile genetic elements play in the stability and metabolic adaptation of individual isolates, can be helpful for regulatory assessments.

The chromosomes of *P. putida* and *P. aeruginosa* have been described in detail by Holloway and Morgan (1986) and by Ratnaningsih et al. (1990), Romling et al. (1989) and Holloway et al. (1994). Holloway et al. (1990a) provide genetic maps of these two species, which are useful in locating the relative positions of important genes and provide a good summary of other chromosomal and extrachromosomal features. The sizes of the chromosomes for *P. putida* and *P. aeruginosa* vary from approximately 4,400 to 5,400 kb, with *P. aeruginosa* strain PAO having a genome size (5,400 kb) significantly larger than the 4,700 kb *E. coli* chromosome. Analysis of the distribution of chromosomal genes in pseudomonads shows that those involved in biosynthesis are not contiguous as with the enterobacteria. The genes for catabolic functions tend to be clustered on the chromosome, but are also not contiguous. Many catabolic functions are located on plasmids (e.g. Table 2); these genes, such as TOL (toluene degradation) and NAH (napthalene degradation), tend to be contiguous. This genome configuration allows for many diverse substrains within a species, each adapted to a particular environment.

Pseudomonas species contain a large variety of plasmids, insertion sequences, and transposons. The diversity of plasmids involved in degradation of organic compounds, drug resistance, and phytopathogenicity is indicated in Tables 2, 4 and 7. Insertion sequences (IS elements) and transposons are mobile within the genome of gram-negative bacteria, and can act as new promoters or as terminators,

causing polar mutations. If two IS elements are located near each other in the appropriate orientation, they can be transposed to a second genome as a unit along with any intervening genes.

These three classes of mobile genetic elements (plasmids, insertion sequences, and transposons) can potentially interact within the same isolate, causing shifts in the positions of key catabolic genes. An example is the NAH plasmid napthalene degradative genes, which are nested within a defective but mobilizable transposon on the plasmid (Tsuda and Iino, 1990). Such shifts can result in a variable stability for some traits. For example, *P. syringae* pv. *savasatoni* mutations causing IAA deficiency were identified to have resulted from the action of two IS elements. In another case, a 150 kb plasmid (able to integrate into the chromosome) from *P. syringae* pv *phaseolicola,* when excised from the chromosome, resulted in the formation of a series of plasmids that either contained chromosomal DNA or were deletion mutants of the plasmid. These events were associated with a common repeated sequence (RS) (Coplin, 1989). In a reverse situation, components of the TOL plasmid have been shown to integrate into both the *P. putida* and *P. aeruginosa* chromosomes (Holloway et al., 1990b), thereby potentially stabilizing degradative genes in the genome of the isolates.

Besides affecting the stability of certain traits, mobile genetic elements allow pseudomonads to recruit new genes from replicons such as plasmids, which can lead to new metabolic capabilities. Specific examples have been given by Chakrabarty (1995) of *P. putida*'s ability to recruit new degradative genes on a transposable element. These new genes allow the organism to degrade new chemicals without the need to evolve completely new degradative pathways. This species has been able to acquire the genes needed to degrade 3-chlorobenzoate to the intermediate protocatechuate, which then is further degraded by resident chromosomal genes. In a similar fashion, the same species has been able to degrade phenol by acquisition of two genes, *pheA* and *pheB*, whose products can convert phenol to intermediates which are metabolized by a chromosomally-encoded *ortho* pathway (Chakrabarty, 1995). A transposon-like mobile element encoding a dehalogenase function has also been recently described in *P. putida* (Thomas et al., 1992). In the well-characterised *P. putida* mt-2 plasmid pWW0, the TOL-degradative enzymes are encoded on a 56 kb transposon which is itself part of a 70 kb transposon (Tsuda et al., 1989), giving rise to a family of TOL plasmids (Assinder and Williams, 1990). In addition to acquisition of degradative genes, pseudomonads can also acquire genes whose products aid in waste degradation.

The chlorosis-inducing phytotoxin coronatine, produced by *P. syringae* pvs. *tomato* and *atropurpurea* is plasmid encoded (Coplin, 1989). Other toxins (e.g. phaseolotoxin, syringomycin and tabtoxin) have been shown to be chromosomally encoded. *P. syringae* pv. *savasatoni* produces abnormal growths due to an imbalance of cytokinin and auxin plant hormones. The genes for their biosynthesis are plasmid encoded in oleander, but not olive pathovars. The majority of *P. solanacearum* strains contain a large (700-1000 kb) megaplasmid that contains genes for host range and pathogenicity.

8. Genetic transfer capability

The ability of pseudomonads to develop new metabolic pathway capabilities is often dependent on an isolate's ability to acquire DNA from other bacteria, which is then integrated into the genome in a manner dependent on the organism's environment. The three common systems for gene transfer in bacteria, namely conjugation, transduction and transformation, have been observed among members of the genus *Pseudomonas.* All three gene transfer mechanisms have been observed under laboratory and natural conditions. Gene transfer by all three mechanisms is affected by biological factors such as the nature and host range of the mobile genetic element, its transfer frequency, the concentrations of recipient and donor organisms, and the presence of other organisms which prey on donors and recipients. Abiotic factors such

as temperature, moisture, and the presence of physical substrates which allow survival and/or gene transfer also affect the transfer frequency.

Even if the DNA is transferred to a new recipient, it may not be expressed. Sayre and Miller (1990) provide a detailed summary of factors associated with transposons and plasmids, the donors and recipients, and other biotic and abiotic conditions which affect gene transfer rates.

Conjugation

The acquisition of genetic material via conjugative plasmids represents an important evolutionary mechanism in the production of strains resistant to antibiotics and heavy metals, and with the ability to mineralise xenobiotics in selective environments. Gene transfer events may even affect the pathology of certain phytopathogens. Changes in cultivar-specificity and a loss of ability to produce fluorescent pigments of *P. syringae* pv. *pisi* were found to result upon the acquisition of IncP1 replicons such as plasmid RP4. Curing the RP4 plasmid from the strain maintained the new phenotype (Moulton et al.*,* 1993). Walter et al. (1987) developed a combined mating technique to measure the conjugal transfer potential of conjugative plasmids that uses four different standard mating techniques (colony cross streak, broth mating, combined spread plate, and membrane filtration), since no one technique worked best for the tested combinations of plasmids and recipients.

Conjugation between pseudomonads has been detected in both soil and aquatic environments. The transfer of conjugative plasmids has been demonstrated to occur between pseudomonads in a number of non-rhizosphere and rhizosphere soil environments, both in microcosms and *in situ* (van Elsas et al.*,* 1988; Trevors and Berg, 1989; Lilley et al.*,* 1994).

Transfer frequencies were found to be enhanced by two orders of magnitude, that is, up to 10^{-2} per recipient organism, on the rhizoplane of sugarbeet *in situ* (Lilley et al., 1994). Soil components (such as clay, silt, organic matter and plant roots) provide excellent surfaces for the cell-to-cell contact required for bacterial conjugation (Trevors and Berg, 1989; Stotzky et al.*,* 1991). In wheat plant root (van Elsas et al.*,* 1988) and sugarbeet (Lilley et al., 1994) conjugation studies, survival of the donor and recipient, as well as frequency of plasmid transfer, decreased with increasing distance from the plant root. Transfer frequencies are also affected by soil moisture, with frequencies for R-plasmid transfer between *E. coli* isolates shown to be optimal at 60 to 80% soil moisture holding capacity (Trevors and Starodub, 1987). Conjugal transfer of broad host range plasmids between *P. aeruginosa* donor and recipient strains in lake water has been observed to occur at a lower rate in the presence of the natural microbial community (O'Morchoe et al., 1988). Plasmids incapable of conjugation themselves have been shown to be mobilised from a laboratory strain of *E. coli* in a laboratory-scale wastewater treatment facility by mobilizer and recipient *E. coli* strains of both laboratory and wastewater origin (Mancini et al., 1987).

The TOL plasmid pWWO can be transferred to other microorganisms, and its catabolic functions for the metabolism of alkylbenzoates are expressed in a limited number of gram-negative bacteria, including members of the rRNA group I pseudomonads and *E. coli* (Ramos-Gonzalez et al., 1991). Transfer of the recombinant plasmid to *Erwinia chrysanthemi* was observed, but transconjugants failed to grow on alkylbenzoates because they lost catabolic functions. Pseudomonads belonging to rRNA groups II, III, and IV, *Acinetobacter calcoaceticus*, and *Alcaligenes* sp. could not act as recipients for TOL, either because the plasmid was not transferred or because it was not stably maintained. Under optimal laboratory conditions, the frequency of transfer of pWWO from *P. putida* as a donor to pseudomonads belonging to rRNA group I was on the order of 1 to 10^{-2} transconjugants per recipient, whereas the frequency of intergeneric transfer ranged from 10^{-3} to 10^{-7} transconjugants per recipient. Intra-species, but not inter-species transfer of TOL in soils has been reported (Ramos et al., 1991), but it was affected by the type of

soil used, the initial inoculum size, and the presence of chemicals that could affect the survival of the donor or recipient bacteria (Ramos-Gonzalez et al., 1991).

The *P. putida* TOL plasmid pWWO and the wide host range RP4 plasmid are able to mediate chromosomal mobilisation in the canonical unidirectional way (i.e. from donor to recipient cells) and bi-directionally [i.e. donor to recipient to donor (retrotransfer)] (Lejeune and Mergeay, 1980; Mergeay et al., 1987; Top et al., 1992; Ramos-Gonzales et al., 1994). Transconjugants are recipient cells that have received DNA from donor cells, whereas retrotransconjugants are donor bacteria that have received DNA from a recipient. The TOL plasmid pWWO and the pRP4 plasmid are able to directly mobilise and retromobilise a chromosomal marker integrated into the chromosome of the other *Pseudomonas* strains, and this process probably involves a single conjugational event. The rate of retrotransfer (as well as direct transfer) of chromosomal markers is influenced by the location of the marker on the chromosome, and it ranges from 10^{-3} to less than 10^{-8} retrotransconjugants per donor (transconjugants per recipient). The mobilised DNA is incorporated into the chromosome of the retrotransconjugants (transconjugants) in a process that seems to occur through recombination of highly homologous flanking regions. No interspecific mobilisation of the chromosomal marker in matings involving *P. putida* and the closely related *P. fluorescens* was observed.

It seems clear that pseudomonads can acquire plasmids from other bacteria in the environment. This premise is supported by the array of plasmids that have been recovered from members of *Pseudomonas,* some of which are listed in Tables 2, 4 and 7. The boundaries to gene transfer events are illustrated by plasmid RP4, originally isolated in *P. aeruginosa,* which has been shown to be transmissible to all gram-negative bacteria tested (Riley, 1989). *E. coli* has been shown to transfer plasmid-borne genetic information to over 40 genera (Stotzky et al., 1991). Direct evidence of pseudomonad isolate acquisition of plasmids from other bacteria in the environment is also available: Bale et al. (1988) showed that an introduced *P. putida* recipient acquired mercury resistance plasmids from an intact lotic epilithic bacterial community at frequencies up to 3.75×10^{-6} per recipient.

Transduction/bacteriophage mediated gene transfer

Two characteristics of a bacteriophage (phage) which are important in determining its ability to broadly distribute DNA were summarised in Sayre and Miller (1990). First, the host range of most phages is restricted to one species or a small number of related taxa, although broad host range phages such as phages P1 and Mu are known. Second, phages which undergo specialised transduction are likely to transfer chromosomal genes which are in close proximity to the phage integration site, while generalised transducing phages can transfer any of the bacterial genome's sequences with approximately equal frequency.

Many different lytic and temperate phages have been identified in *Pseudomonas*, and the morphological diversity among phages is at least as great as for any other bacterial genera. Transduction by temperate phage of *P. aeruginosa* chromosomal DNA has been demonstrated in fresh water microcosms (Morrison et al., 1979; Saye et al., 1987; 1990) and the phylloplane of bean and soy bean plants (Kidambi et al., 1994).

P. aeruginosa has been frequently reported as subject to lysogeny, the process by which the phage chromosome becomes integrated into the bacterial host chromosome and is stably replicated with it, as a prophage. Lysogeny may lead to increased fitness of bacterial strains in the natural environment, by increasing the size and flexibility of the gene pool available to natural populations of bacteria via horizontal gene transfer. Approximately 45% of *Pseudomonas* field isolates tested positive in colony hybridisations when probed with phage isolated from the same area (Miller et al., 1990a). The prophage

appears to contribute a major source of phage in the natural environment. In addition to mediating the transfer of genetic material within and between species, the induction of certain prophages results in transposition and mutagenesis events within the host genome.

Transformation

Both chromosomal and plasmid DNA are subject to natural transformation in the environment, a natural physiological process which is different from the artificial transformation techniques used in the laboratory (Stewart, 1990). In order for transformation of a cell to result in expression of the new DNA sequence, DNA must: 1) be excreted or lost from a donor cell; 2) persist in the environment; 3) be present in sufficient concentrations for efficient transformation to occur; 4) come in contact with a recipient cell which is naturally competent to receive the donor DNA; 5) be able to evade any recipient cell defences which degrade foreign DNA; and 6) integrate into a stably-maintained replicon in the recipient. Marine and soil environments have been shown to contain biologically significant levels of dissolved DNA (Paul et al., 1987; Lorenz et al., 1988). Soil environments offer protection from nuclease digestion for chromosomal and plasmid DNA (otherwise available for transformation) when bound to clay and sediment matter (Lorenz and Wackernagel, 1991; Romanowski et al., 1991; Khanna and Stotzky, 1992).

Natural transformation was found for *P. stutzeri, P. mendocina, P. alcaligenes and P. pseudoalcaligenes,* but not for *P. aeruginosa, P. putida, P. fluorescens*, and *P. syringae strains* (Carlson et al., 1983).

B. Human Health Considerations

9. Diseases caused and mechanism of pathogenicity including invasiveness and virulence

9.0 General considerations

Included amongst the rRNA group I fluorescent pseudomonads are species pathogenic to humans. Those that do cause infection are generally opportunists, promoting a variety of conditions ranging from endocarditis to dermatitis (Pollack, 1992; Artenstein and Cross, 1993; Berger et al. 1995; Jarvis and Skipper, 1994). Individuals most at risk from *Pseudomonas* infection are the immunocompromised, in particular individuals with AIDS, patients with cystic fibrosis, and those suffering major trauma or burns (Artenstein and Cross, 1993; Neu, 1985; Bodey et al., 1983; Moayyedi et al., 1994; Schuster and Norris, 1994).

It should be stressed that only some of the Pseudomonads have been implicated in human disease and that proponents should not base assessment criteria on *P. aeruginosa,* the most problematic member of the genus. Proponents should also be aware that not all strains of a given species have been shown to promote disease, and that assessment criteria should be based upon the strain being reported rather than the species as a whole.

9.1 *P. aeruginosa*

The predominant pseudomonad isolated from clinical sources is *P. aeruginosa*. Evaluation of the pathogens causing nosocomial infections in hospitals in the United States (Jarvis and Martone, 1992) indicated that *P. aeruginosa* was the fourth most common pathogen isolated (10.1%) in hospital-wide surveillance and the most common (12.4%) in intensive care units. In more recent surveys, *P. aeruginosa* was found to be the fifth most common pathogen (9%) (Emori and Gaynes, 1993) and increased to 29% in intensive care facilities [European Prevalence of Infection in Intensive Care Study (EPIC) (Spencer, 1994)]. The ability of *P. aeruginosa* to persist in a number of hospital disinfectants and pharmaceuticals (Dominik et al., 1995; Gilardi, 1991) and in sanitary facilities (Bobhammer et al., 1996; Döring et al., 1991; Zembrzuska-Sadkowska et al., 1995) probably contributes to its prevalence in the hospital environment. *P. aeruginosa* causes a wide range of syndromes, involving nearly all body systems, that vary from sub-acute to chronic (Artenstein and Cross, 1993; Pollack, 1992).

Bacteremia associated with *P. aeruginosa* is restricted mainly to immunocompromised individuals with significant underlying disease (Artenstein and Cross, 1993; Askamit, 1993; Spencer, 1994). Symptoms are indistinguishable from gram-negative sepsis caused by other bacteria (Pollack, 1993). Factors associated with bacteremia include malignancy, transplants (solid organ, bone marrow), diabetes, cirrhosis of the liver, renal failure, burns, trauma, intravenous drug abuse, corticosteroid therapy, cytotoxic chemotherapy, cardiopulmonary resuscitation, immunoglobulin deficiency, AIDS, broad spectrum antibiotics, and invasive instrumentation (IV catheters, endoscopes, mechanical ventilation, etc.) (Askamit, 1993; Artenstein and Cross, 1993; Dropulic et al., 1995; Mallolas et al., 1990; Nensey et al., 1990). Major entry portals for *P. aeruginosa* bacteremia appear to be following infection (or colonisation) of the respiratory tract (Gallagher and Watanakunakorn, 1990; Artenstein and Cross, 1993) and the genitourinary tract (Gallagher and Watanakunakorn, 1990; Aksamit, 1993).

. *P. aeruginosa* septicemia is primarily a condition of debilitated, immunocompromised adults and of infants. It is usually acquired in hospital, with prior broad-spectrum antibiotic therapy as a predisposing factor (Bodey et al., 1983; Richet et al., 1989), although community-acquired infections in apparently healthy children (Ros, 1989) and adults (Ishihara et al., 1995) have been reported. Mortality is high in immunocompromised patients (up to 50%) (Artenstein and Cross, 1993; Bisbe et al., 1988), with a better prognosis in “normal healthy” individuals (Ishihara et al., 1995).

Endocarditis due to *P. aeruginosa* has been associated with two major predisposing factors (Artenstein and Cross, 1993): the use of prosthetic heart valves and the use of illicit parenteral drugs. In drug abusers, endocarditis usually occurs in the right side of the heart and is sub-acute, although a complication, septic pulmonary emboli involving the tricuspid valve, can occur (Pollack, 1992).

Nosocomial pneumonia is the second most common hospital-acquired infection in the United States (Aksamit, 1993; Emori and Gaynes, 1993). *P. aeruginosa* is the most prevalent etiological agent for both poly- and mono-microbial pneumonia (Emori and Gaynes, 1993). Community-acquired pneumonia has also been attributed to this organism; its occurrence is rare (Artenstein and Cross, 1993), but it is more frequently recognised in AIDS patients (Dropulic et al., 1995; Schuster and Norris, 1994). Colonisation of the oropharyngeal and/or the upper gastrointestinal tracts is an important precursor to nosocomial pneumonia, although colonisation does not always imply infection. However, susceptibility to pneumonia is inversely related to a patient’s basic health (Aksamit, 1993; Artenstein and Cross, 1993, Dick et al., 1988; Dropulic et al., 1995). Conditions predisposing to *P. aeruginosa* pneumonia are similar to those mentioned for bacteremia.

Bacteremic pneumonia, with organisms isolated from both the lung and bloodstream, resembles bacteremia and pneumonia in clinical presentation. Prognosis is bleak with this syndrome. Mortality rates of 80-100% are observed, compared to 27-50% for bacteremia and 30-60% for pneumonia (Aksamit, 1993).

Chronic pulmonary colonisation by *P. aeruginosa* in the lungs of patients with cystic fibrosis results in frequent acute episodes of pneumonia and chronic bronchiectasis, but rarely in bacteremic pneumonia (Aksamit, 1993). Chronic infection leads to the obstruction of the airways, respiratory distress, and eventually death (Gilligan, 1991; Romling et al., 1994).

Otolaryngologic infections due to *P. aeruginosa* range from superficial and self-limiting to life-threatening (Artenstein and Cross, 1993). The most serious ear infection due to this organism is malignant otitis externa, usually resulting from a failure of topical therapy, and resulting in an invasive disease-destroying tissue which may progress to osteomyelitis at the base of the skull and possible cranial nerve abnormalities (Artenstein and Cross, 1993). Other ear infections associated with *P. aeruginosa* include external otitis (swimmer's ear), otitis media, chronic suppurative otitis media, and mastoiditis (Artenstein and Cross, 1993; Legent et al., 1994; Kenna, 1994; Pollack, 1992).

P. aeruginosa is the leading cause of gram-negative ocular infections, presenting as keratitis or endophthalmitis (Holland et al., 1993; Chatterjee et al., 1995; Bukanov et al., 1994). Predisposing factors include the use of contact lenses (in particular their cleaning and storage solutions), trauma, burns, ocular irradiation, compromised host defences, and systemic infections (Holland et al., 1993; Pollack, 1992; Imayasu et al., 1994; Stapleton et al., 1995).

Moisture is the paramount defining factor in *P. aeruginosa* growth. Normal dry skin does not support growth, whereas moist skin enables the organism to flourish. For this reason, dermatologic infections with *P. aeruginosa* tend to be more prevalent in moist tropical and subtropical climates (Bodey et al., 1983) or to be associated with the use of swimming pools, hot tubs or whirlpools (Gustafson et al., 1983; Trueb et al., 1994; Vesaluoma et al., 1995). The use of contaminated "loofah" cosmetic sponges is another source of *P. aeruginosa* infection (Bottone and Perez, 1993; 1994; Fisher, 1994). Folliculitis, pyoderma, cellulitis and ecthyma gangrenosum are all dermatologic infections in which *P. aeruginosa* has been implicated (Pollack, 1992; Artenstein and Cross, 1993; Gustafson et al., 1983; Fisher, 1994; Noble. 1993).

P. aeruginosa is a frequent isolate from wounds, particularly those contaminated with soil, plant material or water. Its presence may reflect colonisation as opposed to infection, which is a consequence of its ubiquitous distribution in nature (Artenstein and Cross, 1993; Pollack, 1992). Puncture wounds, particularly those penetrating to bone, may result in osteomyelitis or osteochondritis. The former is common in intravenous drug abusers (Artenstein and Cross, 1993) and the latter in puncture wounds to the foot in children and diabetics (Lavery et al., 1994; Pollack, 1992; Jarvis and Skipper, 1994). The wearing of tennis shoes (sneakers) at the time of puncture injury increases the chance of *P. aeruginosa* infection (Pollack, 1992; Lavery et al., 1994; Fisher et al., 1985).

In rare cases, *P. aeruginosa* has been associated with meningitis or brain abscess (Pollack, 1992) and infection of the gastro-intestinal tract (Artestein and Cross, 1993). Both conditions are nosocomially acquired, occurring in patients suffering from malignancies, invasive procedures or neutropenia (Pollack, 1992; Artenstein and Cross, 1993).

9.2 *P. fluorescens*

P. fluorescens has occasionally been associated with human infection. The inability of most strains to grow at normal human body temperature (Palleroni, 1992a) restricts invasion and subsequent disease promotion. This organism has the ability to grow at 4°C (Gilardi, 1991). This characteristic, along with the observation that it is isolated from the skin of a small percentage of blood donors, makes it an occasional contaminant of whole blood and blood products (Puckett et al., 1992; Stenhouse and Milner, 1992). Pseudobacteremia may result from the infusion of contaminated products (Scott et al., 1988; Simor et al., 1985; Gottlieb et al., 1991; Foreman et al., 1991) or from the use of contaminated equipment (Anderson and Davey, 1994).

P. fluorescens has been occasionally isolated from patients with AIDS (Franzetti et al., 1992; Roilides et al., 1992), where it caused bacteremia and urinary tract, ocular and soft tissue infections. Chamberland et al. (1992), in their across-Canada survey of septicemia, found that 1.5% of isolates were *P. fluorescens*. It is apparent that *P. fluorescens* can be an opportunistic pathogen in cancer patients and in others who are severely immunocompromised, but that it is of little concern to immunocompetent individuals. *P. fluorescens* is occasionally found in sputa of patients with cystic fibrosis, although its role as a pathogenic factor has yet to be resolved.

9.3 *P. fragi*

P. fragi is one of the pseudomonads associated with food spoilage (Barrett et al., 1986; Drosinos and Board, 1995; Greer, 1989) and is commonly isolated from milk products, pork and lamb. A search of the literature dating back to 1966 failed to reveal any association between *P. fragi* and human disease.

9.4 *P. putida*

P. putida is a rare opportunistic pathogen in immunocompromised individuals. Like *P. fluorescens*, this organism can grow at 4°C in whole blood and blood products and is consequently an occasional source of pseudobacteremia (Pitt, 1990; Taylor et al., 1984; Tabor and Gerety, 1984). Septicaemia and septic arthritis due to *P. putida* in immunocompromised patients have been reported (MacFarlane et al., 1991; Madhavan et al., 1973) and bacteremia in AIDS patients can occur at low frequency (Roilides et al., 1992). All syndromes appear to be associated with breaching of the patient's mechanical defences, either associated with transfusion or following placement of in-dwelling catheters.

9.5 *P. chlororaphis, P. syringae, P. tolaasii*

A search of the literature dating back to 1966 failed to reveal any association between these species and human disease. The possibility does exist that an incomplete identification has failed to speciate these organisms, and that they are reported in the literature as *Pseudomonas* sp.

10. Communicability

P. aeruginosa, the species of most concern in the rRNA group I pseudomonads, has a ubiquitous distribution at a low frequency in nature (Romling et al., 1994a). Outside of the hospital environment, 20 to 30% of people harbour faecal *P. aeruginosa*. This frequency increases during hospitalisation as a result of contact with an environment in which the organism is more common. Both healthy individuals and patients with P. aeruginosa infections may serve as reservoirs for infection in hospitals.

P. aeruginosa is an important cause of nosocomial infections. It is particularly a problem in burn units, neonatal units, and wards housing leukemia and other cancer patients (Bergen, 1981). Nosocomial infections may spread by transmission 1) directly between patients; 2) via medical personnel; 3) via inanimate objects which may serve as reservoirs or vectors; and 4) from the normal flora of the patient (i.e. autoinfection).

Most types of hospital equipment or utensils can serve as a source of infection, including pharmaceutical products, disinfectants, water jugs, table tops, trays, urine bottles, urethral catheters, anaesthetic equipment, and respiratory apparatus. Transmission may also occur via food stuffs such as strawberries, plums and other fruit, vegetables, frozen poultry, refrigerated eggs, lemonade, raw milk, and any equipment or utensil involved in the preparation or serving the food.

11. Infective dose

Infective dose for the fluorescent pseudomonads is not really relevant, since infection usually occurs in immunosuppressed individuals. Most patients suffering from cystic fibrosis acquire a *P. aeruginosa* infection at some stage of their lives, resulting in frequent, recurrent bouts of pneumonia. Mortality in such cases may reach 100%.

12. Host range, possibility of alteration

P. aeruginosa has a broad host range which includes humans, animals, and some plants. It converts from a non-mucoid state to a mucoid, alinate-producing variant in the lungs of CF patients. The mucoid form is almost exclusive to colonisation of this site. Upon *in vitro* propagation, the mucoid strains isolated from CF lungs may undergo a spontaneous reversion to the non-mucoid form (Maharaj et al., 1992).

13. Capacity for colonisation

Fluorescent pseudomonads may be found in the normal bacterial flora of the intestines, mouth or skin of humans or animals. Colonisation is harmless under normal circumstances. In immunosuppressed or immunocompromised patients the capacity for colonisation by *P. aeruginosa* is high.

14. Possibility of survival outside the human host

rRNA group I fluorescent pseudomonads do not require human or animal hosts for survival. Most are common residents of soil, rhizosphere, sediment, and aquatic habitats. These generally moist environments provide natural reservoirs for the organisms. The pseudomonads have modest nutritional demands and can survive for months in tap water, distilled water, sink drains, or any other moist environment.

15. Means of dissemination

The fluorescent pseudomonads are ubiquitous microorganisms. Anyone (not only infected individuals), or anything, may serve as a source or vector for dissemination (refer also to 10 and 26).

16. Biological stability

In *P. aeruginosa* infections of the CF lung, a transition from a non-mucoid to a mucoid, alginate producing variant is observed, indicating the pleomorphic nature of this organism. Furthermore, the level of toxin production varies with the isolate, suggesting that expression levels of chromosomally encoded genes are subject to strain differences. Recent studies indicate that this variation is attributable to the variable position of the genes on the chromosome, due at least in part to chromosome reassortment and the movement of IS-like sequences (Vasil et al., 1990).

17. Antibiotic-resistance patterns

17.1 *P. aeruginosa*

P. aeruginosa is naturally resistant to many widely used antibiotics. Resistance in part is thought to be the result of an impermeable outer membrane and the production of extracellular polysaccharides (Quinn, 1992). The organism is usually resistant to low levels of kanamycin, penicillins (with the exception of the anti-pseudomonal penicillins: carbenicillin, ticarcillin, piperacillin), most of the first and second generation cephalosporins, chloramphenicol, nalidixic acid, tetracyclines, erythromycin, vancomycin, sulfonamide, trimethoprim and clindamycin (Wiedemann and Atkinson, 1991). Antibiotic resistance is often due to the presence of plasmids (Table 4). Individual strains may be resistant to antibiotics to which the species is generally susceptible. For this reason, antibiotic resistance patterns should not be relied on for species verification, but should be assessed on a case-by-case basis.

17.2 *P. fluorescens* and *P. putida*

Antibiotic resistance patterns for *P. fluorescens* and *P. putida* are difficult to assess, since only small numbers of isolates have been tested in controlled studies. The organisms tested are susceptible to low levels of kanamycin and resistant to carbenicillin and gentimicin, two of the antibiotics still in use against *P. aeruginosa* (Pitt, 1990). Again the use of antibiotic resistance/susceptibility profiles should be regarded with caution, since variation within a species may be great.

17.3 *P. chlororaphis*, *P. fragi*, *P. syringae*, *P. tolaasii*

Antibiotic susceptibility patterns for these species were not found in the literature searched.

18. Toxigenicity

The pathogenicity of *P. aeruginosa* is accredited to the wide array of virulence-associated factors produced by some if not all strains. Pili act as adhesins to a variety of cell types and enable the organism to colonise epithelial surfaces (Prince, 1992). Once established, the bacteria secrete a number of extracellular products capable of tissue damage and facilitating dissemination of the bacteria (Plotkowski

et al., 1994). Proteases (including elastase), exotoxin-A, exoenzyme-S, phospholipase-C, exolipase, rhamnolipid, alginate, cytotoxin, high molecular weight leukocidin and endotoxin have all been implicated in pathogenesis (Fick, 1993; Govan and Nelson, 1992; Holder, 1993; Holland et al., 1993; Jaeger et al., 1991; McCubbin and Fick, 1993; Kudoh et al., 1994; Lutz et al., 1991; Noda et al., 1991).

Exotoxin-A (ETA) and exoenzyme-S are ADP-ribosyltransferases which inhibit protein synthesis in the eukaryotic cell. ETA is produced during the decline of the *P. aeruginosa* growth cycle. Its synthesis is dependent on the iron concentration in the growth medium (Stephen and Pietrowski, 1986). The levels of both ETA and exoenzyme-S vary with the isolate examined.

Phospholipase-C (PLC) is another extracellular enzyme produced by *P. aeruginosa* which is toxic in micro or sub-microgram levels. PLC preferentially degrades phospholipids, which are plentiful in the eukaryote cell. In addition, one of the substrate products of lipid degradation by PLC (diacylglycerol) can have toxic effects on the host animal by inducing the production of potent substances (arachronic acid metabolites and protein kinase C). These by-products alter eukaryotic cell metabolism and incite inflammatory responses.

Elastase, one of the extracellular proteases, degrades elastin, collagen, human immunoglobulin and serum α-1-proteinase inhibitor (Iglewski et al., 1990), activities which help evade the immune response and sponsor tissue invasion. Alkaline protease, another of the extracellular proteases, is active on IgA, cytokines (TNF-α; IFN-γ; IL-2), lactferrin and transferrin, fibrinogen, and fibrin (Shibuya et al., 1991; Doring et al., 1988; Frick et al., 1985; Parmely et al., 1990). These enzymatic activities promote disruption of respiratory cilia and increased vascular permeability, which probably contribute to establishment in the lung and resulting pneumonia.

The toxigenic potential of other species of *Pseudomonas* is less well studied. Proteases and phospholipases have been detected in some strains of *P. fluorescens* and *P. putida,* but their significance in human infection has yet to be elucidated.

19. Allergenicity

Fluorescent pseudomonads have not been described as potent allergens. However, they do possess endotoxin (lipopolysaccharide), which may precipitate an allergic response in some individuals.

Table 4

Examples of plasmids encoding for drug resistances in *P. aeruginosa*

Plasmid	Resistances encoded
RP1	carbenicillin, kanamycin, neomycin, tetracycline
RP1-1	carbenicillin
R9169	carbenicillin, kanamycin, neomycin, tetracycline
R6886	carbenicillin, kanamycin, neomycin, tetracycline
RP8	carbenicillin, kanamycin, neomycin, tetracycline
R2-72	carbenicillin, streptomycin, kanamycin
R38-72	tetracycline, streptomycin
R39-72	tetracycline, streptomycin
R931	tetracycline, streptomycin
R679	streptomycin, sulphonamide
R1162	streptomycin, sulphonamide
R3108	streptomycin, sulphonamide, tetracycline
R209	streptomycin, sulphonamide, gentamicin
R130	streptomycin, sulphonamide, gentamicin
R716	streptomycin
R503	streptomycin
R5265	streptomycin, sulphonamide
R64	ampicillin, carbenicillin, sulphonamide, gentimicin, kanamycin
R40a	ampicillin, anamycin, paromycinin, sulphonamide

taken from Trevors (1991)

20. Availability of appropriate prophylaxis and therapies

20.1 *P. aeruginosa*

Antibiotic therapy for *P. aeruginosa* depends upon the site of infection and the relative susceptibility of the particular strain to the antibiotics tested. Generally, the species is susceptible to very few antibiotics. Ceftazidime, cefsulodin, imipenem, ticarcillin-clavulanic acid, azlocillin, piperacillin, the aminoglycosides, colistin and ciprofloxacin are some of the antibiotics with a high percentage of susceptible isolates (Chamberland et al., 1992; Wiedemann and Atkinson, 1991; Legent et al., 1994). Combination therapy using two effective antibiotics may increase the clinical cure rate in some infections (Lucht et al., 1994), and synergistic combinations of an aminoglycoside with a β-lactam (that has activity against *Pseudomonas*) have continued to be effective (Sepkowitz et al., 1994). The particular antibiotic regime selected will depend, however, on the strain in question and cannot be answered in a generic manner.

20.2 *P. fluorescens* and *P. putida*

Ceftazidime (Jones et al., 1989; Watanabe et al., 1988), imipenem (Jones et al., 1989) and meropenem (Jones et al., 1989) have been described as active against *P. fluorescens*. Antibiotics active against *P. putida* are ceftazidime, carbapenems, aminoglycosides, tetracyclines and polymixin B (Kropec et al., 1994; Bergen, 1981; Papapetropoulou et al., 1994). Any possible treatment regime should be proposed for the strain in question and not based on generic information for the species.

20.3 *P. chlororaphis*, *P. fragi*, *P. syringae*, *P. tolaasii*

No antibiotic susceptibility data for these species were found in the literature searched.

C. Environmental and Agricultural Considerations

21. Natural habitat and geographic distribution. Climatic characteristics of original habitats

21.0 General considerations

. *Pseudomonas* species have been isolated from a wide variety of habitats including soils, fresh or sea water, clinical specimens and laboratory reagents (including distilled water), food stuffs and wastes, flowers, fruit, vegetables, and diseased and healthy plants and animals. Many species appear to have a global distribution. For example, beneficial colonizers and *Pseudomonas*-incited plant diseases (such as *P. syringae*) are worldwide in distribution and involve representatives of most major groups of common plants.

Although pseudomonads are often considered to be ubiquitous, there are also many reports of niche specialisation. For instance, the number of epiphytic bacteria (such as *P. syringae*) present on the leaves of newly emerged plants is very low, indicating that the soil does not appear to serve as an important source of inoculum (Lindow, 1992) or habitat. As well, many phytopathogenic pseudomonads

can only be isolated from the diseased host. For example, *P. syringae* isolates are generally only found in association with live plants or propagative material, and in these niches they appear as virtually homogeneous populations (Schroth et al., 1981). At present, the distribution of these pathogens in the absence of the host is unclear.

21.1 *P. aeruginosa*

. *P. aeruginosa* is widely distributed in soil (Bradbury, 1986) and water (Palleroni, 1984). It is occasionally isolated from both healthy and diseased plants (Bradbury, 1986). Experiments conducted with lettuce and bean under varying conditions of temperature and humidity indicated that *P. aeruginosa* can colonise these plants under conditions of high temperature and humidity (27ºC, 80-95% humidity) (Green et al., 1974). The occurrence declined in lettuce and bean when the temperature and humidity were lowered (16ºC, 55-75% humidity).

Cho et al. (1975) studied the occurrence of *P. aeruginosa* on the foliage and in the soil of potted ornamental plants in order to determine their importance as a disseminating agent in hospital environments. They concluded that although potted plants are potential carriers for introduction of the species to hospital environments, there is no evidence that these plants constitute a primary source of bacteria for hospital infections. Results of a study to determine the prevalence of bacteria in passerines and woodpeckers suggest that *Pseudomonas* spp., including *P. aeruginosa,* are not uncommon in the gut flora of omnivorous and granivorous birds (Brittingham et al., 1988).

21.2 *P. chlororaphis*

P. aureofaciens (*P. chlororaphis*) was one of the most commonly occurring bacteria in soil, and on roots and leaves of both sugarbeet and spring wheat, during the growing season (De Leij et al., 1994). *P. chlororaphis* has also been isolated from water and from dead larvae of cockchafer, a large European beetle (Palleroni, 1984).

21.3 *P. fluorescens*

P. fluorescens is commonly found on plant surfaces, as well as in decaying vegetation, soil and water (Bradbury, 1986). It can be isolated from soil, water, plants, animals, the hospital environment, and human clinical specimens. It is commonly associated with spoilage of foodstuffs such as fish and meat (Gilardi, 1991). The presence of *P. fluorescens* in the rhizosphere of plants has been widely reported. For example, Milus and Rothrock (1993) found *P. fluorescens* to be a very good coloniser of wheat roots, and Lambert et al. (1990) found *P. fluorescens* to be one of the most frequently occurring bacteria on root surfaces in young sugar beet plants in Belgium and Spain.

P. marginalis (*P. fluorescens*) is ubiquitous in soil and is often an internal resident of plant tissues (Schroth et al., 1992). Cuppels and Kelman (1980) detected *P. marginalis* in a Wisconsin river and lake, field soils, root zones of potato plants, washwater from a potato chip processing plant, and decaying carrot and cabbage heads. Strains were found in Wisconsin soils just after the spring thaw, and thus probably overwintered there.

21.4 *P. fragi*

P. fragi has been found associated with refrigerated meat and dairy products (Jay, 1992).

21.5 *P. putida*

P. putida is very common in soils and plant rhizospheres (Palleroni, 1984). Gilardi (1991) indicated the species can be isolated from soil, water, plants, animal sources, the hospital environment, and human clinical specimens. It can be isolated from soil and water after enrichment in mineral media with various carbon sources.

P. putida appears to have a broad global distribution. Sisinthy et al. (1989) isolated the species from soil samples collected in, and around, a lake in Antarctica. However, particular strains may have a more restricted distribution. Chanway and Holl (1993) studied strains obtained from spruce seedling rhizospheres at two different locations in British Columbia, Canada, and found two distinct strains based upon analysis of fatty acids. When the origin of the spruce seed was matched with that of the inoculated *P. putida* strain, a significant increase in the amount and rate of seedling emergence was detected compared to unmatched tests of seedling emergence, suggesting ecotype specificity of strains.

21.6 *P. syringae*

P. syringae occurs naturally among the microflora that inhabit the leaf surface of plants that are typically found in temperate and Mediterranean climates (Wilson and Lindow, 1994; Bradbury, 1986). *P. syringae* survives in association with the host plant and propagative material from the host plant. There is little evidence to suggest that these bacteria survive in soil. They may, however, survive in soil in association with residues of diseased plants, having some capacity to colonise root systems (both host and non-host plant). Stone or pome fruit pathogens, such as *P. syringae,* exist in lesions, cankers or tumours. Inoculum is therefore available for dissemination under favourable environmental conditions. Most of the *P. syringae* group appears to have the capacity to survive as epiphytes on protected parts of healthy leaves, in the buds of the host, and even on non-host plants.

21.7 *P. tolaasii*

P. tolaasii is a natural inhabitant of peat and lime used for casing material in the production of commercial mushrooms, and can be easily isolated from compost after pasteurisation (Howard et al., 1994). In the commercial production of mushrooms, high relative humidity and surface wetness encourage the expression of symptoms of brown blotch caused by *P. tolaasii* (Howard et al., 1994). Symptoms of brown blotch occur more frequently on mushrooms that remain wet for a long time, and in places where they touch one another (Howard et al., 1994). Brown blotch, the mushroom disease caused by *P. tolaasii*, has been reported on all continents except Africa (Bradbury, 1986; Suyama and Fujii, 1993).

22. Significant involvement in environmental processes, including biogeochemical cycles and potential for production of toxic metabolites

Pseudomonads can have a significant involvement in a variety of environmental processes, including important biogeochemical cycles. For example, certain *Pseudomonas* species have the capacity for denitrification producing dinitrogen gas from nitrate. These species include *P. aeruginosa, P. fluorescens* (biotypes I and III) and *P. chlororaphis* (Palleroni, 1984).

Toxic metabolites of hazardous wastes

The microbial degradation of a hazardous waste may result in mineralization of the parent waste, or in partial degradation of the parent waste to products which may be toxic. Some microorganisms may

not initially produce problematic metabolites. However, loss of a lower portion of a degradative pathway due to genetic instability may result in the generation of toxic metabolites. These toxic metabolites may result in death of the cell, thus limiting the metabolite's production. The metabolite may also be released from the cell to soil or water and become rapidly inactivated or mineralised by other physical or biological processes. On the other hand, some metabolites may be released from the cell, remain stable in the environment, and have toxic effects equivalent to, or greater than, the parent hazardous waste. There is also a possibility that a microorganism will not produce a metabolite of concern when presented with a single waste, but will produce toxic metabolites in the presence of a complex mixture of related compounds.

Many examples of hazardous waste metabolites have been detected in laboratory experiments, but no well-documented field studies on metabolite formation have been conducted. In many instances, the metabolites produced by one organism will be degraded further or mineralised by others in the immediate environment. For example, TCE epoxide and phosgene are likely degradation products from methanotrophic degradation of trichloroethylene and chloroform, respectively (Alvarez-Cohen and McCarty, 1991). Although these compounds are toxic in mammalian systems, both are also highly reactive and would likely react intracellularly and/or not persist in the environment once released from the cells. Examples of the potential for hazardous metabolites include the production and accumulation of formamide from cyanide as a result of cyanide degradation by *P. fluorescens* strain NCIMB 11764 (Kunz, et al., 1992). As well, Castro and Belser (1990) demonstrated that *P. putida* PpG-786 can dehalogenate 1,1,2-trichloroethane by two pathways under aerobic conditions. The dominant pathway is oxidative and leads to chloroacetic acid and glyoxylic acid. However, a competitive reductive pathway occurs simultaneously and yields vinyl chloride exclusively.

Complex mixtures can result in dead-end metabolite production, or failure to degrade one of the parent compounds, even though the individual wastes can be mineralised individually. Benzene, toluene, and *p*-xylene (BTX) are common contaminants of drinking water, and each individual BTX compound can be mineralised by naturally occurring organisms. However, a combination of the three cannot be mineralised naturally, and can result in accumulation of 3,6-dimethylcatechol from *p*-xylene and a lack of degradation of benzene (Lee et al., 1995).

23. Pathogenicity – host range, infectivity, toxigenicity, virulence, vectors

23.0 General considerations

The fluorescent rRNA group I pseudomonads exhibit a range of pathogenicity characteristics. Some species have not been implicated in animal or plant disease. Other species may be opportunistic pathogens for weakened individuals. The fluorescent rRNA group I pseudomonads also include plant pathogens.

23.1 *P. aeruginosa*

Pathogenicity to animals

P. aeruginosa may be found as part of the normal bacterial flora of the intestines, mouth or skin of animals (e.g. cattle, dogs, horses, pigs). It has a broad host range among animals, which may also extend to plants. Under normal circumstances, colonisation is harmless and infection only occurs when

local or general defence mechanisms are reduced. *P. aeruginosa* is usually associated with disease in individuals with low resistance to infection.

In susceptible hosts *P. aeruginosa* may cause infection at any site, particularly wounds and the respiratory tract. It can cause endocarditis, meningitis, pneumonia, otitis, vaginitis and conjunctivitis. Host defence mechanisms against *P. aeruginosa* are very low in mink and chinchilla, in which the bacterium can spread rapidly, causing fatal disease (Bergen, 1981).

P. aeruginosa has been associated with disease in pigs, sheep and horses (Hungerford, 1990), as well as cattle (Hamdy et al., 1974). Sheep inoculated epicutaneously with *P. aeruginosa,* and then wetted, can rapidly develop a bacterial exudative dermatitis (Hungerford, 1990). *P. aeruginosa* has been reported as the etiological agent in outbreaks of acute infectious disease in mink (Wang, 1987) and was the suspected etiological agent in a report of fatal bronchopneumonia and dermatitis in an Atlantic bottle-nosed dolphin (Diamond and Cadwell, 1979). It has also been reported to be associated with pathogenicity in ducks (Safwat et al., 1986), turkeys (Hafez et al., 1987), Japanese ptarmigan (Sato et al., 1986), and pheasant chicks (Honich, 1972) and to be the causal agent of a disease in broiler fowl in several countries.

P. aeruginosa was reported as one of the causative agents of infectious stomatitis or "mouthrot" in snakes (Draper et al., 1981), although it has been suggested that it is an opportunistic invader rather than an exogenous pathogen in snakes (Draper et al., 1981; Jacobson et al., 1981). Frogs (*Rana pipiens*) that were intraperitoneally injected with high doses (10^4-10^6 bacteria) of *P. aeruginosa* showed significant mortality under stressful conditions (Brodkin et al., 1992). *P. aeruginosa* has been associated with pathogenicity in Nile fish (Youssef et al., 1990) and catfish (*Clarias batrachus*) (Manohar et al., 1976) and as the etiological agent of fin rot in *Rhamdia sapo* (Angelini and Seigneur, 1988).

P. aeruginosa has been associated with pathogenicity in the tobacco hornworm (*Manduca sexta*) (Horohov and Dunn, 1984) and seven species of Lepidoptera, including the silkworms *Pericallia ricini* and *Bombyx mori* (Som et al., 1980). Experimental inoculation of honeybees, by dipping in a bacterial suspension of *P. aeruginosa,* resulted in a 70% death rate within 50 hours (Papadopoulou-Karabela et al., 1992). Dorn (1976) reported *P. aeruginosa* to be responsible for disease outbreaks in laboratory populations of the milkweed bug *Oncopeltus fasciatus*.

The abundant extracellular products of *P. aeruginosa* are thought to contribute to its adverse effects. These products include toxin A, alkaline protease, alkaline phosphatase, lipase, phospholipases and elastase. Toxin A is toxic to animals, with a mean lethal dose in mice of about 0.2 µg when injected intraperitoneally or 0.06 µg when injected intravenously (Nicas and Iglewski, 1986). Toxin A is produced by about 90% of clinical isolates, and a chromosomal location has been established for the structural gene (Nicas and Iglewski, 1986). Most strains produce several extracellular proteases. For mice injected intravenously, the LD_{50} of the alkaline protease and the elastase is 375 and 300 µg respectively (Nicas and Iglewski, 1986). *P. aeruginosa* proteases are reported to be toxic to insects (*Galleria mellonella)* (Lysenko, 1974). *P. aeruginosa* also produces the haemolytic extracellular product phospholipase C, which causes hepatic necrosis and pulmonary edema when injected interperitoneally, and rhamnolipid, which has an LD_{50} of 5 mg when injected interperitoneally into mice (Nicas and Iglewski, 1986).

Pathogenicity to plants

P. aeruginosa has been described as an opportunistic invader of plants (Bradbury, 1986). It has been reported to cause blight disease in bean plants (El Said et al., 1982), and to have caused a lethal palm blight (Bradbury, 1986). Slow soft rot has been produced in plant tissue upon inoculation with strains of *P. aeruginosa* isolated from both animals and plants, and lesions and some necrosis have been found in

tobacco leaves when inoculated with the bacterium (Bradbury, 1986). In a study involving 46 strains of *P. aeruginosa* isolated from human, plant and soil sources, the ratio of pathogenic to non-pathogenic strains for vegetables was 5:1 (Lebeda et al., 1984).

More recently, two strains of *P. aeruginosa* (a clinical isolate and a plant isolate) have been found to elicit severe soft rot symptoms in the leaves of inoculated *Arabidopsis thaliana* plants from certain ecotypes but not others (Rahme et al., 1995). These authors suggested that a strain that exhibited ecotype specificity would most likely be a true plant pathogen, in contrast to a strain that has no capacity to be a plant pathogen under natural settings but infects plants as a consequence of the artificial environment of a laboratory. The same two strains of *P. aeruginosa* were found to cause significant mortality in a mouse burn model. The authors identified genes encoding three virulence factors (*toxA, plcS* and *gacA*) that were required for the full expression of pathogenicity in both plants and animals.

23.2 *P. chlororaphis*

Pathogenicity to animals

A strain of *P. chlororaphis* has been reported to cause disease in salmon fry (*Oncorhynchus rhodurus*) and to kill trout, carp and eel, when inoculated (Egusa, 1992). This strain was judged to be pathogenic to fish (Hatai et al., 1975). *P. chlororaphis* has also been reported to inhibit egg hatch of the nematode, *Criconemella xenoplax*, at a concentration of $2x10^8$ cfu/ml (Westcott and Kluepfel, 1993). Shahata et al. (1988) reported that *P. chlororaphis* infected chickens.

Pathogenicity to plants

P. chlororaphis has been reported as the causal agent for a disease in straw mushrooms (*Volvariella volvacea*) in Puerto Rico, characterised by basal soft rot, internal water-soaking and discoloration (Hepperly and Ramos-Davila, 1986).

23.3 *P. fluorescens*

Pathogenicity to animals

P. fluorescens can infect a wide range of animals including horses (Sarasola et al., 1992), chickens (Lin et al., 1993), marine turtles (Glazebrook and Campbell, 1990), and many fish and invertebrate species. However, because it is unable to grow at elevated temperatures like that of the human body (Palleroni, 1992a), it is unlikely to be more than a rare opportunistic pathogen for warm-blooded animals.

P. fluorescens is considered a secondary invader of damaged fish tissues, but may also be a primary pathogen (Roberts and Horne, 1978; Stoskopf, 1993). The species causes bacterial tail rot and can affect freshwater and saltwater fishes throughout the world (Stoskopf, 1993). *P. fluorescens* releases extracellular proteases upon invasion of the fish host (Li and Flemming, 1968), and morbidity can be quite high. Outbreaks of bacterial tail rot have been reported in goldfish, silver carp, bighead, tench, grass carp, black carp, golden shiner, rainbow trout, European eels, paradise fish, and other labyrinth fishes. Egusa (1992) reviewed *Pseudomonas* diseases in fish and indicated that, in the United States, the disease termed *Pseudomonas* septicemia, due to infection by bacteria related to *P. fluorescens* (AFS-FHS, 1975), is seen in comparatively large numbers in warm-water fish.

Adverse effects associated with *P. fluorescens* in fish species often appear to be linked to stress from transportation or cultivation of fish. For example, *P. fluorescens* has been associated with disease in the cultivation of rainbow trout, *Oncorhynchus mykiss* (Barros et al., 1986), Atlantic salmon, *Salmo salar* (Carson and Schmidtke, 1993), chinook salmon, *Oncorhynchus tshawytscha* (Newbound et al., 1993), sea bream, *Evynnis japonica* (Kusuda et al., 1974), bighead carp, *Aristichthys nobilis*, and silver carp, *Hypophthalmichthys molitrix* (Petrinec et al., 1985), catfish and carp (Gatti and Nigelli, 1984), tench (Ahne et al., 1982), and tilapia species (Okaeme, 1989; Miyashita, 1984; Miyazaki et al., 1984).

Barker et al. (1991) found that exposure of high numbers of *P. fluorescens* to egg surfaces of rainbow trout (*Oncorhynchus mykiss*) during the initial stages of incubation poses a threat to egg survival. Conversely, *P. fluorescens* was not pathogenic when injected into brown trout (Smith and Davey, 1993) or silver mullet fish (*Mugil curema*) (Alvarez and Conroy, 1987).

P. fluorescens has also been implicated in pathogenicity to some invertebrates. James and Lighthart (1992) determined an LC_{50} for the 1st, 2nd, 3rd and 4th instar larvae of a coleopteran insect (*Hippodamia convergens*) (4.8 x 10^{9}, 2.8 x 10^{10}, 3.9 x 10^{9}, and 3.2 x10^{11} CFU/ml, respectively) and concluded that *P. fluorescens* is a weak bacterial pathogen. *P. fluorescens* has also been reported to be associated with pathogenicity in the mosquitoes *Culex quinquefasciatus*, *Anopheles stephensi*, and *Aedes aegypti* (Murty et al., 1994) and in the field slug *Deroeras reticulatum* (Wilson et al., 1994). However, Genthner et al. (1993) studied the effects of *P. fluorescens* on eastern oysters (*Crassostrea virginica*) and found no signs of infectivity or pathogenicity.

Pathogenicity to plants

P. fluorescens is generally considered to be a saprophyte rather than a plant pathogen (Bradbury, 1986), although Ormrod and Jarvis (1994) considered it to be an opportunistic pathogen causing soft rot in plants. *P. fluorescens* biovar 2 (*P. marginalis*), however, is actively pectinolytic, causing soft rot of various plants, and is considered a plant pathogen (Tsuchiya et al., 1980; Hildebrand, 1989; Membre and Burlot, 1994; Brock et al., 1994). Bradbury (1986) recognised three pathovars in *P. marginalis* which cause soft rot in a wide range of vegetables and other plants.

A number of studies have reported adverse effects associated with *P. fluorescens* and plants (Gaudet et al., 1980; Anson, 1982; Hwang et al., 1989; Richardson, 1993; Ozaktan and Bora, 1994). Tranel et al. (1993) found that *P. fluorescens* strain D7 inhibited root growth of downy brome (*Bromus tectorum*) by production of a phytotoxin. Sellwood et al. (1981) confirmed pathogenicity experimentally for an atypical *P. fluorescens* biotype I on chicory plants and suggested that the group *P. fluorescens* does not solely comprise saprophytes. However, other studies have found no adverse effects on plants from inoculations with *P. fluorescens* (Arsenijevic, 1986; Arsenijevic and Balaz, 1986; Surico and Scala, 1992). At present, the epidemiology of pathogenic strains of *P. fluorescens* is not well understood (Hildebrand, 1994).

Liao (1991) has cautioned that, at present, it is not known for sure that *P. fluorescens* strains are non-pathogens and do not cause deleterious effects on plants. Liao indicated that the *pel* gene encoding production of pectate lysase (an enzyme which contributes ability to cause soft rot in plants) is well conserved in fluorescent pseudomonads, and may exist and remain repressed in certain strains or species which exhibit non-pectolytic phenotypes under laboratory conditions. Liao further indicated that saprophytic strains of *P. fluorescens* can be induced to become pathogenic and raised a concern about the safety of using the species as a biocontrol agent.

23.4 *P. fragi*

Pathogenicity to animals

No reports were found of *P. fragi* as an animal pathogen.

Pathogenicity to plants

No reports were found of *P. fragi* as an plant pathogen.

23.5 *P. putida*

Pathogenicity to animals

P. putida can infect a variety of animals including goats (Hungerford, 1990), koala (Ladds et al., 1990), turkey (Ononiwu, 1980) and fish (Kusuda and Toyoshima, 1976). However, because it is unable to grow at elevated temperatures like that of the human body (Palleroni, 1992a), it is unlikely to be more than a rare opportunistic pathogen for warm-blooded animals.

Kusuda and Toyoshima (1976) reported *P. putida* to be a pathogen to cultivated yellowtail fish. However, there have been no reports on the epizootiology, symptoms, or histological or pathological findings, and the disease has not been well-defined (Egusa, 1992). Austin and McIntosh (1991) considered *P. putida* to be one of a variety of gram-negative bacteria pathogens of potential concern to farmed and wild fish. *P. putida* has also been associated with pathogenicity in the snail, *Biomphalaria glabrata* (Cheng, 1986), the crayfish (Boemare and Vey, 1977), and the olive fly (Haniotakis and Avtizis, 1977).

Pathogenicity to plants

. *P. putida* was included in the *Guide to Plant Pathogenic Bacteria* solely because its multiplication in the rhizosphere of paddy rice plants has been implicated in “suffocation disease”, which arises under conditions of poor drainage (Bradbury, 1986). Studies have reported that *P. putida* is not pathogenic to mushrooms (Ozakatan and Bora, 1994) or crucifer plants (Shaw and Kado, 1988).

Liao (1991) has cautioned that, at present, it is not known for sure that *P. putida* strains are non-pathogens and do not cause deleterious effects on plants. Liao indicated the *pel* gene encoding production of pectate lysase (an enzyme which contributes ability to cause soft rots in plants) is well conserved in fluorescent pseudomonads and may exist and remain repressed in certain strains or species which exhibit non-pectolytic phenotypes under laboratory conditions. Homologous sequences were found in strains of *P. putida,* and Liao raised a concern about the safety of using *P. putida* as a biocontrol agent.

23.6 *P. syringae*

Pathogenicity to animals

No reports were found of *P. syringae* as an animal pathogen.

Pathogenicity to plants

P. syringae is principally an assemblage of foliar pathogens, although it occurs as both pathogenic and epiphytic (non-pathogenic) strains. The species has a broad range of potential plant hosts (Table 5). Pathogenic strains can exhibit both pathogenic (i.e. disease-causing) and epiphytic behaviours on susceptible hosts (Crosse, 1959). The initiation of infection results when a threshold level of bacteria is

reached on the leaf surface; in the case of *P. syringae* pv. *syringae* this is reported to be 10^4 cfu gm^{-1} tissue (Hirano and Upper, 1983).

The association between rain and the onset of foliar blights caused by *P. syringae* is well recognised. Rain appears to stimulate the differential growth of pathogenic *P. syringae* isolates from the heterogeneous populations (pathogenic and non-pathogenic strains). Rain-triggered growth of *P. syringae* results in the establishment of large pathogenic populations required for disease development (Hirano and Upper, 1992).

There appears to be a distinctive set of symptoms associated with each causal agent. *Pseudomonas syringae* pv. *savasatoni* incites tumourous outgrowths on stems and leaves of oleander and olive under natural conditions. These symptoms have been found to be associated with the production of the auxin, indole acetic acid (IAA), in tissues infected with the bacterium. Furthermore, chlorosis, a common symptom when plants are infected by a number of pathogens belonging to the *P. syringae* group, is indicative of production of a toxin. For example, halo blight of beans caused by *P. syringae* pv. *phaselicola* is mediated by the toxin, phaseolotoxin. Other phytopathogenic pseudomonads producing toxins are illustrated in Table 6.

The *Dictionary of Natural Products* (Chapman and Hall, 1995) lists the following toxins produced by various strains of *P. syringae*: 1H-Indole-3-carboxaldehyde, octicidin (phytotoxin), phaseolotoxin (phytotoxin), N-Phosphosulfamylornithine (phytotoxin), syringomycin (phytotoxin), syringostatin A (phytotoxin), syringostatin B (phytotoxin), syringotoxin B (phytotoxin), tagetitoxin (phytotoxin), coronafacic acid (induces chlorosis in plants), halotoxin (phytotoxin), tabtoxin (phytotoxin). Coronatine (phytotoxin) is also produced by certain strains of *P. syringae* (Cuppels and Ainsworth, 1995). Gross (1985) determined that syringomycin production was stimulated by iron and suppressed by inorganic phosphate, that production occurred between 15 and 27°C, and that a slow growth rate of *P. syringae* favours toxin production.

Table 5

Range of plant species susceptible to infection with *P. syringae*

Plant (genus, species)	*P. syringae* pathovar
Acer spp.	aceris
Aesculus indica	aesculi
Antirrhinum majus	antirrhini
Apium graveolens	apii
Beta spp., *Heleanthus annuus*, *Tropaeolum majus*	aptata
Avena sativa, *Hordeum vulgare*, *Triticum aestivum*	atrofaciens
Agropyron spp., *Agrostis* spp., *Bromus* spp., *Dlymus* spp., *Festuca* spp., *Lolium* spp., *Phleum pratense*, *Stipa* spp.	atropurpurea
Corylus avellana	avellanae
Berberis spp.	bereridis
Cannabis sativa	cannabina
Ceratonia siliqua	ciccaronei
Avena spp., *Arrhenatherum elatius*, *Calamogrostis montanensis*, *Deschampsia caespitosa*, *Koeleria cristata*, *Phelum partense*, *Triticum* X *Secale*, *Trisetum spicatum*, *Zea mays*	coronafaciens
Delphinium spp.	delphinii
Dysoxylum spectabile	dysoxyli
Eriobotrya japonica	eriobotryae
Ficus palmata	fici
Coffea arabica	garcae
Glycine max	glycinea
Helianthus spp.	helianthi
Hordeum vulgare, Triticum aestivum	japonica
Citrullum lanatus, Cucumis spp., *Cucurbita* spp.	lachrymans
Zea mays, Sorghum bicolor	lapsa
Brassica spp., *Raphus sativus*	maculicola
Nicotiana tabacum	mellea
Morus spp.	mori
Prunus spp.	morsprunorum
Myrica rubra	myricae
Oryza sativa	oryzae

Table 5

Range of plant species susceptible to infection with *P. syringae* (cont.)

Plant (genus, species)	***P. syringae* pathovar**
Panicum miliaceum	panici
Malus pumila, Pyrus communis	papulans
Passiflora edulis	passiflorae
Prunus persica	persicae
Phaseolus spp., *Pisum sativum, Pueraria lobata*	phaseolicola
Philadelphus coronarium	philadelphi
Photinia glabra	photiniae
Lathrus spp., *Pisum* spp., *Vicia* spp.	pisi
Allium porrum	porri
Primula spp.	primulae
Protea cynaroides	proteae
Ribes aureum	ribicola
Forsythia intermedia, Fraxinus spp., Ligustrum spp.,	
Nerium oleander, *Olea* spp., *Nicotiana tabacum*	savastanoi
Sesamum indicum	sesami
Avena sativa, *Triticum* X *Secale*	striafaciens
many hosts	syringae
Glycine max, *Nicotiana tabacum*	tabaci
Ambrosia artemisiifolia, *Helianthus* spp., *Tagetes* spp.	tagetis
Camellia sinensis	theae
Capsicum anum, *Lycopersicon esculentum*	tomato
Ulmus spp.	ulmi
Viburnum spp.	viburni
Pseudostuga menziesii	

adapted from Bradbury (1986) and Chanway and Holl (1992)

Table 6

Some toxins produced by phytopathogenic *Pseudomonas* sp.

Pseudomonad	Toxin(s)	Mechanism or site of action	Host plant(s)
P. syringae			
pv. *atropurpurea*	coronatine		Italian rye grass
pv. *coronafaciens*	tabtoxin-β-lactam	glutamine synthetase	oat
pv. *garcae*	tabtoxin-β-lactam	glutamine synthetase	coffee
pv. *glycinea*	coronatine/ polysaccharide		soybean
pv. *lachrymans*	extracellular polysaccharides		cucumber
pv. *maculicola*	coronatine		crucifers
pv. *morsprunorum*	coronatine		sour cherry
pv. *phaseolicola*	phaseolotoxin	ornithine transcarbamoylase	bean, kudzu
pv. *savasatoni*	IAA & cytokinins	plant growth regulators	olive, oleander
pv. *syringae*	syringomycins syringopeptins syringotoxins	plasma membrane	peach, maize
pv. *tabaci*	tabtoxin-β-lactam	glutamine synthase	tobacco
pv. *tagetis*	tagetitoxin	chloroplastic RNA polymerase	marigold
pv. *tomato*	coronatine		tomato
P. tolaasii	tolaasin	plasma membrane	mushroom

taken from Durbin (1996)

23.7 *P. tolaasii*

Pathogenicity to animals

No reports were found of *P. tolaasii* as an animal pathogen.

Pathogenicity to plants

P. tolaasii causes Brown blotch (bacterial blotch), the most common bacterial disease of the commercial button mushroom, *Agaricus bisporus* (Howard et al., 1994). This disease can result in serious economic losses. *P. tolaasii* has also been found to cause disease in the oyster mushroom, *Pleurotus ostreatus,* and the shiitake mushroom, *Lentinus edodes* (Suyama and Fujii, 1993).

P. tolaasii produces a haemolytic lipodepsipeptide toxin, tolaasin, which is a potent biosurfactant (Hutchison and Johnstone, 1993) and the primary disease determinant of its pathogenicity to the cultivated mushroom, *Agariculs bisporus* (Rainey et al., 1993). Tolaasin is phytotoxic when infiltrated into tobacco leaves (Rainey et al., 1991). Synthesis of tolaasin is chromosomally determined, and it is known that at least five genetic loci are required for tolaasin synthesis (Rainey et al., 1993).

24. Interactions with and effects on other organisms in the environment

24.1 *P. aeruginosa*

Certain *P. aeruginosa* strains are antagonistic to plant pathogens such as damping-off fungi (Bradbury, 1986; Buysens et al., 1994). Duffy and Defago (1995) found that clinical and plant isolates of *P. aeruginosa* suppressed root diseases of cucumber, maize and wheat caused by soilborne fungi *Gaeumannomyces graminis* var. *tritici*, *Phomopsis sclerotiodes*, *Pythium ultimum* and *Rhizoctonia solani*. A soil isolate of *P. aeruginosa* suppressed foliar disease on wheat caused by *Septoria tritici* (Flaishman et al., 1990).

P. aeruginosa can have a synergistic effect on the survival of salmonellae, enabling them to survive more than 140 days in double-distilled water (Warburton et al., 1994). It has also been suggested that *P. aeruginosa* may act synergistically with pectolytic bacteria that colonise vegetables, such as *P. marginalis* (*P. fluorescens*) and *Erwinia cartovora* (Bradbury, 1986). A protective immunity against *P. aeruginosa* infection has been reported in mice vaccinated with heat-killed *Lactobacillus casei* (Miake et al., 1985).

P. aeruginosa is known to produce 1-phenazinecarboxamide (the amide of 1-phenazinecarboxylic acid), which is active against some phytopathogenic fungi and *Candida albicans.* A related compound, 1-phenazinol, which is active against gram-positive bacteria and fungi, and which shows some viral activity, is also produced by *P. aeruginosa*. 1-phenazinol has an LD_{50} of 500 mg/kg in mice dosed intraperitoneally. Pyoluteorin and its 3'-nitro derivative are produced by *P. aeruginosa*. Both compounds have antibacterial, antifungal and herbicidal properties. The LD_{50} of the pyoluteorin to mice is 125 mg/kg (Chapman and Hall, 1995). The antibiotic, 2-heptyl-4-hydroxyquinoline N-oxide, is a metabolite of *P. aeruginosa* and is a potent 5'- lipoxygenase inhibitor, with an LD_{50} of 40 mg/kg in mice dosed intraperitoneally (Chapman and Hall, 1995).

24.2 *P. chlororaphis*

P. chlororaphis has been widely investigated for its ability to enhance plant growth through suppression of deleterious root-colonising bacteria. Compounds known as siderophores are produced by *P. chlororaphis*. These compounds chelate iron, thereby depriving certain root-colonising plant pathogens of iron necessary for their growth (Smirnov et al., 1991).

Many studies have indicated that *P. chlororaphis* has the ability to suppress plant disease. For example, *P. aureofaciens* has been investigated as a biocontrol agent to suppress take-all, the wheat root fungal disease. The ability of *P. aureofaciens* to inhibit *Gaeumannomyces graminis* var. *tritici*, the causal agent of take-all, was demonstrated *in vitro* and *in vivo* (Harrison et al., 1993). It is believed that disease suppression is largely due to the production of phenazine antibiotics (Thomashow and Pierson, 1991). Carruthers et al. (1995) tested the ability of *P. aureofaciens* to suppress root rot of *Asparagus officinalis* caused by *Phytophthora megasperma* var. *sojae*. *P. aureofaciens* significantly reduced the level of infection and disease severity. Other tests suggested that *P. aureofaciens* had a direct growth stimulatory effect on asparagus, independent of antibiotic production (Carruthers et al., 1995). Berg and Ballin (1994) found *P. chlororaphis* inhibited the growth of the phytopathogenic fungus *Verticillium dahliae.*

When Douglas fir seed was inoculated with *P. aureofaciens,* and grown in pasteurised soil, shoot biomass increased significantly when compared with non-inoculated controls (Chanway and Holl, 1992). *P. aureofaciens* has been found to inhibit mycelial growth of *Rhizoctonia solani* in dual culture between 15 and 30°C (Lee et al., 1990). Inoculation of rice seeds was found to control rice sheath blight in the early growth stages, and seedling blight caused by *R. solani, Fusarium moniliforme* and *Pythium ultimum* was suppressed by seed treatment and soil incorporation of *P. aureofaciens* (Lee et al., 1990). In another experiment, the emergence of sweet corn seedlings from soil infested with *Pythium ultimum* was greatly enhanced by coating the seed with *P. aureofaciens* (Mathre et al., 1994). *P. aureofaciens* has also been evaluated for its ability to suppress *Pythium ultimum* damping off of cucumber seedlings (Sugimoto et al., 1990).

P. aureofaciens was antagonistic to *Clavibacter michiganensis* subsp. *sepedonicus*, the bacteria implicated in potato ring rot in greenhouse trials with potato seedlings (de la Cruz et al., 1992). *P. aureofaciens* significantly reduced populations of, and infection by, the ring rot bacteria (de la Cruz et al., 1992). Fukui et al. (1994) investigated the relationship between pericarp colonisation by *Pythium ultimum* in sugar beets and the growth of pseudomonads in the spermosphere. They found a positive correlation between the incidence of pericarp colonisation by *Pythium ultimum* and the length of the lag phase of the strain used to inoculate the seeds. England et al. (1993) investigated the nodulation of whitebean (*Phaseolus vulgaris* L.) by *Rhizobium phaseoli* in the presence of *P. aureofaciens*. No significant difference was found in the numbers of nodules produced in the presence of *P. aureofaciens* as a result of the symbiotic relationship between *Rhizobium phaseoli* and whitebean roots in vermiculite.

P. chlororaphis was observed to interfere with the growth of shiitake mushrooms in field experiments with shiitake cultivated logs (Raaska and Mattila-Sandholm, 1991). Siderophores were produced, however the addition of iron to *in vitro* cultures did not entirely neutralize the growth inhibition of mycelia by *P. chlororaphis.* It was concluded that although iron-binding plays an important role, it is not the only factor involved in the inhibition of shiitake by *P. chlororaphis* (Raaska and Mattila-Sandholm, 1991). A siderophore extracted from *P. aureofaciens* was found to inhibit uptake of ferric iron by maize and pea, and the synthesis of chlorophyll in these plants was reduced (Becker et al., 1985).

P. aureofaciens is reported to produce an antibiotic-like compound in iron-rich conditions that inhibits the growth of the plant fungal pathogen *Aphanomyces euteiches* (Carruthers et al., 1994). Mazzola

et al. (1992) suggested that the production of phenazine antibiotics contributes to the ecological competence of *P. aureofaciens,* and that reduced survival of strains unable to produce the antibiotics is due to diminished ability to compete with the resident microflora. Thomashow et al. (1990) found that suppression of take-all is related directly to the presence of the antibiotic phenazine-1-carboxylic acid in the rhizosphere of wheat. In another experiment, 2-hydroxyphenazine-1-carboxylic acid and 2-hydroxyphenazine were also found to be responsible for take-all suppression in wheat (Pierson and Thomashow, 1992). Pyrrolnitrin [3-chloro-4-(3-chloro-2-nitrophenyl)-1H-pyrrole] is an antifungal compound produced by *P. chlororaphis*; its LD50 in mice dosed orally is 1 g/kg (Chapman and Hall, 1995). The antifungal compound, 1,3,6-trihydroxy-2,4-diacetophenone, has also been isolated from culture media (Harrison et al., 1993).

24.3 *P. fluorescens*

P. fluorescens has been recognised as beneficial to plant growth (Weller and Cook, 1986; Kloepper et al., 1988). It can enhance plant growth through production of siderophores, which efficiently complex environmental iron, making it unavailable to other components of the soil microflora. Increased plant yields achieved through the inoculation of plant roots have been mimicked by the application of the siderophore, pseudobactin, isolated from *P. fluorescens*. Antibiotic production by *P. fluorescens* has been recognised as an important factor in its ability to suppress phytopathogens. *P. fluorescens* has also been found to significantly promote nodulation, growth and nitrogen accumulation in faba beans (*Vicia faba*) (Omar and Abd-Alla, 1994). Heat-killed cells had no effect.

Certain strains of *P. fluorescens* can promote the formation of ice crystals in water at temperatures near 0°C (Lindow and Panopoulos, 1988; Lindow, 1992). Large populations of these ice^{+} bacteria on plant surfaces can cause frost injury. Only 0.01 to 40% of the total bacteria on plant surfaces are sufficient to cause frost injury. In the absence of these bacteria, water on plants can cool to -40°C.

Smith and Davey (1993) found that *P. fluorescens* strains were able to inhibit *Aeromonas salmonicida* that was isolated from Atlantic salmon with furunculosis. Pre-smolts asymptomatically infected with *A. salmonicida* and bathed in a solution containing *P. fluorescens* strains were less likely to develop stress-induced furunculosis than non-treated fish. It was concluded that *P. fluorescens* inhibits *A. salmonicida* by competing for free iron, and that it protects against stress-induced furunculosis by inhibiting *A. salmonicida* on external locations. Kimura et al. (1990) found that a strain of *P. fluorescens* biovar I (46NW-04) isolated from the aquatic environment produced an antiviral substance that was effective against fish viruses.

24.4 *P. fragi*

Monitoring of microbial flora succession on minced lamb meat revealed that *P. fragi* was the dominant climax species (Drosinos and Board, 1995). Another study indicated that *P. fragi* dominated the flora on lamb carcasses at both 7 and 30°C (Prieto et al., 1992).

24.5 *P. putida*

P. putida is very common in soils and plant rhizospheres, where it seems to have a stimulating effect on plant growth (Palleroni, 1984). *P. putida* has been shown to suppress a variety of plant pathogens and to reduce the incidence of plant disease (Liao, 1989; Gamliel and Katan, 1993; Duijff et al., 1994; Freitas et al., 1991; Defago and Hass, 1990). This may be due in part to its inhibition of plant pathogenic microorganisms by sequestering iron or producing metabolites with antibiotic properties. Formation of a

siderophore complex by the plant may also be involved (Defago and Hass, 1990). Siderophore-mediated competition for iron was indicated as the mechanism of suppression of Fusarium wilt of carnation by *P. putida* (WCS358r) (Duijff et al., 1994) and suppression of phytopathogens to winter wheat (Freitas et al., 1991).

Al-Achi et al. (1991) found that, when introduced as pairs into irradiated, sterile soils, a *P. fluorescens* strain prevented optimum colonisation by a *P. putida* strain. The addition of *P. putida* to sterile soil already populated by *P. fluorescens* impeded growth of *P. putida* in that soil. However, adding *P. fluorescens* to soil populated by *P. putida* did not prevent growth of *P. fluorescens* and caused a decrease in *P. putida*. These results suggested competition for similar niches in soils, and that *P. fluorescens* was the more competitive species studied.

24.6 *P. syringae*

Some strains of *P. syringae* have the ability to cause ice nuclei to form at temperatures just below 0°C, thus inducing freezing injury to susceptible plants and allowing disease development to occur (Lindow, 1983). Nutritional starvation for nitrogen, phorphorous,sulphur or iron at 32°C, followed by a shift to 14-18°C, led to the rapid induction (from non-detectable to 100% in 2 to 3 h) of type I ice nuclei (Nemecek-Marshall et al., 1993).

Replacement series experiments on bean leaves between *P. syringae* and epiphytic *P. fluorescens*, *Pantoea agglomerans* (*Erwinia herbicola*), *Stenotrophomonas maltophilia* (*Xanthomonas maltophilia*) and *Methylobacterium organophilum* have demonstrated that the epiphytes were all capable of higher levels of coexistence with *P. syringae* than was observed with another *P. syringae* strain. The level of coexistence with the epiphytes was inversely correlated with the ecological similarity of the strains and with a differential preference for amino acids, organic acids and carbohydrates (Wilson and Lindow, 1994).

The invasion and exclusion abilities of 29 strains of *P. syringae* were studied on leaves in 107 pairwise combinations in which each strain was inoculated on day 0, and the second (challenge) was inoculated on the same leaf on day 3 (Kinkel and Lindow, 1993). The presence of an established population often significantly reduced the growth of the second strain when quantified on day 6; successful invaders (challenge) were significantly less likely to exclude challenge populations than were non-successful invaders. Hirano and Upper (1993) determined that an introduced antibiotic-resistant strain of *P. syringae* spread but did not persist when applied to bean plants grown in the field; it was concluded that the introduced strain was less fit than the pool of indigenous species. Competition between indigenous soil bacteria and single cells of *P. syringae* pv. *syringae* engineered with bioluminescence genes from *Vibrio harveyi* can be monitored using charge-coupled enhanced microscopy (Silcock et al., 1992).

Defreitas et al. (1993) determined that *P. syringae* R25 inoculated on field peas (*Pisum sativum*) did not affect plant growth in plastic growth pouches but, in soil, did inhibit nitrogenase activity of nodules formed by indigenous rhizobia; *P. syringae* R25 inhibited the growth of field beans (*Phaseolous vulgaris*) in both plastic growth pouches and in soil. When peas were inoculated with both *P. syringae* R25 and *Rhizobium leguminosarum,* there was an increase in plant biomass in growth pouches but no effect was observed in soil; when beans were inoculated with both *P. syringae* R25 and *Rhizobium phaseoli*, there were severe deleterious effects on seedling emergence, plant biomass and nodulation in both growth pouches and soil.

Table 7

Phytopathogenic strains of *P. syringae* containing plasmids

Pathovar	**Reference**
P. syringae pv. *angulata*	Piwowarski and Shaw, 1982
P. syringae pv. *atrpurpurea*	Sato et al., 1983
P. syringae pv. *coronafaciens*	Piwowarski and Shaw, 1982
P. syringae pv. *glycinea*	Curiale and Mills, 1983
P. syringae pv. *lachrymans*	Coplin, 1989
P. syringae pv. *papulans*	Burr et al., 1988
P. syringae pv. *phaseolicola*	Quant and Mills, 1984
P. syringae pv. *savastanoi*	Comai et al., 1982
P. syringae pv. *striafaciens*	Beck-Von Bodmann and Shaw, 1987
P. syringae pv. *syringae*	Gonzales et al., 1984
P. syringae pv. *tabaci*	Obukowicz and Shaw, 1983; 1985
P. syringae pv. *tomato*	Denny, 1988; Bender and Cooksey, 1986

24.7 *P. tolaasii*

P. tolaasii produces a haemolytic lipodepsipeptide toxin, tolaasin, which is a potent biosurfactant (Hutchison and Johnstone, 1993) and the primary disease determinant of its pathogenicity to the cultivated mushroom, *Agariculs bisporus* (Rainey et al., 1993). Tolaasin is also active against a range of basidiomycetes and gram-positive bacteria (Rainey et al., 1991).

The nematode *Caenorhabditis elegans* is reported to decrease the spread of *P. tolaasii* in mushroom growth chambers (Grewal, 1991). *P. fluorescens* biovar *reactans* was frequently isolated from the gut of *C. elegans* along with mushroom sporophores. All the isolates of *P. fluorescens* biovar *reactans* isolated from nematodes were antagonists to *P. tolaasii*. It was suggested that, as *C. elegans* selects *P. fluorescens* biovar *reactans* rather than *P. tolaasii* as a food, it probably spreads the antagonist in the mushroom crop and may contribute to the control of mushroom blotch (Grewal, 1991). *P. fluorescens* has also been described by other researchers as antagonistic to *P. tolaasii* (Khanna and Olivier, 1989; Munjal et al., 1989; Nair and Fahy, 1972). Nair and Fahy (1972) reported *Enterobacter aerogenes* to be antagonistic to *P. tolaasii*.

Thorn and Tsuneda (1992) report that 23 species of wood-decay basidiomycetes attacked or lysed *P. tolaasii* when tested. Attack took the form of increased hyphal branching within the bacterial colonies, often preceded by directional growth toward them.

25. Ability to form survival structures (e.g. spores, sclerotia)

Pseudomonads are asporogenous, that is, they do not form spores or other survival structures. Pseudomonads are, however, pleomorphic and represent a tremendously diverse group of strains able to tolerate extreme environmental conditions, including the extremes of temperature.

Bacteria that do not form survival structures like spores and cysts are suspected to have other survival strategies. A number of researchers have reported the existence of dwarf or ultramicrobacteria in nutrient-stressed environments (Rosak and Colwell, 1987). These cells have been described from seawater (Amy and Morita, 1983) and soil (Casida, 1977). Cells are able to develop to their full size, once exposed to an abundant supply of nutrients.

26. Routes of dissemination, physical or biological

Physical

Pseudomonads may be disseminated by air or water currents. For example, Trevors et al. (1990) used soil-core microcosms to study the movement of a *P. fluorescens* isolate through soil planted with wheat and unplanted. In the absence of ground water flow, limited movement was detectable along the soil column planted with wheat, while no movement was detected in the unplanted soil. In contrast, movement of the strain through the column was dependent on the flow rate of the water and the number of times the columns were flushed through. Water flow also affected the distribution of the inoculant along the wheat roots. Bacterial cell size has been related to the movement of cells through a soil column, with smaller bacterial cells (< 1.0μm) moving fastest through the column (Gannon et al., 1991).

Rain may also be an important source of inoculum and means of dispersal for pseudomonads. Rain splash has been attributed to move pseudomonads colonising leaf surfaces down the plant canopy and into the soil (Hirano and Upper, 1992; Butterworth and McCartney, 1992; McCartney and Butterworth, 1992). Large drops of artificial rain were more effective in dispersing bacteria than smaller drops (Butterworth and McCartney, 1992). Humidity correlated positively with the consequent survival of pseudomonads dispersed by rain splash (McCartney and Butterworth, 1992). However, dispersal is short range (one or a few metres) (Constantidou et al., 1990). Pseudomonads (including *P. syringae* Ice^+ strains) have been found to leave plant surfaces in an aerosol-stable state and enter the troposphere during dry, warm weather (Lindemann et al., 1982; Lindemann and Upper, 1985). They are then transported and washed downwards during rainfall (Constantidou et al., 1990).

Biological

Pseudomonads are motile bacteria characterised by the presence of at least one flagellum. While there is no convincing evidence that the bacteria are flagellated in soil (Stotzky et al., 1991), flagella appear to confer increased epiphytic fitness on *P. syringae* strains in association with moisture on leaf surfaces (Haefele and Lindow, 1987). The potential for certain fluorescent pseudomonads to colonise plant surfaces has been attributed to the presence of pili (Vesper, 1987; de Groot et al., 1994), surface charge properties (James et al., 1985), the production of agglutinin, a glycoprotein complex, released from root surfaces (Anderson, 1983), and the ability of certain saprophytic pseudomonads to adhere to the agglutinin of specific plant species (Glandorf et al., 1993; 1994).

Earthworms moving through soil have been implicated in the dissemination of bacteria over short distances. As well, Johnson et al. (1993) have demonstrated the ability of honey bees to disseminate a biological control strain of *P. fluorescens* used against the fireblight pathogen, *Erwinia amylovora,* in apple and pear blossoms. Honey bees carrying approximately 10^4 to 10^6 cfu per bee effectively inoculated fruit tree blossoms with bacteria.

27. Containment and decontamination

Containment plans have been proposed for microbial releases, although few of them have been used, and their efficacy is yet to be demonstrated. It is likely to be difficult to eliminate all the bacteria from a site of introduction. Many of the proposed chemical treatments have gross rather than localised effects; hence their application may have considerable impact on the natural flora, fauna and microflora at the site. Pseudomonads will colonise many laboratory and hospital disinfectants, and may exhibit broad spectrum resistance to a number of widely used antibiotics. Disinfectants based on quaternary ammonium compounds and chlorhexidine solutions have been found to be contaminated with pseudomonads. Disinfectant contaminants include *P. aeruginosa, P. fluorescens,* and *P. cepacia* (Bergen, 1981).

P. putida strains that degrade alkylbenzoates have been modified to carry a fusion of the P (lac) promoter to the gef gene, which encoded a killing protein (Molin et al., 1993; Ramos et al., 1994). Expression from P (lac) was controlled through a regulatory cascade, so that P (lac) was switched on or off by the absence or presence of alkylbenzoates respectively. Similar uncontained strains were also constructed and tested as a control. Contained and uncontained strains were genetically stable, and their survival and functionality in soil microcosms were as expected. Both contained and uncontained strains survived well in soils supplemented with alkylaromatics, whereas survival of the contained strain in soil microcosms without methylbenzoates was markedly reduced in contrast to the control strain, which survived in these soils in the absence of alkylbenzoates (Jensen et al., 1993; Ronchel et al., 1995).

28. Description of detection and monitoring techniques, including specificity, sensitivity and reliability

28.1 Techniques employed in the laboratory and/ or environment for detecting the presence of, and for monitoring, numbers of the organism

Information on detection and monitoring techniques is provided in this information element as well as in information element 2 and Table 8. Each of the well-described detection methods has limitations as well as advantages for enumeration and/or detection (Drahos, 1992). For example, under certain conditions an approach which provides reasonable sensitivity by culturing a microorganism (e.g. the viable plate count) may give reliable data for culturable populations. Furthermore, many approaches are complementary; methods utilising nutritional, antibiotic and enzymatic markers rely on the ability of the target organism to express the marker genes during the selection or reculturing process. However, expression of these traits may not always be optimal, for instance under conditions of severe environmental stress. In these situations, a direct method of detection could be used.

Selective plating

Selective plating has been used widely in combination with selectable phenotypes based on antibiotic resistances (often spontaneous mutations) (Compeau et al., 1988; Fredrickson et al., 1989; Thompson et al., 1990) or introduced genes such as *xylE* (Winstanley et al., 1989; Morgan et al., 1989) l*acZY* (Cook et al., 1991; Drahos et al., 1988), *lux* (Shaw and Kado, 1986) and *mer* gene (Iwasaki et al., 1993; 1994). A number of these genes have been used for marking and tracking pseudomonads. It is important to ensure that the marker is not found in the indigenous microflora of the environment to which the microorganism will be introduced.

Pseudomonads appear to be highly culturable on laboratory media and may be isolated from environmental samples using viable plating (Drahos, 1992). Generally, 1 g of environmental sample is homogenised or shaken in 9 ml of an appropriate dilutent such as ¼ strength Ringer's solution or physiological saline. The homogenate is serially diluted 1 in 10, 100 µl aliquots spread onto selective agar, and the plates incubated at 28°C. A number of selective media are available commercially, such as *Pseudomonas* selective agar (Oxoid) and *Pseudomonas* agar F (Difco). Both media have a low iron content, promoting the production of the iron-chelating, fluorescent siderophores. Selective agars are supplemented with antibiotics. A commercially prepared cocktail of cephalorodine, fucidin acid and cetrimide (Oxoid) is available which may be supplemented with ampicillin and the antifungal agent, cyclohexamide. Microorganisms may be detected at or above a detection limit of 10^2 (i.e. one cell may be detected when a minimum of 100 are present per g of sample) (Trevors and van Elsas, 1989). Sensitivity may be increased by plating larger volumes or by using smaller dilutions, i.e. 1 in 2 instead of 1 in 10.

Most probable number

Most probable number (MPN) methods (Alexander, 1982) have been used to attain greater sensitivity. A serial dilution of the sample is made in an appropriate dilutent to an extinction point (Atlas, 1982). Three to ten replicates of each dilution are made and the pattern of positive and negative scores recorded (i.e. growth or no growth). Statistical tables are used to determine the MPN of microorganisms present in the sample. MPNs like the viable plate count require growth and reproduction of the strains, and may be less accurate since an MPN is established with confidence limits (Jain et al., 1988).

Table 8

Examples of identification and detection techniques

Method	**Reference**	**Sensitivity/reliability**
DNA extraction followed by Polymerase Chain Reaction (PCR)	Stefan and Atlas, 1988	100 *P. cepacia* cells $100g^{-1}$ sediment, against a background of 10 non-target organisms
	Pillai et al., 1991	1 to 10 *E.coli* (with *Tn*5 insert) colony forming unit (cfu)g^{-1} soil
	Tsai and Olson, 1992	3 cells *E.coli* g^{-1} soil; primers directed at 16S rRNA
	Tushima et al., 1995	10 cells g^{-1} water
hybridization using radio-labelled probes	Holben et al., 1988; Stefan and Atlas, 1988	10^3 to 10^4 cells g^{-1} soil
	Jain et al., 1988; Blackburn et al., 1987	10^2 cells g^{-1} soil (similar to viable plate count)
direct microscopy using immuno-fluorescence	Schmidt, 1974; Bohool and Schmidt, 1980; Ford and Olson, 1988	10^6 to 10^7 cells g^{-1} soil
enzyme-linked immunosorbent assays (ELISA)	Morgan et al., 1991; Scholter et al., 1992	10^3 cells g^{-1} soil; 10-10^2 cells g^{-1} soil
selective viable plating	Trevors and van Elsas, 1989; Iwasaki et al., 1993,1994	10^2 cfu g^{-1} soil; 1 cfu ml^{-1} water; 10 cfu g^{-1} soil
most probable number (MPN) viable counts	Alexander, 1982; De Leij et al., 1993	< 10^2 cfu g^{-1} soil; <10^1 cfu g^{-1} soil

Simple chemotaxonomical approach

A simple chemotaxonomical approach which avoids isolation and cultivation of microorganisms has been used. For example, quinone profiles (Hiraishi et al., 1991) or polyamine patterns (Auling et al., 1991) have been used as biomarkers for a survey of pseudomonads (and acinetobacters) in activated sludge from sewage treatment facilities.

Immunological methods

SDS-PAGE coupled with immunological probes have been applied to identify fluorescent pseudomonads of environmental origin (Sorenson et al., 1992). Other possibilities for detecting pseudomonads in environmental samples include the application of phylogenetic probes applied in in situ hybridisations (DeLong et al., 1989), or strain or species-specific monoclonal antibodies labelled with fluorescent dyes (Bohlool and Schmidt, 1980; Conway de Macario et al., 1982). Blair and McDowell (1995) describe an ELISA method for detecting extracellular proteinase of P. fragi.

Microscopic examination and direct enumeration of microorganisms *in situ* can also be used, although this type of approach is not sensitive. To detect one bacterium at a magnification of 1000, the cell density must be 10^6 to 10^7 per g soil. The approach does, however, provide information about the spatial distribution of a strain colonising an environmental substrate, and can be used to enumerate non-culturable microorganisms.

Ramos-Gonzalez et al. (1992) produced highly specific monoclonal antibodies against surface lipopolysaccharides (LPS) of *P. putida* 2440 and developed a semi-quantitative dot blot immunoassay for bacteria in liquid media. This allowed the authors to detect, in complex samples, as few as 100 cells per spot by using peroxidase-conjugated antibody against the antibody that recognised *P. putida* 2440. An intrinsic limitation of this technique is the turbidity of the samples, which may limit maximum assay volume. This assay is also of limited use for bacteria introduced into soils or sediments because of intrinsic fluorescent backgrounds. *P. putida* 2440 (pWWO) released in lake mesocosms have been successfully tracked with monoclonal antibodies (Brettar et al., 1994; Ramos-Gonzalez et al., 1992).

Nucleic acid probes and primers

Nucleic acid probes and/or PCR primers may be used for the detection of gene sequences in the environment. A number of sequence hybridization techniques including Southern, slot-blot, dot-blot, and colony hybridization have been used for environmental isolates. These approaches would be particularly applicable to strains with traits that are not widely distributed throughout the environment under study, and against which specific probes and primers may be designed. The sensitivity of the hybridization approach is variable and for the most part strain-specific. Generally, radioactively labelled probes provide for more sensitivity than non-radioactive probes. Sensitivity can be enhanced using PCR. However, the increased efficiency of the amplified signal obtained by the PCR assay is countered by the inefficient extraction of nucleic acids from environmental samples (Bramwell et al., 1994). For example, soils contain positively charged cations which are sandwiched between layers of clay, and which are able to bind negatively charged nucleic acids, making their retrieval difficult. Caution is required in using PCR as a method for the enumeration of bacteria, as the extreme sensitivity of this procedure renders quantification by target dilution difficult (Drahos, 1992). Thiem et al. (1994) and Zhou and Tiedje (1995) point out the complexity of using molecular techniques for monitoring pseudomonads used for subsurface bioremediation.

Denaturing gradient gel electrophoresis (DGGE) of DNA is a suitable method for those species which are difficult to culture on growth media. This method has been used by Muyzer et al. (1993). Whole DNA is isolated. Using two primers, one with a GC-rich end, a fragment of 16s rDNA is amplified by PCR. This results in a mixture of DNA fragments, equal in size but different in sequence, corresponding to the various organisms in the sample. The mixture is fractionated by DGGE, resulting in one band for each organism type. The bands are sequenced, and based on the sequences, the rRNA-group can be determined.

Polymerase chain reaction (PCR) based sequence amplification

A technique that is finding increasing application for specific identification of microorganisms is the technique referred to as REP-PCR (based on PCR amplification between repetitive sequences commonly found in bacteria). This technique relies on development of adequate databases, but is used with increasing frequency (De Bruijn, 1992). Other approaches are to follow the expressed phenotype attributed to the introduction of a marker gene (e.g. bioluminescent genes) (Prosser, 1994), and to use competitive PCR based on introduction of an internal standard during the PCR amplification (Leser, 1995).

Arbitrary PCR primers

Identification can be facilitated based on the analysis of DNA produced from total DNA, using PCR and arbitrary primers (Welsh and McClelland, 1990; Williams et al., 1990).

Specific PCR primers

P. aeruginosa can be identified using PCR amplification of the 16S-23S rDNA internal transcribed spacer region (Tyler et al., 1995).

28.2 Specificity, sensitivity, reliability

The specificity of identification/monitoring methodologies will generally require some sort of experimental study to demonstrate that the method distinguishes the introduced inoculant from indigenous relatives. An approximate estimate of sensitivity for a number of methods is given in Table 8. However, sensitivity of detection is a function of the organism and of the habitat.

A problem with applying any method of detection is its dependence on extraction efficiencies. Problems are exemplified in soil. Traditionally, bacteria have been recovered from soils through the mechanical shaking of the soil in an appropriate dilutent. The ease of extracting cells or nucleic acids varies between soil types, with extraction efficiencies being higher in sand as opposed to clay-based soils. Strong chemical and physical interactions may occur between microorganisms and the particulate matter of soil. These associations may be ionic, since bacteria are negatively charged and clay soil minerals contain positively charged cations. Dispersion of soil aggregates has been considered important, as entrapment of microorganisms in soil aggregates is considered to be one of the most significant means by which microorganisms are retained in soil (Hopkins et al*.,* 1991). Attempts to disrupt these soil-microbe associations to extract bacteria have utilised homogenisation, chemical dispersants, cation exchange resins, and differential centrifugation (Faegri et al*.,* 1977; Bakken, 1985; MacDonald, 1986; Herron and Wellington, 1990; Hopkins et al*.,* 1991).

Soil is a highly heterogeneous substrate with a non-uniform spatial distribution of bacterial colonies (Wellington et al*.,* 1990). Sampling strategies should consider the variability of the soil matrix

under study; errors attributable to the difficulties of sampling heterogeneous substrates may be compensated for by taking composite samples (Atlas and Bartha, 1981).

Microorganism themselves will also affect the efficiency of extraction of biological molecules such as DNA. For example, bacteria, even those quite closely related, vary in the conditions required for lysis. Hence methodologies aiming to extract the total DNA from soil will selectively recover DNA from isolates that lyse easily, making representative sampling of environmental substrates difficult.

Similar selective pressures apply to viable plating methodologies, since these methods favour the growth of bacteria that readily grow on agar plates under laboratory conditions. Furthermore, all media are selective to some extent, so that certain bacterial species will appear in different proportions, if at all, on different bacteriological agars. Sorheim et al. (1989) compared the populations recovered from soil on three different non-selective media. Bacterial populations exhibiting the same level of diversity were isolated on all media. Each of the media appeared to select for a different population of isolates, with 30% of the population appearing common to all three media. 20% of the isolates recovered from two of the media were distinct to that particular media, and 60% of isolates on the third media were unique to it.

The sensitivity of the viable plate count has been estimated to be 10^2 cfu/g soil (Trevors and van Elsas, 1989). However, this may be improved by combining methods to extract and concentrate the biomass from environmental material prior to plating. Detection limits as low as 10 streptomycete spores per 100 g sterile soil have been demonstrated (Herron and Wellington, 1990).

Pseudomonads are highly culturable on rich media. Their importance may therefore have been overestimated as a result of over-representation on isolation plates (Miller et al., 1990b; Sorheim et al., 1989). Nutritionally limiting isolation media and lower incubation temperatures with longer incubations may allow a greater diversity of bacterial isolates to be recovered from environmental substrates (Miller et al., 1990b).

Ottawa' 92: The OECD Workshop on Methods for Monitoring Organisms in the Environment (OECD, 1994a) includes a review of the monitoring of microorganisms (including *P. aureofaciens*) in the phyllosphere (Bailey et al., 1994) and a review of the different methods available. A companion document, *Compendium of Methods for Monitoring Organisms in the Environment* (OECD, 1994b), contains 39 methods for detecting or monitoring microorganisms, including the following species of *Pseudomonas*: *P. aureofaciens*, *P. fluorescens*, *P. putida*, *P. aeruginosa* and *P. syringae*.

SECTION V – REFERENCES

AFS-FHS (American Fisheries Society, Fish health Section). 1975. Suggested procedures for the detection and identification of certain infectious diseases. U.S. Fish and Wildlife Service, Washington, D.C., 99p.

Ahne, W., Popp, W. and Hoffman, R. 1982. *Pseudomonas fluorescens* as a pathogen for tench. Bulletin of the European Association of Fish Pathologists 2: 56-57.

Aksamit, T.R. 1993. *Pseudomonas* pneumonia and bacteremia in the immunocompromised patient. In: Fick, R.B. Jr. (ed.). ***Pseudomonas aeruginosa*** the opportunist. Pathogenesis and disease (pp. 177-188). Boca Raton: CRC Press.

Al-Achi, B.J., Platsouka, E. and Levy, S.B. 1991. Competitive colonisation between *Pseudomonas* species in sterile soils. Current Microbiology 23: 97-104.

Alaoukaty, A., Appanna, V.D. and Huang, J. 1992. Aluminum, chromium and manganese detoxification mechanisms in *Pseudomonas syringae* - An X-ray fluorescence study. Microbios 70(282): 13-22.

Alexander, M. 1982. Most probable number method for microbial populations. In: A.L. Page, R.H. Miller and Keeney, D.R. (eds). *Methods of Soil Analysis*, Part 2 (pp. 815-820). ASA and SSA, Madison, WI.

Alford, J.A. and Pierce, D.A. 1961. Lipolytic activity of microoganisms at low and intermediate temperatures. III. Activity of microbial lipases at temperatures below 0° C. Journal of Food Science. 26: 518-524.

Alvarez, J.D. and Conroy, D.A. 1987. The experimental induction of bacterial haemorrhagic septicaemia in silver mullet (*Mugil curema* Val., 1836) held in sea water. Bulletin of the European Association of Fish Pathologists 7: 8-10.

Alvarez-Cohen, L. and P.L. McCarty. 1991. Product toxicity and cometabolic competitive inhibition modelling of chloroform and trichloroethylene transformation by methanotrophic resting cells. Applied and Environmental Microbiology 57: 1031-1037.

Amy, P.S. and Morita, R.Y. 1983. Starvation-survival patterns of sixteen freshly isolated open-ocean bacteria. Applied and Environmental Microbiology 45: 1109-1115.

Anderson, A.J. 1983. Isolation from root and shoot surfaces of agglutinins that show specificity for saprophytic pseudomonads. Canadian Journal Botany 61: 3438-3443.

Anderson, M. and Davey, R. 1994. Pseudobacteraemia with *Pseudomonas fluorescens*. Medical Journal Aust. 160: 233-234.

Andreoni, V. and Bestetti, G. 1986. Comparative analysis of different *Pseudomonas* strains that degrade cinnamic acid. Applied and Environmental Microbiology 52: 930-934.

Angelini, N.M. and Seigneur, G.N. 1988. Fin rot in *Rhamdia sapo*. Isolation of the aetiological agents, *Aeromonas hydophila* and *Pseudomonas aeruginosa*, and experimental infection. Revista Argentina de Microbiologia 20(1): 37-48.

Angle, J.S., Levin, M.A., Gagliardi, J.V. and McIntosh, M.S. 1995. Validation of microcosms for examining the survival of *Pseudomonas aureofaciens* (*lac* ZY) in soil. Applied and Environmental Microbiology 61: 2835-2839.

Anson, A.E. 1982. A Pseudomonad producing orange soft rot disease in cacti. Phytopathologische Zeitschrift 103: 163-172.

Arsenijevic, M. 1986. Some weeds as test plants for the pathogenicity of plant pathogenic *Pseudomonas* species. II. Reaction of *Datura stramonium* L. and *Chenopodium album* L. Zastita Bilja 37: 225-229.

Arsenijevic, M. and Balaz, J. 1986. Wheat (*Triticum aestivum ssp. vulgare* (Vill.Host) MacKey) as a test plant for the pathogenicity of *Pseudomonas syringae* pv. *syringae* Van Hall. Zastita Bilja 37: 201-212.

Artenstein, A.W. and Cross, A.S. 1993. Local and disseminated diseases caused by *Pseudomonas aeruginosa*. In: Campa, M., et al. (eds). *Pseudomonas aeruginosa* as an opportunistic pathogen (pp. 224-244). Plenum Press, New York/London.

Assinder, S.J. and Williams, P.A. 1990. The TOL plasmids: determinants of the catabolism of toluene and xylenes. Advances in Microbial Physiology 31: 1-69.

Atlas, R.M. 1982. Enumeration and estimation of microbial biomass. In: Burns, R.G. and Slater, J.H. (eds). *Experimental Microbial Ecology* (pp. 84-102). Blackwell Scientific Publications, Oxford, UK.

Atlas, R.M. and Baratha, R. 1981. Determination of microbial numbers, biomass and activities. In: *Microbial Ecology: Fundamentals and Applications* (pp.81-132). London: Addison-Wesley, Publishing Company.

Auling, G. 1992. Polyamines, biomarkers for taxonomy and ecology of phytopathogenic and other bacteria. Belgian Journal of Botany. 125: 203-209.

Auling, G., Pilz, F., Busse, H.-J., Karrasch, S., Streichan, M. and Schon, G. 1991. Analysis of the polyphosphate-accumulating microflora in phosphorous-eliminating, anaerobic-aerobic activated sludge systems by using diaminopropane as a biomarker for rapid estimation of *Acinetobacter* spp. Applied and Environmental Microbiology 57: 3585-3592.

Austin, B., and McIntosh, D. 1991. New bacterial fish pathogens and their implications for fish farming. Reviews in Medical Microbiology 2: 230-236.

Babu, G.R.V., Chetty, C.S., Wolfram, J.H. and Chapatwala, K.D. 1994. Microbial metabolism of nitriles and cyanides. Journal of Environmental Science and Health - Part A - Environmental Science and Engineering 29: 1957-1971.

Baggi, G., Boga, M.M., Catelani, D., Galli, E. and Treccani, V. 1983. Styrene catabolism by a strain of *Pseudomonas fluorescens*. Systematic and Applied Microbiology 4: 141-147.

Bailey, M.J., Thompson, I.P. and Lynch, J.M. 1994. Methods for monitoring microorganisms in the phyllosphere, pp. 44-53. In: Ottawa '92: The OECD Workshop on Methods for Monitoring Organisms in the Environment. OECD Environment Monograph No. 90. OECD, Paris.

Bakken, L.R. 1985. Separation and purification of bacteria from soil. Applied and Environmental Microbiology 49: 1482-1487.

Bale, M.J., Fry, J.C. and Day, M.J. 1988. Transfer and occurrence of large mercury resistance plasmids in river epilithon. Applied and Environmental Microbiology 54: 972-978.

Barker, G.A., Smith, S.N. and Bromage, N.R. 1991. Commensal bacteria and their possible relationship to the mortality of incubating salmonid eggs. Journal of Fish Diseases 14: 199-210.

Barrett, E.L., Solanes, R. E., Tang, J.S. and Palleroni, N.J. 1986. *Pseudomonas fluorescens* biovar V: its resolution into distinct component groups and the relationship of these groups to other *P. fluorescens* biovars, to *P. putida*, and to psychrotrophic pseudomonads associated with food spoilage. Journal of General Microbiology 132: 2709-2721.

Barros, G.C., Vianni, M.C.E. and Nogueira, Y.A. 1986. Outbreak of disease in rainbow trout attributed to *Pseudomonas fluorescens*. Revista da Faculdade de Medicina Veterinaria e Zootecnia da Universidade de Sao Paulo 23: 167-173.

B.C.I. (Biotechnology Citation Index), Institute for Scientific Information, CD-ROM. 1991-1995. Philadelphia, U.S.A.

Beattie, G.A. and Lindow, S.E. 1994. Survival, growth and localization of epiphytic fitness mutants of *Pseudomonas syringae* on leaves. Applied and Environmental Microbiology 60(10): 3790-3798.

Beck-Von Bodman, S.B. and Shaw, P.D. 1987. Conservation of plasmids among plant-pathogenic *Pseudomonas syringae* isolates of diverse origins. Plasmid 17: 240-47.

Becker, J.O., Hedges, R.W. and Messens, E. 1985. Inhibitory effect of pseudobactin on the uptake of iron by higher plants. Applied and Environmental Microbiology 49(5): 1090-1093.

Bender, C. L. and Cooksey, D.A. 1986. Indigenous plasmids in *P. syringae* pv. *tomato*: conjugative transfer and role in copper resistance. Journal of Bacteriology 165: 534-541.

Berg, G. and Ballin, G. 1994. Bacterial antagonists to *Verticillium dahliae* kleb. Journal of Phytopathology 141(1): 99-110.

Bergen, T. 1981. Human- and animal-pathogenic members of the genus *Pseudomonas*. In: Starr, M.P., Stolp, H., Truper, H.G., Balows, A. and Schegel, H.G. (eds). *The Prokaryotes - A Handbook on Habitats, Isolation and Identification of Bacteria*. Springer-Verlag, Berlin.

Berger, T.G., Kaveh, S., Becker, D. and Hoffman, J. 1995. Cutaneous manifestations of *Pseudomonas* infections in AIDS. Journal of the American Academy of Dermatology 32(1): 279-280.

Bestetti, G., Galli, E., Ruzzi, M. Baldicci, G., Zennato, E. and Frontali, L. 1984. Molecular characterization of a plasmid from *Pseudomonas fluorescens* involved in styrene degradation. Plasmid 12: 181-188.

Binnerup, S.J., Jensen, D.F., Thordal, C.H. and Sorensen, J. 1993. Detection of viable, but non-culturable *Pseudomonas fluorescens* DF 57 in soil using a microcolony epifluorescence technique. FEMS Microbiology Ecology 12: 97-105.

Bisbe, J., Gateil, J.M., Puig, J., Mallolas, J., Martinez, J.A., Jimenez de Anta, M.T. and Soriano, E. 1988. *Pseudomonas aeruginosa* bacteremia: Univariate and multivariate analysis of factors influencing the prognosis in 133 episodes. Review of Infectious Diseases 10: 629-635.

Blackburn, J.W., Jain, R.K. and Sayler, G.S. 1987. Molecular microbial ecology of a naphthalene degrading genotype in activated sludge. Environmental Scientific Technology 21: 884-890.

Blair, I.S. and McDowell, D.A. 1995. Detection of extracellular proteinase of *Pseudomonas fragi* by enzyme-linked immunosorbent-assay. Current Microbiology 31(3):180-185.

Bodey, G.P., Bolivar, R., Fainstein, V. and Jajeda, L. 1983. Infections caused by *Pseudomonas aeruginosa*. Review of Infectious Diseases 5: 279-313.

Boelens, J., Woestyne, M.V. and Verstraete, W. 1994. Ecological importance of motilty for the plant growth-promoting rhizo*Pseudomonas* strain ANP15. Soil Biology and Biochemistry 26: 269-277.

Boemare, N. and Vey, A. 1977. Study on bacterial strains isolated from the crayfish *Atlantoastacus pallipes* affected by septicemia and hepato-intestinal diseases. Ann. Hydrobiol. 8: 153-162.

Bohool, B.B. and Schmidt, E.L. 1980. The immunofluorescence approach in microbial ecology. Advances in Microbial Ecology 4: 203-241.

Bottone, E.J. and Perez, A.A. 1993. ***Pseudomonas aeruginosa*** folliculitis acquired through use of a contaminated loofah sponge: an unrecognised potential public health problem. Journal of Clinical Microbiology 31: 480-483.

Bottone, E.J. and A.A. Perez. 1994. Loofah sponges as reservoirs and vehicles in the transmission of potentially pathogenic bacterial speciies to human skin. Journal of Clinical Microbiology 32: 469-472.

Bowers, J.H. and Parke, J.L. 1993. Colonisation of pea (*Pisum sativum* L.) taproots by *Pseudomonas fluorescens*: effect of soil temperature and bacterial motility. Soil Biology and Biochemistry 25: 1693-1701.

Boyle, A.W., Silvin, C.J., Hassett, H.P., Nakas, J.P. and Tanenbaum, S.W. 1993. Bacterial PCB degradation. In: Microorganisms to Combat Pollution (pp. 161-174). Kluwer Academic Publishers, Dordrecht, Netherlands.

Bradbury, J.F. 1986. Guide to Plant Pathogenic Bacteria. C.A.B. International Mycological Institute. Kew, Surrey, UK.

Bramwell, P.A., Barallon, R.V., Rogers, H.J. and Bailey, M.J. 1994. Extraction of DNA from the phylloplane. In: Akkermans, A.D.L., van Elsas, J.D. and de Bruijin, F.J. (eds). *Molecular Microbial Ecology Manual.* Kluwer Academic Publishers, in press.

Brettar, I., Ramos-Gonzalez, M.I., Ramos, J.L. and Hofle, M.G. 1994. Fate of *Pseudomonas putida* after release into lake water mesocosms: different survival mechanisms in response to environmental conditions. Microbial Ecology 27: 99-122.

Brittingham, M.C., Temple, S.A. and Duncan, R.M. 1988. A survey of the prevalence of selected bacteria in wild birds. Journal of Wildlife Diseases 24(2): 299-307.

Brock, T.D., Madigan, M.T., Martinko, J.M. and Parker, J. 1994. *Biology of Microorganisms* (7th Edition). Prentice Hall, New Jersey.

Brodkin, M.A., Simon, M.P., DeSantis, A.M. and Boyer, K.J. 1992. Response of *Rana pipiens* to graded doses of the bacterium *Pseudomonas aeruginosa*. Journal of Herpetology 26(4): 490-495.

Bukanov, N., Ravi, V.N., Miller, D., Srivastava, K. and Berg, D.E. 1994. ***Pseudomonas aeruginosa*** corneal ulcer isolates distinguished using the arbitrarily primed PCR DNA fingerprinting method. Current Eye Research 13: 783-790.

Burr, T.J., Norelli, J.L, Katz, B., Wilcox, W.F. and Hoying, S.A. 1988. Streptomycin resistance of *Pseudomonas syringae* pv. *papulans* in apple orchards and its association with a conjugative plasmid. Phytopathology 78: 410-13.

Busse, J. and Auling, G. 1988. Polyamine pattern as a chemotaxonomic marker within the *Proteobacteria*. Systematic and Applied Microbiology 11: 1-8.

Busse, J., El-Banna, T. and Auling, G. 1989. Evaluation of different approaches for identification of xenobiotic degrading pseudomonads. Applied and Environmental Microbiology 55: 1578-1583.

Butterworth, J. and McCartney, H.A. 1991. The dispersal of bacteria from leaf surfaces by water splash. Journal of Applied Bacteriology 71(6): 484-496.

Butterworth, J. and McCartney, H.A. 1992. Effect of drop size on the removal of *Bacillus subtilis* from foliar surfaces by water splash. Microbial Releases 1: 177-185.

Buysens, S., Poppe, J. and Hofte, M. 1994. Role of siderophores in plant growth stimulation and antagonisms by *Pseudomonas aeruginosa* 7NSK2. In: Ryder, M.H., Stephens, P.M., and Bowen, G.D. (eds). *Improving Plant Productivity with Rhizosphere Bacteria*, pp. 139-141, CSIRO Division of Soils, Glen Osmond, S. Australia.

C.A.B. Abstracts CD-ROM, C.A.B. International. 1972-1995. Wallingford, Oxon, UK.

Cabral, J.P.S. 1995. Viability and respiration activity of *Pseudomonas syringae* cells starved in buffer. Canadian Journal of Microbiology 41(4-5): 372-377.

Carlson, C., Pierson L., Rosen J. and Ingraham J. 1983. *Pseudomonal stutzeri* and related species undergo natural transformation. Journal of Bacteriology 153: 93-99.

Carruthers, F.L., Conner, A.J. and Mahanty, H.K. 1994. Identification of a genetic locus in *Pseudomonas aureofaciens* involved in fungal inhibition. Applied and Environmental Microbiology 60(1): 71-77.

Carruthers, F.L., Shumthomas, T., Conner, A.J. and Mahanty, H.K. 1995. The significance of antibiotic production by *Pseudomonas aureofaciens* Pa147-2 for biological control of *Phytophthora megasperma* root rot of asparagus. Plant and Soil 170(2): 339-344.

Carson, J. and Schmidtke, L.M. 1993. Opportunistic infection by psychrotrophic bacteria of cold-comprised Atlantic salmon. Bulletin of the European Association of Fish Pathologists 13: 49-52.

Casida, L.E. 1977. Small cells in pure cultures of *Agromyces ramosus* and in natural soil. Canadian Journal of Microbiology 23: 214-216.

Castro, C.E. and Belser, N.O. 1990. Biodehalogenation: oxidative and reductive metabolism of 1,1,2-trichloroethane by *Pseudomonas putida* - biogeneration of vinyl chloride. Environmental Toxicology and Chemistry 9: 707-714.

Chakrabarty, A. 1995. Microbial degradation of hazardous chemicals: evolutionary considerations and bioremediative developments. In: *Bioremediation: the Tokyo '94 Workshop* (pp. 71-80). OECD, Paris.

Chamberland, S., L'Ecuyer, J., Lessard, C., Bernier, M., Provencher, P., Bergeron M.G. and the Canadian Study Group. 1992. Antibiotic susceptibility profiles of 941 gram-negative bacteria isolated from septicemic patients throughout Canada. Clinical Infectious Diseases 15: 615-628.

Chanway, C.P. and Holl, F.B. 1992. Influence of soil biota on Douglas-fir (*Pseudotsuga menziesii*) seedling growth: the role of rhizosphere bacteria. Canadian Journal of Botany 70: 1025-1031.

Chanway, C.P. and Holl, F.B. 1993. Ecotypic specificity of spruce emergence-stimulating *Pseudomonas putida*. Forest Science 39: 520-527.

Chapman and Hall, 1995. Dictionary of Natural Products on CD-ROM, Release 4:1. London, UK.

Chatterjee, D.K. and Chakrabarty, A.M. 1983. Genetic homology between independently isolated chlorobenzoate-degradative plasmids. Journal of Bacteriology 153: 532-534.

Chatterjee, A., Kwartz, J., Ridgway, A.E. and Storey, J.K. 1995. Disposable soft contact lens ulcers: a study of 43 cases seen at Manchester Royal Eye Hospital. Cornea 14(2): 138-141.

Cheng, T. 1986. Biological control studies of bacteria associated with moribund *Biomphalaria glabrata* mollusca in the laboratory. Journal of Invertebrate Pathology 47: 219-224.

Cho, J.J., Schroth, M.N., Kominos, S.D. and Green, S.K. 1975. Ornamental plants as carriers of *Pseudomonas aeruginosa.* Phytopathology 65: 425-431.

Christensen, H., Boye, M., Poulsen, L.K. and Rasmussen, O.F. 1994. Analysis of fluorescent pseudomonads based on 23S ribosomal DNA sequences. Applied and Environmental Microbiology 60: 2196-2199.

Comai, L., Surico, G. and Kosuge, T. 1982. Relation of plasmid DNA to indolacetic acid production in different strains of *Pseudomonas syringae* pv. *savasatoni.* Journal of General Microbiology 128: 2157-63.

Compeau, G., Al-Achi, B.J., Plastouka, E. and Levy, S.B. 1988. The survival of rifampicin-resistant mutants of *Pseudomonas putida* and *Pseudomonas fluorescens* in soil systems. Applied and Environmental Microbiology 54: 2432-2438.

Constantinidou, H.A., Hirano, S.S., Baker, L.S. and Upper, C.D. 1990. Atmospheric dispersal of ice-nucleation bacteria: the role of rain. Phytopathology 80: 934-937.

Conway de Macario, E.A., Macario, J.L. and Kandler, O. 1982. Monoclonal antibodies for immunological analysis of methanogenic bacteria. Journal of Immunology 129: 1670-1674.

Cook, R.J., Weller, D.M., Drahos, D.J., Kovacevich, P.A., Hemming, B.C., Barnes, G. and Peirson, E.I. 1991. Establishment, monitoring and termination of field tests with genetically altered bacteria applied to wheat for biological control of take-all. In: Mackenzie, D.R. (ed.), *Biotechnology Field Results*. Academic Press.

Cooksey, D.A. 1990. Genetics of bactericide resistance in plant pathogenic bacteria. Annual Review of Phytopathology 28: 201-219.

Coplin, D.L. 1989. Plasmids and their role in the evolution of plant pathogenic bacteria. Annual Review of Phytopathology 27: 187-212.

Crosse, J.E. 1959. Bacterial canker of stone-fruits. IV. Investigation of a method for measuring the inoculum potential of cherry trees. Annals of Applied Biology 47: 306-317.

Cuppels, D. and Ainsworth, T. 1995. Molecular and physiological characterization of *Pseudomonas syringae* pv. *tomato* and *Pseudomonas syringae* pv. *maculicola* strains that produce the phytotoxin coronatine. Applied and Environmental Microbiology 61(10): 3530-3536.

Cuppels, D.A. and Kelman, A. 1980. Isolation of pectolytic fluorescent pseudomonads from soil and potatoes. Phytopathology 70: 1110-1115.

Curiale, M.S. and Mills, D. 1983. Molecular relatedness among cryptic plasmids in *Pseudomonas syringae* pv. *glycinea.* Phytopathology 73: 1440-43.

Dandurand, L.M., Morra, M.J., Chaverra, M.H. and Orser, C.S. 1994. Survival of *Pseudomonas* spp. in air-dried mineral powders. Soil Biology and Biochemistry 26: 1423-1430.

De Bruijn, F.J. 1992. Use of repetitive (repetitive extragenic palindromic and enterobacterial repetitive intergeneric consensus) sequences and the polymerase chain reaction to fingerprint the genomes of *Rhizobium meliloti* isolates and other soil bacteria. Applied and Environmental Microbiology 58: 2180-2187.

De Groot, A., Heijnen, I., de Cock, H., Filloux, A. and Tommassen, J. 1994. Characterization of type IV pilus genes in plant growth-promoting *Pseudomonas putida* WCS358. Journal of Bacteriology 176: 642-650.

De la Cruz, A.R., Poplawsky, A.R. and Wiese, M.V. 1992. Biological suppression of potato ring rot by fluorescent pseudomonads. Applied and Environmental Microbiology 58(6): 1986-1991.

De Leij, F.A.A.M., Bailey, M.J., Lynch, J.M. and Whipps, J.M. 1993. A simple most probable number technique for the sensitive recovery of a genetically engineered *Pseudomonas aureofaciens* from soil. Letters in Applied Microbiology 16: 307-310.

De Leij, F.A.A.M., Sutton, E.J., Whipps, J.M. and Lynch, J.M. 1994. Effect of genetically modified *Pseudomonas aureofaciens* on indigenous microbial populations of wheat. FEMS Microbiology Ecology 13: 249-258.

De Leij, F.A.A.M., Sutton, E.J., Whipps, J.M., Fenelon, J.S. and Lynch, J.M. 1995. Impact of field release of genetically modified *Pseudomonas fluorescens* on indigenous microbial populations of wheat. Applied and Environmental Microbiology 61: 3443-3453.

Defago, G. and Hass, D. 1990. Pseudomonads as antagonists of soilborne plant pathogens: modes of action and genetic analysis. In: Bollag, J.M. and Stotsky, G. (eds). *Soil Biochemistry* (pp. 249-291), Vol. 6. Marcel Dekker Inc, New York, N.Y.

Defreitas, J.R., Gupta, V.V.S.R. and Germida, J.J. 1993. Influence of *Pseudomonas syringae* R25 and *P. putida* R105 on the growth the N_2 fixation (acetylene-reduction activity) of pea (*Pisum sativum* L) and field bean (*Phaseolus vulgaris* L). Biology and Fertility of Soils 16(3): 215-220.

DeLong, E.F., Wickham, G.S. and Pace, N.R. 1989. Phylogenetic stains: ribosomal RNA-based probes for the detection of single cells. Science 243: 1360-1363.

Denny, T.P. 1988. Phenotypic diversity in *Pseudomonas syringae* pv. *tomato.* Journal of General Microbiology 134: 1939-48.

Diamond, S.S. and Cadwell, G.A. 1979. Fatal bronchopneumonia and dermatitis caused by *Pseudomonas aeruginosa* in Atlantic Bottle-Nosed Dolphin. Journal of the American Veterinary Medical Association 175: 984-987.

Dick, J.D., Shull, V.B., Karp J.E. and Valentine, J. 1988. Bacterial and host factors affecting ***Pseudomonas aeruginosa*** colonization versus bacteremia in granulocytopenic patients. EJCCO 24: 547.

Dominik, R.H., Segebade, I.E. and Taenzer, V. 1995. Risk of microbial contamination of iodinated contrast media on multiple use of large-volume bottles. European Journal of Radiology 19(3): 198-205.

Dorn, A. 1976. *Pseudomonas aeruginosa* causes epidemic disease in the milkweed bug, *Oncopeltus fasciatus* Dalla (Insecta, Heteroptera). Experientia 32(5): 599-601.

Doyle, J.D., Short, K.A., Stotsky, G., King, R.J., Seidler, R.J. and Olsen, R.H. 1991. Ecologically significant effects of *Pseudomonas putida* PPO301 (pRO103), genetically engineered to degrade 2,4-dichlorophenoxyacetate, on microbial populations and processes in soil. Canadian Journal of Microbiology 37: 682-691.

Drahos, D.J. 1992. Methods for the detection, identification and enumeration of microbes. In: Andrews, J.H. and Hirano, S.S. (eds). *Microbial Ecology of Leaves.* Springer-Verlag, New York.

Drahos, D.J., Barry, G.F., Hemming, B.C., Brandt, E.J., Skipper, H.D., Kline, E.L., Klupfel, D.A., Hughes, T.A. and Gooden, D.T. 1988. Pre-release testing procedures: U.S. field test of a *lac*ZY-engineered soil bacterium, pp. 181-191. In: Sussman, M., Collins, C.H., Skinner, F.A. and Stewart-Tull, D.E. (eds). *The Release of Genetically-Engineered Microorganisms*. Academic Press, London.

Draper, C.S., Walker, R.D. and Lawler, H.E. 1981. Patterns of oral bacterial infection in captive snakes. Journal of the American Veterinary Medical Association 179: 1223-1226.

Dropulic, L.K., Leslie, J.M., Eldred, L.J., Zenilman, J. and Sears, C.L. 1995. Clinical manifestations and risk factors of *Pseudomonas aeruginosa* infection in patients with AIDS. Journal of Infectious Diseases 171(4): 930-937.

Drosinos, E.H. and Board, R.G. 1995. Microbial and physicochemical attributes of minced lamb: Sources of contamination with pseudomonads. Food Microbiology 12: 189-197.

Duffy, B.K. and Defago, G. 1995. Fungal inhibition and suppression of root disease using clinical isolates of *Pseudomonas aeruginosa*. Phytopathology 85: 1188.

Duijff, B.J., Baker, P.A.H.M. and Schippers, B. 1994. Suppression of Fusarium wilt of carnation by *Pseudomonas putida* WCS358 at different levels of disease incidence and iron availability. Biocontrol Science and Technology 4: 279-288.

Durbin, R.D. 1992. Role of toxins for plant-pathogenic pseudomonads. In: *Pseudomonas. Molecular Biology and Biotechnology* (pp. 43-55). American Society for Microbiology, Washington, D.C.

Eaton, R.W. and Timmis, K.N. 1986. Characterization of a plasmid-specified pathway for catabolism of isopropylbenzene in *Pseudomonas putida* RE204. Journal of Bacteriology 168: 123-131.

Egusa, S. 1992. *Infectious Diseases of Fish*. A.A. Balkema Publishers, Bookfield, USA.

El Aalam, S., Pauss, A. and Lebeault, J. 1993. High efficiency styrene biodegradation in a biphasic organic/water continuous reactor. Applied Microbiology and Biotechnology 39: 696-699.

El Said, H.M., Gewaily, E.M., Salem, S.H. and Tohamy, M.R. 1982. Biological and chemical control of *Pseudomonas aeruginosa*, the causal organism of blight disease of bean plants in Egypt. Egyptian Journal of Microbiology 17: 65-80.

Emori, T.G. and Gaynes, R.P. 1993. An overview of nosocomial infections, including the role of the microbiology laboratory. Clinical Microbiology Reviews 6: 428-442.

England, L.S., Lee, H. and Trevors, J.T. 1993. Recombinant and wild-type *Pseudomonas aureofaciens* strains in soil: survival, respiratory activity and effects on nodulation of whitebean *Phaseolus vulgaris* L. by *Rhizobium* species. Molecular Ecology 2: 303-313.

Ensley, B.D. 1994. Designing pathways for environmental purposes. Current Opinion in Biotechnology 5: 247-248.

Faegri, A., Torsvik, V.L. and Goksoyr, J. 1977. Bacterial and fungal activities in soil: Separation of bacteria and fungi by a rapid fractionation centrifugation technique. Soil Biology and Biochemistry 9: 105-112.

Fava, F., Gioia, D.D. and Marchetti, L. 1993. Characterization of a pigment produced by *Pseudomonas fluorescens* during 3-chlorobenzoate co-metabolism. Chemosphere 27: 825-835.

Festl, H.W., Ludwig, W. and Schleifer, K.H. 1986. A DNA hybridization probe for the *Pseudomonas fluorescens* group. Applied and Environmental Microbiology 51: 1190-1194.

Fick, R.B., Jr. 1993. *Pseudomonas aeruginosa* - the microbial hyena and its role in disease: An introduction. In: Fica, R.B., Jr. (ed.). *Pseudomonas aeruginosa the opportunist: Pathogenesis and disease* (pp. 1-5). CRC Press, Boca Raton, Fla.

Fisher, A.A. 1994. Folliculitis from the use of a “loofah” cosmetic sponge [news]. Cutis 54: 12-13.

Fisher, M.C., Goldsmith, J.F. and Gilligan, P.H. 1985. Sneakers as a source of *Pseudomonas aeruginosa* in children with osteomyelitis following puncture wounds. Journal of Pediatrics 106: 607-609.

Flaishman, M., Eyal, Z., Voisard, C. and Haas, D. 1990. Suppression of *Septoria tritici* by phenazine or siderophore-deficient mutants of *Pseudomonas*. Current Microbiology 20: 121-124.

Ford, S.F. and Olsen, B. 1988. Methods for detecting genetically engineered microorganisms in the environment. Advances in Microbial Ecology 10: 45-79.

Foreman, N.K., Wang, W.C., Cullen, E.J., Stidham, G.L., Pearson, T.A. and Shenep, J.L. 1991. Endotoxic shock after transfusion of contaminated red blood cells in a child with sickle cell disease. Pediatric Infectious Disease Journal 10: 624-626.

Franzetti, F., Cernuschi, M., Esposito, R. and Moroni, M. 1992. *Pseudomonas* infections in patients with AIDS and AIDS-related complex. Journal of Internal Medicine 231(4): 437-443.

Fredrickson, J.K., Bentjen, S.A., Bolton, H. Jr. and Van voris, P. 1989. Fate of Tn*5* mutants of growth-inhibiting *Pseudomonas* sp. in intact soil-core microcosms. Canadian Journal of Microbiology 35: 867-873.

Freitas, De, J.R. and Germida, J.J. 1990. Plant growth promoting rhizobacteria for winter wheat. Canadian Journal of Microbiology 36: 265-272.

Freitas, De, J.R., Germida, J.J. and De Freitas, J.R. 1991. *Pseudomonas cepacia* and *Pseudomonas putida* as winter wheat inoculants for biocontrol of *Rhizoctonia solani*. Canadian Journal of Microbiology 37: 780-784.

Fujita, M., Ike, M., Hioki, J.I., Kataoka, K. and Takeo, M. 1995. Trichloroethylene degradation by genetically engineered bacteria carrying cloned phenol catabolic genes. Journal of Fermentation and Bioengineering 79: 100-106.

Fukui, R., Schroth, M.N., Hendson, M., Hancock, J.G. and Firestone, M.K. 1994. Growth patterns and metabolic activity of *Pseudomonas* in sugar beet spermospheres: relationship to pericarp colonization by *Pythium ultimum*. Phytopathology 84(11): 1331-1338.

Gallagher, P.G. and Watanakunakorn, C. 1990. *Pseudomonas* bacteremia in a community teaching hospital, 1980-1984. Review of Infectious Diseases 11(6): 846-852.

Galli, E., Barbiere, P. and Bestetti, G. 1992. Potential of pseudomonads in the degradation of methylbenzenes. In: Galli, E., Silver, S. and Witholt, B. (eds). *Pseudomonas Molecular Biology and Biotechnology*. American Society of Microbiology.

Gamliel, A. and Katan, J. 1992. Chemotaxis of fluorescent pseudomonads towards seed exudates and germinating seeds in solarized soil. Phytopathology 82: 328-332.

Gamliel, A. and Katan, J. 1993. Suppression of major and minor pathogens by fluorescent pseudomonads in solarized and nonsolarized soils. Phytopathology 83: 68-75.

Gannon, J.T., Manial, V.B. and Alexander, M. 1991. Relationship between cell surface properties and transport of bacteria through the soil. Applied and Environmental Microbiology 57: 190-193.

Gatti, R. and Nigrelli, D. 1984. Haemorrhagic septicaemia in catfish. Pathogenicity of the strains isolated and reproducibility of the disease. Obbiettivi e Documenti Veterinari 5: 61-63.

Gaudet, D.A., Sands, D.C., Mathre, D.E. and Ditterline, R.L. 1980. The role of bacteria in the root and crown rot complex of irrigated sainfoin in Montana. Phytopathology 70: 161-167.

Genthner, F.J., Ross, S.S., Campbell, R.P. and Fournie, J.W. 1993. Fate and survival of microbial pest control agents in nontarget aquatic organisms. Diseases of Aquatic Organisms 16: 157-162.

Gilardi, G.L. 1991. *Pseudomonas* and related genera. In: Balows, A., Hausler, W.J., Herrmann, K.L., Isenberg, H.D. and Shadomy, H.D. (eds). *Manual of Clinical Microbiology* (fifth edition). American Society for Microbiology, Washington, D.C.

Gilligan, P.H. 1991. Microbiology of airway disease in patients with cystic fibrosis. Clinical Microbiology Reviews 4:35-51.

Glandorf, D.C.M., Peters, L.G.L., van der Sluis, I., Bakker, P.A.H.M. and Schippers, B. 1993. Crop specificity of rhizosphere pseudomonads and the involvement of root agglutinins. Soil Biology and Biochemistry 25: 981-989.

Glandorf, D.C.M., van der Sluis, I., Anderson, A.J., Bakker, P.A.H. and Schippers, B. 1994. Agglutination, adherence and root colonization by fluorescent pseudomonads. Applied and Environmental Microbiology 60: 1726-1733.

Glazebrook, J.S. and Campbell, R.S.F. 1990. A survey of the diseases of marine turtles in northern Australia. I. Farmed turtles. Diseases of Aquatic Organisms 9: 83-95.

Gonzalez, C.F., Layher, S.K., Vidaver, A.K. and Olsen, R.H. 1984. Transfer, mapping and cloning of *Pseudomonas syringae* pv. *syringae* plasmid pcg131 and assessment of its role in virulence. Phytopathology 74: 1245-50.

Gottlieb, T, Funnell, G. and Gosbell, I. 1991. *Pseudomonas fluorescens* pseudobacteraemia. Med. J. Aust. 155: 854-855.

Green, S.K., Schroth, M.N., Cho, J.J., Kominos, S.D. and Vitanza-Jack, V.B. 1974. Agricultural plants and soil as a reservoir for *Pseudomonas aeruginosa*. Applied Microbiology 28(6): 987-991.

Greer, G.G. 1989. Bacteria and meat quality. Canadian Institute of Food Science and Technology Journal 22(2): 116-117.

Grewal, P.S. 1991. Effects of *Caenorhabditis elegans* (Nematoda: Rhabditidae) on the spread of the bacterium *Pseudomonas tolaasii* in mushrooms *(Agaricus bisporus*). Annals of Applied Biology 118(1): 47-55.

Gross, D. 1985. Regulation of syringomycin synthesis in *Pseudomonas syringae* pv. *syringae* and defined conditions for its production. Journal of Applied Bacteriology 58: 167-174.

Gustafson, T.L., Band, L.D., Hutcheson, R.H. and Schaffer, W. 1983. *Pseudomonas folliculitis*: an outbreak and review. Review of Infectious Diseases 5:1-8.

Haefele, D.M. and Lindow, S.E. 1987. Flagella motility confers epiphytic fitness advantages upon *Pseudomonas syringae.* Applied and Environmental Microbiology 53: 2528-2533.

Hafez, H.M., Woernle, H. and Heil, G. 1987. *Pseudomonas aeruginosa* infections in turkey poults and treatment trials with apramycin. Berliner und Munchen Tierarztliche Wochenschrift 100(2): 48-51.

Hamdy, A.H.A., Farag, Y.A. and Micheal, F.F. 1974. *Pseudomonas aeruginosa* infection as a possible cause of infertility in Friesan bulls. Journal of the Egyptian Veterinary Medical Association 34: 612-622.

Handley, B.A. and Webster, A.J.F. 1993. Some factors affecting airborne survival of *Pseudomonas fluorescens* indoors. Journal of Applied Bacteriology 75: 35-42.

Haniotakis, G. and Avtizis, N. 1977. Mortality in *Dacus oleae* through infection with *Pseudomonas putida*. Ann. Zool. Ecol. Animal 9: 299-312.

Harrison, L.A., Letendre, L., Kovacevich, P., Pierson, E. and Weller, D. 1993. Purification of an antibiotic effective against *Gaeumannomyces graminis* var. *tritici* produced by a biocontrol agent, *Pseudomonas aureofaciens*. Soil Biology and Biochemistry 25(2): 215-221.

Hartel, P.G., Fuhrman, J.J., Johnson, W.F. and Lawrence, E.G. 1994. Survival of *lac* ZY-containing *Pseudomonas putida* strain under stressful abiotic soil conditions. Soil Science Society of America Journal 58: 770-776.

Hatai, K. and Willoughby, L.G. 1988. *Saprolegnia parasitica* from rainbow trout inhibited by the bacterium *Pseudomonas fluorescens*. Bulletin of the European Association of Fish Pathologists 8: 27-29.

Hatai, K., Egusa, S., Nakajima, M., and Chikahata, H. 1975. *Pseudomonas chlororaphis* as a fish pathogen. Bulletin of the Japanese Society of Scientific Fisheries 41(11): 1203.

Heijnen, C.E., Koh-A-Hin, C.H. and van Elsas, J.D. 1993. Root colonization by *Pseudomonas fluorescens* introduced into soil amended with bentonite. Soil Biology and Biochemistry 25: 239-246.

Hepperly, P.R. and Ramos-Davila, E. 1986. Bacterial basal rot of straw mushrooms. Journal of Agriculture of the University of Puerto Rico 70: 219-221.

Herald, P.J. and Zottola, E.A. 1989. Effect of various agents upon the attachment of *Pseudomonas fragi* to stainless steel. Journal of Food Science 54(2): 461-464.

Herron, P.R. and Wellington, E.M.H. 1990. New method for extraction of streptomycete spores from soil and application to the study of lysogeny in sterile amended and non sterile soil. Applied and Environmental Microbiology 56: 1406-1412.

Hickey, W.J. and Focht, D.D. 1992. Degradation of mono-, di-, and trihalogenated benzoic acids by *Pseudomonas aeruginosa* JB2. Applied and Environmental Microbiology 56: 3842-3850.

Hildebrand, D.C., Palleroni, N.J., Hendson, M., Toth, J. and Johnson, J.L. 1994. *Pseudomonas flavescens* sp. nov., isolated from walnut blight cankers. International Journal of Systematic Bacteriology 44: 410-415.

Hildebrand, P.D. 1989. Surfactant-like characteristics and identity of bacteria associated with broccoli head rot in Atlantic Canada. Canadian Journal of Plant Pathology 11: 205-214.

Hildebrand, P.D. 1994. Head rot. In: Howard, R.J., Garland, J.A. and Seaman, W.L. (eds). *Diseases and Pests of Vegetable Crops in Canada* (pp. 94-95). The Canadian Phytopathological Society and Entomological Society of Canada. Ottawa, Canada.

Hirano, S.S. and Upper, C.D. 1983. Ecology and epidemiology of foliar bacterial plant pathogens. Annual Review of Phytopathology 21:243-269.

Hirano, S.S. and Upper, C.D. 1990. Population biology and epidemiology of *Pseudomonas syringae*. Annual Review of Phytopathology 28: 155-177.

Hirano, S.S. and Upper, C.D. 1992. Population dynamics of *Pseudomonas syringae* in the phyllosphere. In: Galli, E., Silver, S. and Witholt, B. (eds). *Pseudomonas*. *Molecular Biology and Biotechnology*. American Society for Microbiology, Washington, DC.

Hirano, S.S. and Upper, C.D. 1993. Dynamics, spread, and persistence of a single genotype of *Pseudomonas syringae* relative to those of its conspecifics on populations of snap bean leaflets. Applied and Environmental Microbiology 59(4): 1082-1091.

Hiraishi, A., Morishima, Y. and Takeuchi, J.-I. 1991. Numerical analysis of lipoquinone patterns in monitoring bacterial community dynamics in wastewater treatment systems. Journal of General and Applied Microbiology 37: 57-70.

Holben, W.E., Jansson, J.K., Chelm, B.K. and Tiedje, J.M. 1988. DNA probe method for the detection of specific microorganisms in the soil bacterial community. Applied and Environmental Microbiology 54: 703-711.

Holder, I.A. 1993. *Pseudomonas aeruginosa* virulence-associated factors and their role in burn wound infections. In: Fica, R.B. (ed.). *Pseudomonas aeruginosa the opportunist. Pathogenesis and disease* (pp. 235-245). CRC Press: Boca Raton.

Holland, S.P., Pulido, J.S., Shires, T.K. and Costerton, J.W. 1993. *Pseudomonas aeruginosa* ocular infections. In: Fica, R.B. (ed.). *Pseudomonas aeruginosa the opportunist. Pathogenesis and disease* (pp. 159-176). CRC Press: Boca Raton.

Holloway, B.W. and Morgan, A.F. 1986. Genome organisation in *Pseudomonas.* Annual Review of Microbiology 40: 79-105.

Holloway, B.W., Dharmsthiti, S., Johnson, C., Kearney, A., Krishnapillai, V., Morgan, A.F., Ratnaningsih, E., Saffrey, R., Sinclair, M., Strom, D. and Zhang, C. 1990a. Chromosome organisation in *Pseudomonas aeruginosa* and *Pseudomonas putida.* In: Silver, S., Chakabarty, A.M., Iglewski, B. and Kaplan S. *Pseudomonas*: *Biotransformation, Pathogenesis and Evolving Biotechnology*. American Society for Microbiology, Washington, D.C.

Holloway, B.W., Dharmsthiti, S., Krishnapillai, V., Morgan, A., Obeyesekere, V., Ratnaningsih, E., Sinclair, M., Strom, C. and Zhang, C. 1990b. Patterns of gene linkages in *Pseudomonas* species. In: *The Bacterial Chromosome (*pp.97-105). American Society for Microbiology, Washington, D.C.

Holloway, B.W., Romling, U. and Tummler, B. 1994. Genomic mapping of *Pseudomonas aeruginosa PAO*. Microbiology 140: 2907-2929.

Honich, M. 1972. Outbreak of *Pseudomonas aeruginosa* infection among pheasant chicks. Magyar Allatorvosok Lapja 27(6): 329-335.

Hood, S.K. and Zottola, E.A. 1995. Biofilms in food-processing. Food Control 6(1): 9-18.

Hopkins, D.W., Macnaughton, S.J. and O'Donnell, A.G. 1991. A dispersion and differential centrifugation technique for representatively sampling microorganisms from soil. Soil Biology and Biochemistry 23: 227-232.

Horohov, D.W. and Dunn, P.E. 1984. Role of hemocytotoxins in the pathogenicity of *Pseudomonas aeruginosa* in larvae of the tobacco hornworm, *Manduca sexta*. Journal of Invertebrate Pathology 43: 297-298.

Howard, R.J., Garland, J.A. and Seaman, W.L. 1994. *Diseases and Pests of Vegetable Crops in Canada: An Illustrated Compendium*. The Canadian Phytopathological Society and The Entomological Society of Canada. Ottawa, Canada.

Hungerford, T.G. 1990. *Diseases of Livestock* (9th Edition). McGraw-Hill Book Co., Toronto.

Hutchison, M.L. and Johnstone, K. 1993. Evidence for the involvement of the surface active properties of the extracellular toxin tolaasin in the manifestation of brown blotch disease symptoms by *Pseudomonas tolaasii* on *Agaricus bisporus*. Physiological and Molecular Plant Pathology 42(5): 373-384.

Hwang, S.F., Howard, R.J. and Goatcher, L. 1989. Bacteria associated with crown and root rot of sainfoin in southern Alberta. Canadian Plant Disease Survey 69: 5-8.

Iglewski, B.H., Rust, L. and Bever, R.A. 1990. Molecular analysis of *Pseudomonas aeruginosa* elastase. In: Silver, S., Chakabarty, A.M., Iglewski, B. and Kaplan, S. (eds). *Pseudomonas: Biotransformation, Pathogenesis and Evolving Biotechnology.* American Society for Microbiology, Washington, D.C.

Imayasu, M., Petroll, W.M., Jester, J.V., Patel, S.K., Ohashi, J. and Cavanagh, J.D. 1994. The relation between contact lens oxygen transmissibility and binding of *Pseudomonas aeruginosa* to the cornea after overnight wear. Ophthalmology 101: 371-388.

Ishihara, S., Takino, M., Okada, Y. and Mimura, K. 1995. Septic shock due to *Pseudomonas aeruginosa* in a previously healthy woman. Intensive Care Medicine 21(3): 226-228.

Iwasaki, K., Uchiyama, H. and Yagi, O. 1993. Survival and impact of genetically engineered *Pseudomonas putida* harboring mercury resistance gene in aquatic microcosms. Biosci. Biotech. Biochem. 57: 1264-1269.

Iwasaki, K., Uchiyama, H. and Yagi, O. 1994. Survival and impact of genetically engineered *Pseudomonas putida* harboring mercury resistance gene in soil microcosms. Biosci. Biotech. Biochem. 58: 1264-1269.

Jacobson, E., Gaskin, J.M., Page, D., Iverson, W.O. and Johnson, J.W. 1981. Illness associated with paramyxo-like virus infection in a zoologic collection of snakes. Journal of the American Veterinary Medical Association 179(11): 1227-1230.

Jacoby, G.A. 1975. Properties of R plasmids in *Pseudomonas aeruginosa*. In: Schlessinger, D. (ed.). Microbiology 1974. American Society for Microbiology, Washington, D.C.

Jacques, M.A., Kinkel, L.L. and Morris, C.E. 1995. Population sizes, immigration, and growth of epiphytic bacteria on leaves of different ages and positions of field-grown endive (*Cichorium endivia* var latifolia). Applied and Environmental Microbiology 61(3): 899-906.

Jain, D.K., Lee, H. and Trevors, J.T. 1992. Effect of addition of *Pseudomonas aeruginosa* Ug2 inocula or biosurfactants on biodegradation of selected hydrocarbons in soil. Journal of Industrial Microbiology 10(2): 87-93.

Jain, N., Srivastava, S.K. and Srivastava, A.K. 1993. Optimization of culture parameters for degradation of black liquor by *Pseudomonas putida*. Fresenius Environmental Bulletin 2: 705-710.

Jain, R.K., Burlage, R.S. and Sayler, G.S. 1988. Methods for detecting recombinant DNA in the environment. CRC Critical Reviews in Biotechnology 8: 33-84.

James, D.W., Jr., Suslow, T.V. and Steinback, K.E. 1985. Relationship between rapid, firm adhesion and long-term colonization of roots by bacteria. Applied and Environmental Microbiology 50: 392-397.

James, R.R. and Lighthart, B. 1992. The effect of temperature, diet, and larval instar on the susceptibility of an aphid predator, *Hippodamia convergens* (Coleoptera: Coccinellidae), to the weak bacterial pathogen *Pseudomonas fluorescens*. Journal of Invertebrate Pathology 60: 215-218.

Janiyani, K.L., Wate, S.R. and Joshi, S.R. 1993. Morphological and biochemical characteristics of bacterial isolates degrading crude oil. Journal of Environmental Science and Health - Part A - Environmental Science and Engineering 28: 1185-1204.

Janse, J.D., Derks, J.H.J., Spit, B.E. and van der Tuin, W.R. 1992. Classification of fluorescent soft rot *Pseudomonas* bacteria, including *P. marginalis* strains, using whole cell fatty acid analysis. Systematic and Applied Microbiology 15: 538-555.

Jarvis, J.G. and Skipper, J. 1994. *Pseudomonas* osteochondritis complicating puncture wounds in children. J. Pediatr. Orthop. 14(6): 755-759.

Jarvis, W.R. and Martone, W.J. 1992. Predominant pathogens in hospital infections. Journal of Antimicrobial Chemotherapy 29(suppl. A): 19-24.

Jay, J.M. 1992. *Modern Food Microbiology* (4th Edition). Chapman and Hall, New York.

Jensen, L.B., Ramos, J.L., Kaneva, Z. and Molin, S. 1993. A substrate-dependent biological containment system for *Pseudomonas putida* based on the *Escherichia coli* gef gene. Applied and Environmental Microbiology 59: 3713-3717.

Johnson J.L. and Palleroni, N.J. 1989. Deoxyribonucleic acid similarities among *Pseudomonas* species. International Journal of Systematic Bacteriology 39: 230-235.

Johnson, K.B., Stockwell, V.O, Burgett, D.M., Sugar, D. and Loper, J.E. 1993. Dispersal of *Erwinia amylovora* and *Pseudomonas fluorescens* by honey bees from hives to apple and pear blossoms. Phytopathology 83: 478-484.

Jones, R.A., Broder, M.W. and Stotsky, G. 1991. Effects of genetically engineered microorganisms on nitrogen transformations and nitrogen-transforming microbial populations in soil. Applied and Environmental Microbiology 57: 3212-3219.

Jones, R.N., Aldridge, K.E., Allen, S.D., Barry, A.L., Fuchs, P.C., Gerlach, E.H. and Pfaller, M.A. 1989. Multicenter *in vitro* evaluation of Sm-7338, a new carbapenem. Antimicrob. Agents Chemother. 33: 562-565.

Kenna, M.A. 1994. Treatment of chronic suppurative otitis media. Otolaryngol. Clin. North Am. 27: 457-472.

Khalid, A.M. and Aleem, M.I.H. 1991. Application of microorganisms in coal cleaning process. In: Proceedings of an International Symposium on Biotechnology for Energy, Dec. 16-21, 1989 (pp.379-403). Faisalabad, Pakistan.

Khanna, M. and Stotzky, G. 1992. Transformation of *Bacillus subtilis* by DNA bound on montmorillonite and effect of DNase on the transforming ability of bound DNA. Applied and Environmental Microbiology 58: 1930-1939.

Khanna, P. and Olivier, J. M. 1989. Rapid screening of antagonists of blotch bacterium, *Pseudomonas tolaasii.* Indian Phytopathology 42(1): 139-141.

Kidambi, S.P., Ripp, S. and Miller, R.V. 1994. Evidence for phage-mediated gene transfer among *Pseudomonas aeruginosa* strains on the phylloplane. Applied and Environmental Microbiology 60: 496-500.

Kimura, T., Yoshimizu, M., Ezura, Y. and Kamei, Y. 1990. An antiviral agent (46NW-04A) produced by *Pseudomonas* sp. and its activity against fish viruses. Journal of Aquatic Animal Health 2: 12-20.

King, E.O., Ward, M.K. and Raney, D.E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. Journal of Laboratory and Clinical Medicine 44: 301-307.

King, J., Digrazia, P., Applegate, B., Burtage, R., Sanseverino, J., Dunbar, P., Larimer, F. and Sayler, G. 1990. Rapid, sensitive bioluminescent reporter technology for naphthalene exposure and biodegradation. Science 249: 778-780.

Kinkel, L.L. and Lindow, S.E. 1993. Invasion and exclusion among coexisting *Pseudomonas syringae* strains on leaves. Applied and Environmental Microbiology 59(10): 3447-3454.

Kiyohara, H., Torigoe, S., Kaida, N., Asaki, T., Iida, T., Hayashi, H. and Takizawa, N. 1994. Cloning and characterization of a chromosomal gene cluster, pah, that encodes the upper pathway for phenanthrene and naphthalene utilization by *Pseudomonas putida* OUS82. Journal of Bacteriology 176: 2439-2443.

Kloepper, J.W., Hume, D.J., Scher, F.M., Singleton, C., Tipping, B., Laliberte, M., Frauley, K., Kutchaw, T., Simonson, C., Lifshitz, R., Zaleska, I. and Lee, L. 1988. Plant growth promoting rhizobacteria on canola (rapeseed). Plant Disease 72: 42-46.

Kluepfel, D.A. and Tonkyn, D.W. 1991a. Release of soil-borne genetically modified bacteria: biosafety implications from contained experiments. McKenzie, D.R. and Henry, S.C. (eds). *Proceedings of the International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms*, Kiawah Island, South Carolina, November 27, 1990 (pp. 55-65). Bethesda, Maryland: Agricultural Research Institute.

Kluepfel, D.A., Kline, E.L., Hughes, T., Skipper, H., Gooden, D., Drahos, D.J., Barry, G.F., Hemming, B.C. and Brandt, E.J. 1991b. Field testing of a genetically engineered rhizosphere inhabiting pseudomonad: development of a model system. In: McKenzie, D.R. and Henry, S.C. (eds). *Proceedings of the International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms,* Kiawah Island, South Carolina, November 27, 1990 (pp. 189-199). Bethesda, Maryland: Agricultural Research Institute.

Kluepfel, D.A., Kline, E.L., Skipper, H.D., Hughes, T.A., Gooden, D.T., Drahos, D.J., Barry, G.F., Hemming, B.C. and Brandt, E.J. 1991c. The release and tracking of genetically engineered bacteria in the environment. Phytopathology 81(3) 348-352.

Knudsen, G.R. 1989. Model to predict aerial dispersal of bacteria during environmental release. Applied and Environmental Microbiology 55: 2641-2647.

Korfhagen, T.R., Sutton, L. and Jacoby, G.A. 1978. Classification and physical properties of *Pseudomonas* plasmids. In: Sclessinger, D. (ed.). Microbiology 1978, pp. 221-224. American Society for Microbiology, Washington, D.C.

Kropec, A., Lemmen, S., Wursthorn, M. and Daschner, F.D. 1994. Combination effect of meropenem with aminoglycosides and teicoplannin on *Pseudomonas* and enterococci. Infection 22(4): 306-308.

Kudoh, I., Wiener-Kronish, J.P., Hashimoto, S., Pittet, J.F. and Frank, D. 1994. Exoproduct secretions of *Pseudomonas aeruginosa* strains influence severity of alveolar epithelial injury. American Journal of Physiology 267: 551-556.

Kunz, D., Nagappan, O., Silva-Avalos, J. and DeLong, G. 1992. Utilization f-cyanide as a nitrogenous substrate by *Pseudomonas fluorescens NCIMB11764*: evidence for multiple pathways of metabolic conversion. Applied and Environmental Microbiology 58: 2022-2029.

Kusuda, R. and Toyoshima, T. 1976. Properties of pathogenic *Pseudomonas* isolated from cultivated yellowtail. Fish Pathology 11: 133-139.

Kusuda, R., Toyoshima, T. and Nishioka, J. 1974. Properties of pathogenic *Pseudomonas* isolated from cultivated *Evynnis japonica*. Fish Pathology 9: 71-78.

Ladds, P.W., Thomas, A.D., Speare, R. and Brown, A.S. 1990. Melioidosis in a koala. Australian Veterinary Journal 67: 304-305.

Lajoie, C.A., Layton, A.C. and Sayler, G.S. 1994. Cometabolic oxidation of polychlorinated biphenyls in soil with a surfactant-based field application vector. Applied and Environmental Microbiology 60: 2826-2833.

Lambert, B., Joos, H., Dierickx, S., Vantomme, R., Swings, J., Kersters, K. and van Montagu, M. 1990. The identification and plant interaction of a *Phyllobacterium* sp. a predominant rhizobacterium of young sugar beet plants. Applied and Environmental Microbiology 56: 1093-1102.

Lavery, L.A., Harkless, L.B., Felder-Johnson, K. and Mundine, S. 1994. Bacterial pathogens in infected puncture wounds in adults with diabetes. Journal of Foot and Ankle Surgery 33: 91-97.

Lebeda, A., Kudela, V. and Jedlickova, Z. 1984. Pathogenicity of *Pseudomonas aeruginosa* for plants and animals. Acta Phytopathologica Academiae Scientiarum Hungaricae 19: 271-284.

Lee, Y.H., Shim, G.Y., Lee, E.J. and Mew, T.W. 1990. Evaluation of biocontrol activity of fluorescent pseudomonads against some rice fungal diseases in vitro and greenhouse. Korean Journal of Plant Pathology 6(1): 73-80.

Lee, J-Y., Jung, K-H., Choi, S.H. and Kim, H-S. 1995. Combination of the *tod* and *tol* pathways in redesigning a metabolic route of *Pseudomonas putida* for the mineralization of a benzene, toluene, and *p*-xylene mixture. Applied and Environmental Microbiology 61: 2211-2217.

Legard, D.E., McQuilken, M.P., Whipps, J.M., Fenlon, J.S., Fermor, T.R., Thompson, I.P., Bailey, M.J. and Lynch, J.M. 1994. Studies of seasonal changes in the microbial populations on the phyllosphere of spring wheat as a prelude to the release of a genetically modified microorganism. Agriculture, Ecosystems and Environment 50(2): 87-101.

Legent, F., Bordure, P., Beauvillain, C. and Berche, P. 1994. Controlled prospective study of oral ciprofloxacin versus amyoxicillin/clavulanic acid in chronic suppurative otitis media in adults. Chemotherapy 40 (suppl 1): 16-23.

Lejeune, P. and Mergeay, M. 1980. R plasmid-mediated chromosome mobilization in *Pseudomonas fluorescens* G2. Arch. Physiol. Biochim. 80: 289-290.

Leser, T. 1995. Journal of Microbiological Methods 22: 249-262.

Lessie, T.G, Wood, M.S., Bryne, A. and Ferrante, A. 1990. Transposable gene-activating elements in *Pseudomonas cepacia.* In: Silver, S., Chakabarty, A.M., Iglewski, B. and Kaplan, S. (eds). *Pseudomonas: Biotransformation, Pathogenesis and Evolving Biotechnology.* (pp. 279-291). American Society for Microbiology, Washington, D.C.

Li, M.F. and Flemming, C. 1967. A proteolytic pseudomonad from skin lesions of rainbow trout (*Salmo gairdneri*). I. Characteristics of the pathogenic effects and the extracellular proteinase. Canadian Journal of Microbiology 13: 405-416.

Liao, C.H. 1989. Antagonism of *Pseudomonas putida* strain PP22 to phytopathogenic bacteria and its potential use as a biocontrol agent. Plant disease 73: 223-226.

Liao, C.H. 1991. Cloning of pectate lysase gene pel from *Pseudomonas fluorescens* and detection of sequences homologous to pel in *Pseudomonas viridiflava* and *Pseudomonas putida*. Journal of Bacteriology 173: 4386-4393.

Lilley, A.K., Fry, J.C., Day, M.J. and Bailey, M.J. 1994. *In situ* transfer of an exogenously isolated plasmid between *Pseudomonas* spp. in sugar beet rhizosphere. Microbiology 140: 27-33.

Lin, M.Y., Cheng, M.C., Huang, K.J. and Tsai, W.C. 1993. Classification, pathogenicity, and drug susceptibility of hemolytic gram-negative bacteria isolated from sick or dead chickens. Avian Diseases 37: 6-9.

Lindemann, J. and Upper, C.D. 1985. Aerial dispersal of epiphytic bacteria over bean plants. Applied and Environmental Microbiology 50: 1229-1232.

Lindemann, J., Constantinidou, H.A., Barchet, W.R. and Upper, C.D. 1982. Plants as sources of airborne bacteria including ice nucleation-active bacteria. Applied and Environmental Microbiology 44: 1059-1063.

Lindow, S.E. 1983. The role of bacterial ice nucleation in frost injury to plants. Annual Review of Phytopathology 21: 363-384.

Lindow, S.E. 1992. Ice- strains of *Pseudomonas syringae* introduced to control ice nucleation active strains on potato. In: Tjamos, E.S., Papavizas, G.C. and Cook, R.J. (eds). *Biological Control of Plant Diseases*. Plenum Press, New York.

Lindow, S.E. and Panopoulos, N.J. 1988. Field tests of recombinant Ice- *Pseudomonas syringae* for biological frost control in potato. In: Sussman, M., Colins, C.H. and Skinner, F.A. (eds). *Proceedings for the First International Conference on Release of Genetically Engineered Microorganisms.* Academic Press, London.

Lindow, S.E., Knudsen, G.R., Seidler, R.J., Walter, M.V., Lambou, V.W., Amy, P.S., Schmedding, D., Prince, V. and Hern, S. 1988. Aerial dispersal and epiphytic survival of *Pseudomonas syringae* during a pretest for the release of genetically engineered strains into the environment. Applied and Enviornmental Microbiology 54(6): 1557-1563.

Lorenz, M.G. and Wackernagel, W. 1991. High frequency of natural genetic transformation of *Pseudomonas stutzeri* in soil extract supplemented with a carbon/energy and phosphorous source. Applied and Environmental Microbiology 57: 1246-1251.

Lorenz, M.G., Aardema, B.W. and Wackernagel, W. 1988. Highly efficient genetic transformation of *Bacillus subtilis* attached to sand grains. Journal of General Microbiology 134: 107-112.

Lucht, R.F., Fresard, A., Berthelot, P., Farizon, F., Aubert, Dorche, G. and Bousquet, G. 1994. Prolonged treatment of chronic *Pseudomonas aeruginosa* osteomyelitis with a combination of two effective antibiotics. Infection 22: 276-280.

Lynch, J.M. 1990. Longevity of bacteria: considerations in environmental release. Current Microbiology 20: 387-389.

Lysenko, O. 1974. Bacterial exoenzymes toxic for insects: proteinase and lecithinase. Journal of Hygiene, Epidemiology, Microbiology and Immunology 18(3): 347-352.

MacDonald, R.M. 1986. Sampling soil microfloras: dispersion of soil by ion exchange and extraction of specific microorganisms from suspension by elutriation. Soil Biology and Biochemistry 18: 399-406.

MacFarlane, L., Oppenheim, B.A. and Lorrigan, P. 1991. Septicaemia and septic arthritis due to *Pseudomonas putida* in a neutropenic patient. Journal of Infection 23(3): 346-347.

Macnaughton, S.J., Rose, D.A. and O'Donnell, A.G. 1992. Growth and survival of genetically engineered *Pseudomonas putida* in soils of varying texture. Journal of General Microbiology 138: 667-673.

Madhavan, T., Fisher, E.J., Cox, F. and Quinn, E.J. 1973. *Pseudomonas putida* and septic arthritis. Annals of Internal Medicine 78: 971-972.

Madsen, E.L. and Alexander, M. 1982. Transport of *Rhizobium* and *Pseudomonas* through soil. Soil Science Society of America Journal 46: 557-560.

Maharaj, R., Zielinski, N.A. and Chakrabarty, A.M. 1992. Environmental regulation of alginate gene expression in *Pseudomonas aeruginosa*. In: *Pseudomonas.* Molecular Biology and Biotechnology (pp. 105-115). American Society for Microbiology, Washington, D.C.

Mallolas, J.H., Gatell, J.M., Miro, J.M., Marco, F. and Soriano, E. 1990. Epidemiologic characteristics and factors influencing the outcome of ***Pseudomonas aeruginosa*** bacteremia. Reviews of Infectious Disease 12: 718-719.

Mancini, P., Fertels, S., Nave, D. and Gealt, M. 1987. Mobilization of plasmid pHSV106 from *Escherichia coli* HB101 in a laboratory-scale waste treatment facility. Applied and Environmental Microbiology 53: 665-671.

Manohar, L., Sheony, M. G., Reddy, T. K. K. and Chadramohan, K. 1976. A new bacterial fish pathogen causing skin disease in catfish *Clarias batrachus* Linn. (*Pseudomonas aeruginosa*). Current Research, University of Agricultural Sciences, Bangalore, India 5(5): 76-77.

Mathre, D. E., Callan, N. W., Johnston, R. H., Miller, J. B. and Schwend, A. 1994. Factors influencing the control of *Pythium ultimum*-induced seed decay by seed treatment with *Pseudomonas aureofaciens* AB254. Crop Protection 13(4): 301-307.

Mazzola, M., Cook, R.J., Thomashow, L.S., Weller, D.M. and Pierson, L.S. 1992. Contribution of phenazine antibiotic biosynthesis to the ecological competence of fluorescent pseudomonads in soil habitats. Applied and Environmental Microbiology 58(8): 2616-2624.

McCartney, H.A. and Butterworth, J. 1992. Effects of humidity on the dispersal of *Pseudomonas syringae* from leaves by water splash. Microbial Releases 1: 187-190.

McCubbin, M. and Fick, R.B. 1993. Pathogenesis of *Pseudomonas* lung disease in cystic fibrosis. In: Fica, R.B. (ed.) *Pseudomonas aeruginosa the opportunist. Pathogenesis and disease* (pp. 189-211). CRC Press: Boca Raton.

Membre, J.M. and Burlot, P.M. 1994. Effects of temperature, pH, and NaCl on growth and pectinolytic activity of *Pseudomonas marginalis*. Applied and Environmental Microbiology 60: 2017-2022.

Mergeay, M., Lejeune, P., Dadouk, A., Gerits, A.J. and Fabry, L. 1987. Shuttle transfer (or retrotransfer) of chromosomal markers mediated by plasmid pULB113. Mol. Gen. Genet. 209: 61-70.

Miake, S., Nomoto, K., Yokokura, T., Yoshikai, Y., Mutai, M., and Nomoto, K. 1985. Protective effect of *Lactobacillus casei* on *Pseudomonas aeruginosa* infection in mice. Infection and immunity 48(2): 480-485.

Miller, H.J., Liljeroth, E., Henken, G. and van Veen, J.A. 1990b. Fluctuations in the fluorescent pseudomonad and actinomycete populations of rhizosphere and rhizoplane during the growth of spring wheat. Canadian Journal of Microbiology 36: 254-258.

Miller, R.V., Kokjohn, T.A. and Sayler, G.S. 1990a. Environmental and molecular characterization of systems which affect genome alteration in *Pseudomonas aeruginosa.* In*: Pseudomonas:* transformations, pathogenesis and evolving biotechnology. American Society for Microbiology, Washington., D.C.

Milus, E.A. and Rothrock, C.S. 1993. Rhizosphere colonization of wheat by selected soil bacteria over diverse environments. Canadian Journal of Microbiology 39: 335-341.

Miyashita, T. 1984. *Pseudomonas fluorescens* and *Edwardsiella tarda* isolated from diseased tilapia. Fish Pathology 19: 45-50.

Miyazaki, T., Kubota, S.S. and Miyashita, T. 1984. A histopathological study of *Pseudomonas fluorescens* infection in tilapia. Fish Pathology 19: 161-166.

Moayyedi, P., Lynch, D. and Axon, A. 1994. *Pseudomonas* and endoscopy. Endoscopy 26(6): 554-558.

Molin, G. and Ternström, A. 1986. Phenotypically based taxonomy of psychrotrophic *Pseudomonas* isolated from spoiled meat, water and soil. International Journal of Systematic Bacteriology 36: 257-274.

Molin, S., Boe, L., Jensen, L.B., Kristensen, C.S., Givskov, M., Ramos, J.L. and Bej, A.K. 1993. Suicidal genetic elements and their use in biological containment of bacteria. Annual Review of Microbiology 47: 139-166.

Morgan, J.A.W., Winstanley, C., Pickup, R.W. and Sanders, J.R. 1991. The rapid immunocapture of *Pseudomonas putida* cells from lake water using bacterial flagella. Applied and Environmental Microbiology 57: 503-509.

Morgan, J.A.W., Winstanley, C., Pickup, R.W., Jones, J.G. and Saunders, J.R. 1989. Direct phenotypic and genotypic detection of a recombinant pseudomonad population in lake water. Applied and Environmental Microbiology 55: 2537-2544.

Morrison, W.D., Miller, R.V. and Sayler, G.S. 1979. Frequency of F116-mediated transduction of *Pseudomonas aeruginosa* in a fresh water environment. Applied and Environmental Microbiology 36: 724-730.

Moulton, P.J., Vivian, A., Hunter, P.J. and Taylor, J.D. 1993. Changes in cultivar-specificity toward pea can result from transfer of plasmid RP4 and other incompatibility group P1 replicons to *Pseudomonas syringae* pv. *pisi.* Journal of General Microbiology 139: 3149-3155.

Munjal, A., Khanna, P.K. and Garcha, H. S. 1989. *In vitro* chemical and biological control of bacterial blotch of *Agaricus bisporus* (Sing.) In: Lange, K. Grabbe, and Hilber, O. (eds). *Proceedings of the Twelfth International Congress on the Science and Cultivation of Edible Fungi*, Braunschweig, Germany, September 1987 (pp. 667-677). Mushroom Science XII, Part II. Braunschweig, Germany: International Society for Mushroom Science.

Murayama, A. and Saburo, T. 1970. Fragin, a new biologically active metabolite of *Pseudomonas*. Part III. Synthesis. Agricultural and Biological Chemistry 34: 122-130.

Murty, M.G., Srinivas, G. and Sekar, V. 1994. Production of a mosquitocidal exotoxin by a *Pseudomonas fluorescens* strain. Journal of Invertebrate Pathology 64: 68-70.

Muyzer, G., De Waal, E.C. and Uitterlinden, A.G. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied and Environmental Microbiology 59: 695-700.

Nair, N.G. and Fahy, P.C. 1972. Bacteria antagonistic to *Pseudomonas tolaasii* and their control of brown blotch of the cultivated mushroom *Agaricus bisporus*. Journal of Applied Bacteriology 35(3): 439-442.

Nashif, S.A. and Nelson F.E. 1953. The lipase of *Pseudomonas fragi*. II. Factors affecting lipase production. Journal of Dairy Science 36: 471-480.

Nemecek-Marshall, M., Laduca, R. and Fall, R. 1993. High-level expression of ice nuclei in a *Pseudomonas syringae* strain is induced by nutrient limitation and low-temperature. Journal of Bacteriology 175(13): 4062-4070.

Nensey, Y.M., Malhotra, S.K., Schubert, T.T. and Zonca, M.S. 1990. *Pseudomonas aeruginosa* bacteremia associated with sphincter of Oddi manometry. American Journal of Gastroenterology 85(4): 478-479.

Neu, H.C. 1985. Ecology, clinical significance and antimicrobial susceptibility of *Pseudomonas aeruginosa*. In: Gilardi, G.L. (ed.). *Nonfermentative gram-negative rods. Laboratory identification and clinical aspects* (pp. 117-158). Marcel Dekker Inc., New York.

Newbound, G.C., Speare, D.J., Hammell, K.L., Kent, M.L., Ostland, V.E. and Traxler, G.S. 1993. Chehalis River disease: a unique gill disease of salmonids. Canadian Journal of Fisheries and Aquatic Sciences 50: 1092-1100.

Nicas, T.I. and Iglewski, B.H. 1986. Toxins and virulence factors of *Pseudomonas aeruginosa.* In: Sokatch, J.R. (ed.), *The Biology of Pseudomonas* (pp. 195-213), The bacteria, Volume X. Academic Press, Toronto.

Noble, W.C. 1993. Gram-negative bacterial skin infections. Semin. Dermatol. 12: 336-341.

O'Morchoe, S.B., Ogaunseitan, O., Sayler, G.S. and Miller, R.V. 1988. Conjugal transfer of R68.45 and FP5 between *Pseudomonas aeruginosa* strains in a freshwater environment. Applied and Environmental Microbiology 54: 1923-1929.

O'Sullivan, D.J. and O'Gara, F. 1992. Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root pathogens. Microbiological Reviews 56: 662-676.

Obukowicz, M. and Shaw, P.D. 1983. Tn*3* labeling of a cryptic plasmid found in the plant pathogenic bacterium *Pseudomonas tabaci* and mobilization of RSF1010 by donation. Journal of Bacteriology 155: 438-42.

Obukowicz, M. and Shaw, P.D. 1985. Construction of Tn*3*-containing plasmids from plant-pathogenic pseudomonads and an examination of their biological properties. Applied and Environmental Microbiology 49: 468-73.

OECD 1986. Recombinant DNA Safety Considerations. OECD, Paris, 1986.

OECD 1994a. Ottawa '92: The OECD Workshop on Methods for Monitoring Organisms in the Environment, Environment Monograph No. 90. OECD, Paris, 1994.

OECD 1994b. Compendium of Methods for Monitoring Organisms in the Environment, Environment Monograph No. 91. OECD, Paris, 1994.

OECD 1995. Analysis of Information Elements Used in the Assessment of Certain Products of Modern Biotechnology, Environment Monograph No. 100. OECD, Paris, 1995.

OECD 1996. Industrial Products of Modern Biotechnology Intended for Release to the Environment, Environment Monograph No. 117. OECD, Paris, 1996.

Okaeme, A.N. 1989. Bacteria associated with mortality in tilapias, *Heterobranchus bidorsalis*, and *Clarias lazera*, in indoor hatcheries and outdoor ponds. Journal of Aquaculture in the Tropics 4: 143-146.

Omar, S.A. and Abd-Alla, M.H. 1994. Enhancement of faba bean nodulation, nitrogen fixation and growth by different microorganisms. *Biologia Plantarum* 36: 295-300.

Ononiwu, J.C. 1980. Transmissable enteritis of turkeys (Bluecomb) in Nigeria. *Bulletin of Animal Health and Protection in Africa* 28: 352-354.

Ormrod, D.J. and Jarvis, W.R. 1994. *Pseudomonas* diseases. In: Howard, R.J., Garland, J.A. and Seaman, W.L. (eds). *Diseases and Pests of Vegetable Crops in Canada* (pp. 146-147). The Canadian Phytopathological Society and Entomological Society of Canada. Ottawa, Canada.

Oyaizu, H. and Komgata, K. 1983. Grouping of *Pseudomonas* species on the basis of cellular fatty acid composition and the quinone system with special reference to the existance of 3-hydroxy fatty acids. Jouurnal of General and Applied Microbiology 29: 17-40.

Ozaktan, H. and Bora, T. 1994. Studies on identification of bacterial microflora of mushroom in Turkey. Journal of Turkish Phytopathology 23: 73-78.

Palleroni, N.J. 1981. Introduction to the family *Pseudomonadaceae*. In: Starr, M.P., Stolp, H., Truper, H.G., Balows, A. and Schegel, H.G. (eds). *The Prokaryotes - A Handbook on Habitats, Isolation and Identification of Bacteria (*pp. 655-665), Chapter 59. Springer-Verlag, Berlin.

Palleroni, N.J. 1984. *Pseudomonadaceae.* In: Kreig, N.R. and Holt, J.G. (eds). *Bergey's Manual of Systematic Bacteriology* (pp. 141-219), Vol. 1. The Williams and Wilkins Co., Baltimore.

Palleroni, N.J. 1992a. Human and animal pathogenic pseudomonads. In: Balows, A., Truper, H.G., Dworkin, M., Harder, W. and Schleifer, K.H. (eds). *The Prokaryotes,* Chapter 161 (pp. 3086-3103) (2nd Edition), Vol. III. Springer-Verlag, New York.

Palleroni, N.J. 1992b. Present situation of the taxonomy of the aerobic pseudomonads In: Galli, E., Silver, S. and Witholt, B. (eds). *Pseudomonas. Molecular Biology and Biotechnology (*pp. 105-115). American Society of Microbiology, Washington, D.C.

Palleroni, N.J. 1992c. *Pseudomonas* classification. Antonie v. Leeuwenhoek 64: 231-251.

Palleroni, N.J., Kunisawa, R., Contopoulou, R. and Doudoroff, M. 1973. Nucleic acid homologies in the genus *Pseudomonas*. International Journal of Systematic Bacteriology 23: 333-339.

Papadopoulou-Karabela, K., Iliadis, N., Liakos, V. and Bourdzy-Hatzopoulou, E. 1992. Experimental infection of honeybees by *Pseudomonas aeruginosa*. Apidologie 23(5): 393-397.

Papapetropoulou, M., Rodopoulou, G., Giannoulaki, E. and Stergiopoulos, P. 1994. Effect of temperature on antimicrobial susceptibilities of *Pseudomonas* species isolated from drinking water. Journal of Chemotherapy 6(6): 404-407.

Paul, J.H., Jeffrey, W.H. and Deflaun, M.E. 1987. Dynamics of extracellular DNA in the marine environment. Applied and Environmental Microbiology 53: 170-179.

Petrinec, Z., Naglic, T., Matasin Z. and Fijan, N. 1985. *Pseudomonas fluorescens* septicaemia in bighead (*Aristichthys nobilis* Rich) following handling. Veterinarski Arhiv. 55: 277-284.

Pierson, L.S. and Thomashow, L.S. 1992. Cloning and heterologous expression of the phenazine biosynthetic locus from *Pseudomonas aureofaciens* 30-84. Molecular Plant Microbe Interactions 5(4): 330-339.

Pillai, S.D., Josephson, K.L., Bailey, R.L., Gerba, C.P. and Pepper, I.L. 1991. Rapid method for processing soil samples for polymerase chain reaction amplification of specific gene sequences. Applied and Environmental Microbiology 57: 2283-2286.

Pitt, T.L. 1990. *Pseudomonas*. In: Wilson, G., Miles, A. and Parker, M.T. (eds). *Topley and Wilson's Principles of Bacteriology, Virology and Immunity (*8th Edition). Edward Arnold, London.

Piwowarski, J.M. and Shaw, P.D. 1982. Characterization of plasmids from plant pathogenic pseudomonads. Plasmid 7: 85-94.

Plotkowski, M.C., Saliba, A.M., Pereira, S.H., Cervante, M.P. and Bajolet-Laudinat, O. 1994. *Pseudomonas aeruginosa* selective adherence to and entry into human epithelial cells. Infection and Immunity 62: 5456-5463.

Pollack, M. 1992. *Pseudomonas*. In: Gorbach, S.L., Bartlett, J.G. and Blackhew, N.R. (eds). *Infectious Diseases* (pp. 1502-1513). W.B. Saunders Co., Philadelphia/London.

Premalatha, A. and Rajakumar, G. S. 1994. Pentachlorophenol degradation by *Pseudomonas aeruginosa*. World Journal of Microbiology and Biotechnology 10: 334-337.

Prieto, M., Garciaarmesto, M.R., Garcialopez, M.L., Alonso, C. and Otero, A. 1992. Species of *Pseudomonas* obtained at 7° C and 30° C during aerobic storage of lamb carcasses. Journal of Applied Bacteriology 73(4): 317-323.

Prince, A. 1992. Adhesins and receptors of *Pseudomonas aeruginosa* associated with infection of the respiratory tract. Microbial Pathology 13: 251-260.

Prosser, J.I. 1994. Molecular marker systems for detection of genetically engineered microorganisms in the environment. Microbiology 140: 5-17.

Puckett, A., Davison, G., Entwistle, C.C. and Barbara, J.A.J. 1992. Post-transfusion septicaemia 1980-1989: importance of donor arm cleansing. Journal of Clinical Pathology 45: 155-157.

Pujar, B.G. and Ribbons, D.W. 1985. Phthalate metabolism in *Pseudomonas fluorescens* PHK: Purification and properties of 4,5-dihydroxyphthalate decarboxylase. Applied and Environmental Microbiology 49: 374-376.

Quant, R.L. and Mills, D. 1984. An integrative plasmid and multiple-sized plasmids of *Pseudomonas syringae* pv. *phaseolicola* have extensive homology. Molecular and General Genetics 193: 459-466.

Quinn, J.P. 1992. Intrinsic antibiotic resistance in *Pseudomonas aeruginosa*. In: Galli, E., Silver, S. and Witholt, B. (eds). *Pseudomonas. Molecular Biology and Biotechnology.* American Society for Microbiology, Washington, D.C.

Raaska, L. and Maffila-Sandholm, T. 1991. The antogonistic activity of *Pseudomonas chlororaphis* and *Pseudomonas fluorescens* against shiitake (*Lentinula edodes*) mycelia. Material und Organismen 26: 287-302.

Rahme, L.G., Stevens, E.J., Wolfort, S.F., Shoa, J., Tompkins, R.G. and Ausubel, F.M. 1995. Common virulence factors for bacterial pathogenicity in plants and animals. Science 268: 1899-1902.

Rainey, P.B., Brodey, C.L. and Johnstone, K. 1991. Biological properties and spectrum of activity of tolaasin, a lipodepsipeptide toxin produced by the mushroom pathogen *Pseudomonas tolaasii*. Physiological and Molecular Plant Pathology 39(1): 57-70.

Rainey, P.B., Brodey, C.L. and Johnstone, K. 1993. Identification of a gene cluster encoding three high-molecular-weight proteins, which is required for synthesis of tolaasin by the mushroom pathogen *Pseudomonas tolaasii*. Molecular Microbiology 8(4): 643-652.

Ramos, J.L., Duque, E. and Ramos-Gonzalez, M.I. 1991. Survival in soils of an herbicide-resistant *Pseudomonas putida* strain bearing a recombinant TOL plasmid. Applied and Environmental Microbiology 57: 260-266.

Ramos, J.L., Diaz, E., Dowling, D., de Lorenzo, V., Molin, S., O'Gara, F., Ramos, C. and Timmis, K.N. 1994. Behavior of designed bacteria for biodegradation. Bio/Technology 12: 1349-1356.

Ramos-Gonzalez, M.I., Duque, E. and Ramos, J.L. 1991. Conjugational transfer of recombinant DNA in cultures and in soils: Host range of *Pseudomonas putida* TOL plasmids. Applied and Environmental Microbiology 57: 3020-3027.

Ramos-Gonzalez, M.I., Ruiz-Cabello, F., Brettar, I., Garrido, F. and Ramos, J.L. 1992. Tracking genetically engineered bacteria: monoclonal antibodies against surface determinants of the soil bacterium *Pseudomonas putida* 2440. Journal of Bacteriology 174: 2978-2985.

Ramos-Gonzalez, M.I., Ramos-Diaz, M.A., Ramos, J.L. 1994. Chromosomal gene capture mediated by the *Pseudomonas putida* TOL catabolic plasmid. Journal of Bacteriology 174: 2978-2985.

Ratnaningsih, E., Dharmsthiti, S., Krishnapillai, A., Morgan, M., Sinclair, M. and Holloway, B.W. 1990. A combined physical and genetic map of *Pseudomonas aeruginosa* PAO. Journal of General Microbiology 136: 2351-2357.

Rattray, E.A.S., Tyrrell, J.A., Prosser, J.I., Glover, L.A. and Killham, K. 1993. Effect of soil bulk density and temperature on wheat rhizosphere colonization by lux-marked *Pseudomonas fluorescens*. European Journal of Soil Biology 29: 73-82.

Richardson, P.N. 1993. Stipe necrosis of cultivated mushrooms (*Agaricus bisporus*) associated with a fluorescent pseudomonad. Plant Pathology 42: 927-929.

Richet, H., Escande, M.C., Marie, J.P., Zittoun, R. and Lagrange, P.H. 1989. Epidemic *Pseudomonas aeruginosa* serotype O16 bacteremia in hematology-oncology patients. Journal of Clinical Microbiology 27(9): 1992-1996.

Riley, M. 1989. Constancy and change in bacterial genomes. In: Poindexter, J.S. and Leadbeater, E.R. (eds). *Bacteria in Nature* (pp. 359-388).

Roberts, R.J. and Horne, M.T. 1978. Bacterial meningitis in farmed rainbow trout, *Salmo gairdneri* Richardson, affected with chronic pancreatic necrosis. Journal of Fish Diseases 1: 157-164.

Roilides, E., Butler, K.M., Husson, R.N., Mueller, B.U., Lewis, L.L. and Pizzo, P.A. 1992. *Pseudomonas* infections in children with human immunodeficiency virus infection. Pediatric Infectious Disease Journal 11(7): 547-553.

Romanowski, G., Lorenz, M.G. and Wackernagel, W. 1991. Adsorption of plasmid DNA to mineral surfaces and protection against DNase I. Applied and Environmental Microbiology 57: 1057-1061.

Romling, U., Grothues, D., Bautsch, W. and Tummler, B. 1989. A physical genome map of *Pseudomonas aeruginosa* PAO. EMBO Journal 8: 4081-4089.

Romling, U., Fiedler, B., Bosshammer, J., Grothues, D., Greipel, J., von der Hardt, J. and Tummler, B. 1994. Epidemiology of chronic *Pseudomonas aeruginosa* infections in cystic fibrosis. Journal of Infectious Diseases 170: 1616-1621.

Romling, U., Wingender, J., Muller, H. and Tummler, B. 1994. A major *Pseudomonas aeruginosa* clone common to patients and aquatic habitats. Applied and Environmental Microbiology 60: 1734-1738.

Ronchel, M.C., Ramos, C., Jensen, L.B., Molin, S. and Ramos, J.L. 1995. Construction and behavior of biologically contained bacteria for environmental applications in bioremediation. Applied and Environmental Microbiology 61: 2990-2994.

Ros, S.P. 1989. *Pseudomonas* bacteremia in a previously healthy 2-year-old boy. Clinical Pediatrics (Phila.) 28(7): 336.

Rosak, D.B. and Colwell, R.R. 1987. Survival strategies of bacteria in the natural environment. Microbiological Reviews 51: 365-379.

Safwat, E.E.A., Awaad, M.H., Ammer, A.M. and El Kinawy, A.A. 1986. Studies on *Pseudomonas aeruginosa*, *Proteus vulgari*s and *Salmonella typhimurium* infection in ducklings. Egyptian Journal of Animal Protection 24: 287-294.

Sarsola, P., Taylor, D.J., Love, S. and McKellar, Q.A. 1992. Secondary bacterial infections following an outbreak of equine influenza. Veterinary Record 131: 441-442.

Sato, M., Nishiyama, K. and Shirata, A. 1983. Involvement of plasmid DNA in the productivity of coranatine by *Pseudomonas syringae* pv. *atropurpurea*. Annual Phytopathology Society, Japan 49: 522-528.

Sato, Y. et. al. 1986. *Pseudomonas aeruginosa* infection with granulomas in a Japanese ptarmigan. Journal of the Japan Veterinary Medical Association 39(8): 516-519.

Saye, D.J., Ogunseitan, O.A., Sayler, G.S. and Miller, R.V. 1987. Potential for transduction of plasmids in a natural freshwater environment: effect of plasmid donor concentration and a natural microbial community on transduction in *Pseudomonas aeruginosa*. Applied and Environmental Microbiology 53: 987-995.

Saye, D.J., Ogunseitan, O.A., Sayler, G.S. and Miller, R.V. 1990. Transduction of linked chromososmal genes between *Pseudomonas aeruginosa* strains during incubation *in situ* in a freshwater habitat. Applied and Environmental Microbiology 56: 140-145.

Sayre, P.S. and Miller, R.V. 1990. Bacterial mobile genetic elements: importance in assessing the environmental fate of genetically engineered sequences. Plasmid 26: 151-171.

Schleifer, K.H., Amann, R., Ludwig, W., Rothemund, C., Springer, N. and Dorn, S. 1992. Nucleic acid probes for the identification and *in situ* detection of pseudomonads. In: Galli, E., Silver, S. and Witholt, B. (eds). *Pseudomonas. Molecular Biology and Biotechnology* (pp. 105-115). American Society for Microbiology, Washington, D.C.

Schmidt, E.L. 1974. Quantitative autecological study of microorganisms in soil by immunofluorescence. Soil Science 118: 141-149.

Scholter, M., Bode, W., Hartmann, A. and Besse, F. 1992. Sensitive chemoluminescence-based immunological quantification of bacteria in soil extracts with monoclonal antibodies. Soil Biology and Biochemistry 24: 399-405.

Schroth, M.N., Hildebrand, D.C. and Starr, M.P. 1981. Phytopathogenic members of the genus *Pseudomonas* pp. 701-718 In: Starr, M.P., Stolp, H., Truper, H.G., Balows, A. and Schegel, H.G. (eds). *The Prokaryotes - a Handbook on Habitats, Isolation and Identification of Bacteria*. Springer-Verlag, Berlin.

Schroth, M.N., Hildebrand, D.C. and Panopoulos, N. 1992. Phytopathogenic Pseudomonads and related plant-associated Pseudomonads. In: Balows, A., Truper, H.G., Dworkin, M., Harder, W. and Schleifer, K.H. (eds). *The Prokaryotes* (pp. 3104-3131), Chapter 162, Vol. III (2nd Edition). Springer-Verlag Inc., New York.

Schuster, M.G. and Norris, A.H. 1994. Community-acquired *Pseudomonas aeruginosa* pneumonia in patients with HIV infection. AIDS 8(10): 1437-1441.

Scott, J., Boulton, F.E., Govan, J.R.W., Miles, R.S., McClelland, D.B.L. and Prowse, C.V. 1988. A fatal transfusion reaction associated with blood contaminated with *Pseudomonas fluorescens.* Vox Sang. 54: 201-204.

Sellwood, J.E., Ewart, J.M. and Buckler, E. 1981. Vascular blackening of chicory caused by a pectolytic isolate of *Pseudomonas fluorescens*. Plant Pathology 30: 179-180.

Sepkowitz, K.A., Brown, A.E. and Armstrong, D. 1994. Empirical therapy for febrile, neutropenic patients: Persistence of susceptibility of gram-negative bacilli to aminoglycoside antibiotics. Clinical Infectious Diseases 19(4): 810-811.

Shahata, M. A., El-Timawy, A. M. and Seddik, I. 1988. Occurrence of *Pseudomonas* infection in fowl in upper Egypt. Assiut Veterinary Medical Journal 20(39): 168-177.

Shaw, J.J. and Kado, C.I. 1986. Development of a *Vibrio* bioluminescence gene-set to monitor phytopathogenic bacteria during the on-going disease process in a non-disruptive manner. Bio/Technology 4: 560-564.

Shaw, J.J. and Kado, C.I. 1988. Whole plant wound inoculation for consistent reproduction of black rot of crucifers. Phytopathology 78: 981-986.

Short, K.A., Doyle, J.D., King, R.J., Seidler, R.J., Stotzky, G. and Olsen, R.G. 1991. Effects of 2,4-dichlorophenol, a metabolite of a genetically engineered bacterium, and 2,4-dichlorophenoxyacetate on some microorganism-mediated ecological processes in soil. Applied and Environmental Microbiology 57: 412-418.

Silcock, D.J., Waterhouse, R.N., Glover, L.A., Prosser, J.I. and Killham, K. 1992. Detection of a single genetically modified bacterial cell in soil by using charge coupled device-enhanced microscopy. Applied and Environmental Microbiology 58(8): 2444-2448.

Simor, A.E., Ricci, J., Lau, A., Bannatyne, R.M. and Ford-Jones, L. 1985. Pseudobacteremia due to *Pseudomonas fluorescens*. Pediatric Infectious Diseases 4: 508-512.

Sisinthy, S., Rao, N.S., Saisree, L., Vipula, S., Reddy, G.N.S. and Bhargava, P.M. 1989. Isolation and identification of *Pseudomonas* spp. from Schirmacher Oasis, Antarctica. Applied and Environmental Microbiology 55: 767-770.

Smirnov, V.V., Kiprianova, E.A., Boiko, O.I., Kolesova, E. A. and Garagulya, A.D. 1991. The effect of iron ions on antifungal activity of *Pseudomonas*. Mikrobiologicheskii Zhurnal 53(3): 80- 87.

Smith, P.R. and Davey, S. 1993. Evidence for the competitive exclusion of *Aeromonas salmonicida* from fish with stress-inducible furunculosis by a fluorescent pseudomonad. Journal of Fish Diseases 16: 521-524.

Som, N.C., Ghosh, B.B., Arora, G.S. and Majumdar, M.K. 1980. Isolation and identification of *Pseudomonas aeruginosa* pathogenic to insect larvae. Indian Journal of Experimental Biology 18(6): 590-593.

Sorenson, J., Skouv, J., Jorgensen, A. and Nybroe, O. 1992. Rapid identification of environmental isolates of *Pseudomonas aeruginosa*, *P. fluorescens* and *P. putida* by SDS-PAGE analysis of whole-cell protein patterns. FEMS Microbiology Ecology 101: 41-50.

Sorheim, R., Torsvik, V.L. and Goksoyr, J. 1989. Phenotypical divergences between populations of soil bacteria isolated on different media. Microbial Ecology 17: 181-192.

Spencer, R.C. 1994. Epidemiology of infection in ICUs. Intensive Care Medicine 20 (suppl 4): S2-6.

Stadt, S.J. and Saettler, A.W. 1981. Effect of host genotype on multiplication of *Pseudomonas phaseolicola*. Phytopathology 71: 1307-1310.

Stapleton, F., Dart, J.K., Seal, D.V. and Matheson, M. 1995. Epidemiology of *Pseudomonas aeruginosa* keratitis in contact lens wearers. Epidemiology and Infection 114: 395-402.

Steffan, R.J. and Atlas, R.M. 1988. DNA amplification to enhance detection of genetically engineered bacteria in environmental samples. Applied and Environmental Microbiology 55: 2185-2191.

Stenhouse, M.A. and Milner, L.V. 1992. A survey of cold-growing gram-negative organisms isolated from the skin of prospective blood donors. Transfus. Med. 2: 235-237.

Stephen, J. and Pietrowski, R.A. 1986. Bacterial Toxins. 2nd edition. American Society for Microbiology, Washington, D.C.

Stewart, G.J. 1990. The mechanism of natural transformation. In: Levy, S.B. and Miller, R.V. (eds). *Gene Transfer in the Environment (*pp. 139-164). McGraw-Hill Publishing Co, New York.

Stewart, G.J. and Carlson, C.A. 1986. The biology of natural transformation. Annual Review of Microbiology 40: 211-235.

Stone, L.S. and Zottola, E.A. 1985. Relationship between the growth phase of *Pseudomonas fragi* and its attachment to stainless steel. Journal of Food Science 50(4): 957-960.

Stoskopf, M.K. 1993. Bacterial diseases of goldfish, koi, and carp. In: Stoskopf, M.K. *Fish Medicine* (pp. 473-475), Chapter 48. W.B. Saunders Co., Philadelphia, PA.

Stotzky, G., Zeph, L.R. and Devanas, M.A. 1991. Factors affecting the transfer of genetic information among microorganisms in soil. In: Ginzburg, L.R. (ed.). *Assessing Ecological Risks in Biotechnology* (pp. 95-112). Butterworth-Heineman.

Sturman, P.J., Jones, W.L. and Characklis, W.G. 1994. Interspecies competition in colonized porous pellets. Water Research 28(4): 831-839.

Stutz, E., Kahr, G. and Defago, G. 1989. Clays involved in suppression of tobacco black rot by a strain of *Pseudomonas fluorescens*. Soil Biology and Biochemistry 21: 361-366.

Sugimoto, E.E., Hoitink, H.A.J. and Tuovinen, O.H. 1990. Oligotrophic pseudomonads in the rhizosphere: suppressiveness to *Pythium* damping-off of cucumber seedlings *(Cucumis sativus* L.). Biology and Fertility of Soils 9(3): 231-34.

Suoniemi, A., Bjorklof, K., Haahtela, K. and Romantschuk, M. 1995. Pili of *Pseudomonas syringae* pathovar syringae enhance initiation of bacterial epiphytic colonization of bean. Microbiology UK 141(Feb, Part 2): 497-503.

Surico, G. and Scala, A. 1992. Carnation - *Pseudomonas caryophylli* interaction - Population dynamics of *P. caryophylli* and other bacteria in carnation leaves and cultured cells. Petria 2: 93-106.

Suyama, K. and Fujii, H. 1993. Bacterial disease occurred on cultivated mushroom in Japan. Journal of Agricultural Science 38(2): 35-50.

Tabor, M. and Gerety, R.J. 1984. Five cases of *Pseudomonas* sepsis transmitted by blood transfusions. Lancet i: 1403.

Taylor, M., Keane, C.T. and Falkiner, F.R. 1984. *Pseudomonas putida* in transfused blood. Lancet ii:107.

Thiem, S., Krumme, M., Smith, R. and Tiedje, J. 1994. Use of molecular techniques to evaluate the survival of a microorganism injected into an aquifer. Applied and Environmental Microbiology 60: 1059-1067.

Thomas, A.W., Slater, J.H. and Weightman, A.J. 1992. The dehalogenase gene *deh1* from *Pseudomonas putida* PP3 is carried on an unusual mobile genetic element designated *DEH*. Journal of Bacteriology 174: 1932-1940.

Thomashow, L.S. and Pierson, L.S. 1991. Genetic aspects of phenazine antibiotic production by fluorescent pseudomonads that suppress take-all disease of wheat. In: Hennecke, H. and Verma, D.P.S. (eds). *Proceedings of the 5th International Symposium on the molecular genetics of plant-microbe interactions,* Interlaken, Switzerland, September, 1990 (pp. 443-449). Advances in molecular genetics of plant-microbe interactions. Vol. 1. Kluwer Academic Publishers, Netherlands.

Thomashow, L.S., Weller, D.M., Bonsall, R.F. and Pierson, L.S. 1990. Production of the antibiotic phenazine-1-carboxylic acid by fluorescent *Pseudomonas* species in the rhizosphere of wheat. Applied and Environmental Microbiology 56(4): 908-912.

Thompson, I.P., Cook, K.A., Letheridge, G. and Burns, R.G. 1990. Survival of two ecologically distinct bacteria (*Flavobacterium* and *Arthrobacter*) in unplanted and rhizosphere soil: Laboratory studies. Soil Biology and Biochemistry 22: 1029-1037.

Thompson, I.P., Bailey, M.J., Fenlon, J.S., Fermor, T.R., Lilley, A.K., Lynch, J.M., McCormack, P.J., McQuilken, M.P., Purdy, K.J., Rainey, P.B. and Whipps, J.M. 1993a. Quantitative and qualitative seasonal changes in the microbial community from the phyllosphere of sugarbeet (*Beta vulgaris*). Plant and Soil 150(2): 177-191.

Thompson, I.P., Bailey, M.J., Ellis, R.J. and Purdy, K.J. 1993b. Subgrouping of bacterial populations by cellular fatty acid composition. FEMS Microbiology Ecology 102: 75-84.

Thompson, S.V., Hanson, D.R., Flint, K.M. and Vandenberg, J.D. 1992. Dissemination of bacteria antagonistic to *Erwinia amylovora* by honey bees. Plant Disease 76: 1052-1056.

Thorn, R.G. and Tsuneda, A. 1992. Interactions between various wood-decay fungi and bacteria: antibiosis, attack, lysis, or inhibition. Reports of the Tottori Mycological Institute 30: 13-20.

Timmis, K.N. 1994. Environmental biotechnology. Current Opinion in Biotechnology 5: 247-248.

Top, E., Vanrolleghem, P., Mergeay, M. and Verstraete, W. 1992. Determination of the mechanism of retrotransfer by mechanistic modelling. Journal of Bacteriology 174: 5953-5960.

Tranel, P.J., Gealy, D.R. and Kennedy, A.C. 1993. Inhibition of downy brome (*Bromus tectorum*) root growth by a phytotoxin from *Pseudomonas fluorescens* strain D7. Weed Technology 7: 134-139.

Trevors, J.T. 1991. Development of a Microbial Profile on Selected Groups of Microorganisms to assist in evaluations under the Canadian Environmental Protection Act for the Genus: *Pseudomonas*. Environment Canada contract report KE144-0623901SS.

Trevors, J.T. and Berg, G. 1989. Conjugal RP4 transfer between pseudomonads in soil and recovery of RP4 plasmid DNA from soil. Systematic and Applied Microbiology 11: 223-227.

Trevors, J.T. and Starodub, M.E. 1987. R-Plasmid transfer in non-sterile agricultural soil. Systematic and Applied Microbiology 9: 312-315.

Trevors, J.T. and van Elsas, J.D. 1989. A review of selected methods in environmental microbial genetics. Microbiological Sciences 5: 132-136.

Trevors, J.T., van Elsas, J.D., Overbeek, L.S. and Starodub, M-E. 1990. Transport of a genetically engineered *Pseudomonas fluorescens* strain through a soil microcosm. Applied and Environmental Microbiology 56: 401-408.

Trueb, R.M., Gloor, M. and Wuthrich, B. 1994. Recurrent *Pseudomonas* folliculitis. Pediatric Dermatology 11: 35-38.

Tsai, Y-L. and Olson, B.H. 1992. Detection of low numbers of bacterial cells in soils and sediments by polymerase chain reaction. Applied and Environmental Microbiology 58: 754-757.

Tsuchiya, Y., Ohata, K.I. and Shirata, A. 1980. Pathogenicity of the causal bacteria of head rot of lettuce, *Pseudomonas cichorii, P. marginalis* and *P. viridiflava*, to various crop plants. Bulletin of the National Institute of Agricultural Sciences 34: 51-73.

Tsuda, M., Minegishi, K.-I. and Iino, T. 1989. Toluene transposons *Tn4651* and *Tn4653* are class II transposons. Journal of Bacteriology 171: 1386-1393.

Tsuda, M. and Iino, T. 1990. Napthalene degrading genes on plasmid NAH7 are on a defective transposon. Molecular and General Genetics 22: 33-39.

Turkovskaya, O.V., Shub, G.M. and Panchenko, L.V. 1993. Microbial purification of nonionic surfactant sewage. pp. 171-183 In: *Effluent Treatment and Waste Minimization Symposium*. Yorkshire Branch of the Institution of Chemical Engineers and the Environmental Protection Subject Group, Leeds, UK, Mar. 31-Apr. 1, 1993. Symposium Series No. 132.

Tushima, S., Hasebe, A., Komoto, Y., Carter, J.P., Miyashita, K. and Pickup, R.W. 1995. Detection of genetically engineered microorganisms in paddy soil using a simple and rapid "nested" polymerase chain reaction method. Soil Biology and Biochemistry 27: 219-227.

Tyler, S.D., Strathdee, C.A., Rozee, K.R. and Johnson, W.M. 1995. Oligonucleotide primers designed to differentiate pathogenic pseudomonads on the basis of the sequencing of 16S-23S genes coding for internal transcribed spacers. Clinical Diagnostic Laboratory Immunology 2: 448-453.

van Dyke, M.I., Gulley, S.L., Lee, H. and Trevors, J.T. 1993. Evaluation of microbial surfactants for recovery of hydrophobic pollutants from soil. Journal of Industrial Microbiology 11: 163-170.

van Elsas, J.D., Dijkstra, A.F., Govaert, J.M. and van Veen, J.A. 1986. Survival of *Pseudomonas fluorescens* and *Bacillus subtilis* introduced into two soils of different texture in field microplots. FEMS Microbiology Ecology 38: 151-160.

van Elsas, J.D., Govaert, J.M. and van Veen, J.A. 1988. Bacterial conjugation between pseudomonads in the rhizosphere of wheat. FEMS Microbiology Ecology 53: 299-306.

van Elsas, J.D., Trevors, J.T. and van Overbeek, L.S. 1991. Influence of soil properties on the vertical movement of genetically marked *Pseudomonas fluorescens* through large soil microcosms. Biology and Fertility of Soils 10: 249-255.

van Elsas, J.D., Trevors, J.T., Jain, D., Wolters, A.C., Heijnen, C.E. and van Overbeek, L.S. 1992. Survival of, and root colonization by, alginate-encapsulated *Pseudomonas fluorescens* cells following introduction into soil. Biology and Fertility of Soils 14: 14-22.

Vancura, V. 1988. Inoculation of plants with *Pseudomonas putida*. In: Vancura, V. and Kunc, F. (eds). *Interrelationships Between Microorganisms and Plants in Soil (*pp. 185-190). Elsevier Science Publishers, Amsterdam, Netherlands.

Vandenbergh, P.A. and Kunka, B.S. 1988. Metabolism of volatile chlorinated aliphatic hydrocarbons by *Pseudomonas fluorescens*. Applied and Environmental Microbiology 54: 2578-2579.

Vandenhove, H., Merckx, R., Wilmots, H. and Vlassak, K. 1991. Survival of *Pseudomonas fluorescens* inocula of different physiological stages in soil. Soil Biology and Biochemistry 23: 1133-1142.

Vasil, M.L., Pritchard, A.E. and Ostroff, R.M. 1990. Molecular biology of exotoxin A and phospholipase C of *Pseudomonas aeruginosa.* In: Silver, S., Chakrabarty, A.M., Igelewski, B. and Kaplan, S. (eds). *Pseudomonas*: *transformations, pathogenesis and evolving biotechnology.* American Society for Microbiology, Washington, D.C.

Vesaluoma, M., Kalso, S., Jokipii, L., Warhurst, D., Ponka, A. and Tervo, T. 1995. Microbiological quality in Finnish public swimming pools and whirlpools with special reference to free living amoebae: a risk factor for contact lens wearers? British Journal of Ophthalmology 79: 178-181.

Vesper, S.J. 1987. Production of pili (fimbrae) by *Pseudomonas fluorescens* and correlation with attachment to corn roots. Applied and Environmental Microbiology 53: 1397-1405.

Walter, M., Porteous, A. and Seidler, R. 1987. Measuring genetic stability in bacteria of potential use in genetic engineering. Applied and Environmental Microbiology 53: 105-109.

Wang, S.Z. 1987. Diagnosis and control of a disease in mink caused by *Pseudomonas aeruginosa*. Chinese Journal of Veterinary Medicine 13(11): 16-17.

Warburton, D.W., Bowen, B. and Konkle, A. 1994. The survival and recovery of *Pseudomonas aeruginosa* and its effect upon salmonellae in water - Methodology to test bottled water in Canada. Canadian Journal of Microbiology 40(12): 987-992.

Warren, G.J. 1987. Bacterial ice nucleation: molecular biology and applications. Biotechnology and Genetic Engineering Review 5: 107-135.

Weissenfels, W., Beyer, M. and Klein, J. 1990. Bacterial degradation of naphthalene, phenathrene, fluorene and fluoranthene by pure strains. In: *DECHEMA Biotechnology Conferences* (pp. 867-870), Vol. 3, Part B. Frankfurt, Germany. May 30-31, 1989.

Weller, D.M. and Cook, R.J. 1986. Increased growth of wheat by seed treatments with fluorescent pseudomonads, implications of *Pythium* control. Canadian Journal of Plant Pathology 8: 328-334.

Wellington, E.M.H., Cresswell, N. and Saunders, V.A. 1990. Growth and survival of streptomycete inoculants and extent of plasmid transfer in sterile and non-sterile soil. Applied and Environmental Microbiology 56: 1413-1419.

Welsh, J. and McClelland. 1990. Fingerprinting genomems using PCR with arbitrary primers. Nucleic Acids Research 18: 7213-7218.

Wendtpotthoff, K., Backhaus, H. and Smalla, K. 1994. Monitoring the fate of genetically engineered bacteria sprayed on the phylloplane of bush beans and grass. FEMS Microbiology Ecology 15(3-4): 279-290.

Westcott, S.W. and Kluepfel, D.A. 1993. Inhibition of *Criconemella xenoplax* egg hatch by *Pseudomonas aureofaciens*. Phytopathology 83(11): 1245-1249.

White, C.P. and Dunn, H.W. 1978. Compatibility and sex specific phage plating characteristics of the TOL and NAH catabolic plasmids. Genetical Research 32: 207-213.

Wiedemann, B. and Atkinson, B.A. 1991. Susceptibility to antibiotics: Species incidence and trends. In: Lorian, V. (ed.). *Antibiotics in Laboratory Medicine*, pp. 962-1208. Williams and Wilkins, Baltimore, Maryland.

Williams, J.G.K., Kubelik, A.R., Livak, A.R., Rafalski, J.A. and Tingey, S.V. 1990. DAN polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18: 6531-6535.

Williams, R.R., Dehdashti, B. and Lightner, D.V. 1992. Performance of an aquatic multispecies system in evaluating the effects of a model microbial pest control agent on nontarget organisms. Journal of Toxicology and Environmental Health 37: 247-264.

Wilson, M. and Lindow, S.E. 1993a. Effect of phenotypic plasticity on epiphytic survival and colonization by *Pseudomonas syringae*. Applied and Environmental Microbiology 59(2): 410-416.

Wilson, M. and Lindow, S.E. 1993b. Release of recombinant microorganisms. Annual Review of Microbiology 47: 913-944.

Wilson, M. and Lindow, S.E. 1994. Coexistence among epiphytic bacterial populations mediated through nutritional resource partitioning. Applied and Environmental Microbiology 60(12): 4468-4477.

Wilson, M.J., Glen, D.M., Hughes, L.A., Pierce, J.D. and Rodgers, P.B. 1994. Laboratory tests of the potential of entomopathogenic nematodes for the control of field slugs (*Deroceras reticulatum*). Journal of Invertebrate Pathology 64: 182-187.

Winstanley, C., Morgan, J.A.W., Pickup, R.W., Jones, J.G. and Saunders, J.R. 1989. Differential regulation of lambda pL and pR promotors by a cI repressor in a broad-host-range thermoregulated plasmid marker system. Applied and Environmental Microbiology 55: 771-777.

Winstanley, C., Seasman, M., Pickup, R.W., Carter, I.P., Morgan, J.A.W. and Saunders, J.R. 1993. A comparison of the survival of stable and unstable chromosomally located xylE marker cassettes as an indicator of cell division within populations of *Pseudomonas putida* released into lake water and soil. Microbial Releases 2: 97-107.

Wong, P.K., Lam, K.C. and Lo, C.M. 1993. Removal and recovery of Cu(II) from industrial effluent by immobilized cells of *Pseudomonas putida* II-11. Applied Microbiology and Biotechnology 39: 127-131.

Yabuuchi, E. and Devos, P. 1995a. Minutes of the meetings of the International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of the Genus *Pseudomonas* and Related Organisms. Prague, Czech Republic, 4 July 1994. International Journal of Systematic Bacteriology 45: 875.

Yabuuchi, E and Devos, P. 1995b. Minutes of the meetings of the International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of the Genus *Pseudomonas* and Related Organisms. Osaka, Japan, 17 and 20 September, 1990. International Journal of Systematic Bacteriology 45: 877-878.

Yang, P., De Vos, P. Kersters, K. and Swings, J. 1993. Polyamine patterns as chemotaxonomic markers for the genus *Xanthomonas*. International Journal of Systematic Bacteriology 43: 709-714.

Youssef, H., Mourad, M.I., Karim, S.A., Hassanein, K.M. and Sadek, A. 1990. Pathogenicity of *Pseudomonas aeruginosa* to Nile fish. Assiut Veterinary Medical Journal 24(47): 108-115.

Zhou, J. and Tiedje, J. 1995. Gene transfer from a bacterium injected into an aquifer to an indigenous bacterium. Molecular Ecology 4: 613-618.

Zilli, M., Converti, A., Lodi, A., del Borghi, M. and Ferraiolo, G. 1993. Phenol removal from waste gases with a biological filter by *Pseudomonas putida*. Biotechnology and Bioengineering 41: 693-699.

Zurrer, D.A., Cook, A. and Leisinger, T. 1987. Microbial desulfonation of substituted napthalene sulfonic acids and benzene sulfonic acids. Applied and Environmental Microbiology 53: 1459-1463.

Appendix:
Considerations from the OECD "Blue Book"

The ***General Scientific Considerations***, ***Human Health Considerations*** and ***Environmental and Agricultural Considerations*** from the OECD "Blue Book" (*Recombinant DNA Safety Considerations*, OECD, 1986) are the basis of the format of the information presented in Section III of this consensus document. These considerations were also used as a reference point in the document *Analysis of Information Elements Used in the Assessment of Certain Products of Modern Biotechnology* (OECD, 1995), which identified commonalities among OECD Member countries with respect to information elements used during regulatory assessments.

The Considerations set out in the OECD "Blue Book" are as follows:

GENERAL SCIENTIFIC CONSIDERATIONS

Characteristics of Donor and Recipient Organisms

1. Taxonomy, identification, source, culture

1a Names and designations;
1b The degree of relatedness between the donor and recipient organisms and evidence indicating exchange of genetic material by natural means;
1c Characteristics of the organism which permit identification and the methods used to identify the organisms;
1d Techniques employed in the laboratory and/or environment for detecting the presence of, and for monitoring, numbers of the organism;
1e The sources of the organisms;
1f Information on the recipient organism's reproductive cycle (sexual/asexual);
1g Factors which might limit the reproduction, growth and survival of the recipient organism.

2. Genetic characteristics of donor and recipient organisms

2a History of prior genetic manipulation;
2b Characterisation of the recipient and donor genomes;
2c Stability of recipient organism in terms of relevant genetic traits.

3. Pathogenic and physiological traits of donor and recipient organisms

3a Nature of pathogenicity and virulence, infectivity, or toxigenicity;
3b Host range;
3c Other potentially significant physiological traits;
3d Stability of these traits.

Character of the Engineered Organism

4a Description of the modification;
4b Description of the nature, function and source of the inserted donor nucleic acid, including regulatory or other elements affecting the function of the DNA and of the vector;
4c Description of the method(s) by which the vector with insert(s) has been constructed;
4d Description of methods for introducing the vector-insert into the recipient organism and the procedure for selection of the modified organism;
4e Description of the structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified organism;
4f Characterisation of the site of modification of the recipient genome. Stability of the inserted DNA;
4g Frequency of mobilisation of inserted vector and/or genetic transfer capability;
4h Rate and level of expression of the introduced genetic material. Method and sensitivity of measurement;
4i Influence of the recipient organism on the activity of the foreign protein.

HUMAN HEALTH CONSIDERATIONS

Characteristics of the Engineered Organism

1. Comparison of the engineered organism to the recipient organism regarding pathogenicity;
2. Capacity for colonisation;
3. If the organism is pathogenic to humans (or to animals if appropriate):

   3a Diseases caused and mechanism of pathogenicity including invasiveness and virulence;
   3b Communicability;
   3c Infective dose;
   3d Host range, possibility of alteration;
   3e Possibility of survival outside of human host;
   3f Presence of vectors or means of dissemination;
   3g Biological stability;
   3h Antibiotic-resistance patterns;
   3i Toxigenicity;
   3j Allergenicity.

Health Considerations Generally Associated with the Presence of Non-viable Organisms or with the Products of rDNA Processes

4. Toxic or allergenic effects of non-viable organisms and/or their metabolic products;
5. Product hazards.

Management of Personnel Exposure

6. Biological Measures:

   6a Availability of appropriate prophylaxis and therapies;
   6b Availability of medical surveillance.

7. Physical and organisational measures.

ENVIRONMENTAL AND AGRICULTURAL CONSIDERATIONS

Ecological Traits relating to the Donor and Recipient

1a Natural habitat and geographic distribution. Climatic characteristics of original habitats;
1b Significant involvement in environmental processes;
1c Pathogenicity - host range, infectivity, toxigenicity, virulence, vectors;
1d Interactions with and effects on other organisms in the environment;
1e Ability to form survival structure (e.g., seeds, spores, sclerotia);
1f Frequency of genotypic and phenotypic change;
1g The role of the genetic material to be donated in the ecology of the donor organism;
1h The predicted effect of the donated genetic material on the recipient organism.

Application of the Engineered Organism in the Environment

2a Geographical location of site, physical and biological proximity to man and/or any other significant biota;
2b Description of site including size and preparation, climate, temperature, relative humidity, etc.;
2c Containment and decontamination;
2d Introduction protocols including quantity and frequency of application;
2e Methods of site disturbance or cultivation;
2f Methods for monitoring applications;
2g Contingency plans;
2h Treatment procedure of site at the completion of application.

Survival, Multiplication and Dissemination of the Engineered Organism in the Environment

Detection, identification and monitoring techniques

3a Description of detection, identification and monitoring techniques;
3b Specificity, sensitivity and reliability of detection techniques;
3c Techniques for detecting transfer of the donated DNA to other organisms.

Characteristics affecting survival, multiplication and dissemination

4a Biological features which affect survival, multiplication or dissemination;
4b Behaviour in simulated natural environments such as microcosms, growth rooms, greenhouses, insectaries, etc.;
4c Known and predicted environmental conditions which may affect survival, multiplication, dissemination.

Interactions of Engineered Organism(s) with Biological Systems

Target and non-target populations

5a Known and predicted habitats of the engineered organism;
5b Description of the target ecosystems and of ecosystems to which the organism could be disseminated;
5c Identification and description of target organisms;
5d Anticipated mechanism and result of interaction between the engineered organism and the target organism(s);
5e Identification and description of non-target organism(s) which might be exposed.

Stability

6a Stability of the organism in terms of genetic traits;
6b Genetic transfer capability;
6c Likelihood of post-release selection leading to the expression of unexpected and undesirable traits by the engineered organism;
6d Measures employed to ensure genetic stability, if any;
6e Description of genetic traits which may prevent or minimise dispersal of genetic material.

Routes of dissemination

7a Routes of dissemination, physical or biological;
7b Known or potential modes of interaction, including inhalation, ingestion, surface contact, burrowing and injection.

Potential Environmental Impacts

Potential effects on target and non-target organisms

8a Pathogenicity, infectivity, toxigenicity, virulence, vector of pathogen, allergenicity, colonisation;
8b Known or predicted effects on other organisms in the environment;
8c Likelihood of post-release shifts in biological interactions or in host range.

Ecosystems effects

9a Known or predicted involvement in biogeochemical processes;
9b Potential for excessive population increase.

QUESTIONNAIRE TO RETURN TO THE OECD

The **Consensus Document on Information Used in the Assessment of Environmental Applications Involving *Pseudomonas*** is one in a series of OECD "consensus documents" containing information for use during a regulatory assessment of a particular microorganism, or of a new plant variety developed through modern biotechnology. These documents have been developed with the intention that they will be updated regularly to reflect scientific and technical developments.

Users of this document are invited to provide the Environmental Health and Safety Division with relevant new scientific and technical information, and to make proposals for additional areas related to this subject which ought to be considered in the future. This questionnaire is pre-addressed (see reverse). Respondents may either mail this page (or a photocopy) to the OECD, or forward the information requested via fax or E-mail.

OECD Environment Directorate
Environmental Health and Safety Division
2, rue André-Pascal
75775 Paris Cedex 16
France

Fax: (33) (1) 45.24.16.75
E-mail: ehscont@oecd.org

For more information about the Environmental Health and Safety Division and its publications (many of which are available electronically at no charge), consult the OECD's World Wide Web site: ***http://www.oecd.org/ehs/***

==

1. Did you find information in this document useful to your work?
   ☐ YES ☐ NO

2. Please specify the type of work you do:
   ☐ REGULATORY ☐ ACADEMIC ☐ INDUSTRY ☐ OTHER (please specify)

3. Please suggest changes or additions that should be considered when this document is updated.

4. Are there other areas related to this subject which should be considered when the document is updated?

Name: ....
Institution or company: ....
Address: ....
City: ....Postal code:.......................... Country: ....
Telephone: ....Fax:.................................... E-mail: ....

FOLD ALONG DOTTED LINES AND SEAL

PLACE
STAMP
HERE

OECD Environment Directorate
Environmental Health and Safety Division
2, rue André Pascal
75775 Paris Cedex 16
France

FOLD ALONG DOTTED LINES AND SEAL