

Pathogen profile

***Pantoea ananatis*: an unconventional plant pathogen**

TERESA A. COUTINHO* AND STEPHANUS N. VENTER

*Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa***SUMMARY**

Pantoea ananatis causes disease symptoms in a wide range of economically important agricultural crops and forest tree species worldwide. It is regarded as an emerging pathogen based on the increasing number of reports of diseases occurring on previously unrecorded hosts in different parts of the world. Its unconventional nature lies in the fact that, unlike the majority of plant pathogenic microbes, *P. ananatis* is capable of infecting humans and occurs in diverse ecological niches, such as part of a bacterial community contaminating aviation jet fuel tanks and contributing to growth promotion in potato and pepper.

Taxonomy: Bacteria; *Gammaproteobacteria*; family *Enterobacteriaceae*; genus *Pantoea*.

Microbiological properties: Gram-negative; facultatively anaerobic; most strains are motile and produce a yellow pigment in culture; indole positive.

Biology: *Pantoea ananatis* is a common epiphyte; it also occurs endophytically in hosts where it has been reported to cause disease symptoms and in hosts where no such symptoms have been described. Some strains are ice-nucleating, a feature which has been used as a biological control mechanism against some insect pests of agricultural crops and by the food industry.

Disease symptoms: *Pantoea ananatis* infects both monocotyledonous and dicotyledonous plants. The symptoms are diverse depending on the host infected, and include leaf blotches and spots, die-back, and stalk, fruit and bulb rot.

Biological control agent: *Pantoea ananatis* has both anti-fungal and antibacterial properties. These characteristics have the potential of being exploited by biological control specialists.

INTRODUCTION

The appearance of new and re-emerging plant diseases is having a significant constraint on agricultural productivity worldwide (Bandyopadhyay and Frederiksen, 1999; Bright, 1998; Palm, 1999).

*Correspondence: Tel.: +27-12-420 3934; Fax: +27 12 420 3960; E-mail: teresa.coutinho@fabi.up.ac.za

The extent of this threat has increased dramatically over the past two decades as a result of increased movement of agricultural products between countries. The threat of these emerging diseases is, however, not restricted to cultivated plants, as native and wild plants are also at risk (Anderson *et al.*, 2004). This is of considerable importance in terms of biodiversity conservation.

An emerging plant pathogen is considered to be one which has shown an increase in incidence, host and geographical range (Anderson *et al.*, 2004). Since its initial discovery on pineapple, causing fruitlet rot in the Philippines in 1928 (Serrano, 1928), *Pantoea ananatis* has been found to cause a wide range of symptoms on both monocotyledonous and dicotyledonous plants. Its occurrence in these hosts leads to sporadic disease outbreaks, resulting in severe economic losses. From 1983 to date, the host range of *P. ananatis* has increased to eight, and the pathogen has now been reported to cause plant disease symptoms in at least 11 countries. The increase in geographical range is best illustrated by the distribution of palea browning of rice. This disease was initially reported from Japan (Tabei *et al.*, 1988), but now also occurs in Korea (Kim *et al.*, 1989), the Philippines (Xie, 1996 cited by Xie, 2001), China (Xie, 2001) and, more recently, Italy (Cortesi and Pizzatti, 2007).

Pantoea ananatis is a ubiquitous bacterium which, in itself, is not unique as a number of other bacterial plant pathogens share this characteristic. However, *P. ananatis*, when it is not associated with plants as an epiphyte, endophyte, pathogen or symbiont, also occupies diverse and unusual ecological niches. Here it may function as a saprophyte. In the case of humans, *P. ananatis* has also been reported to cause bacteraemia (De Baere *et al.*, 2004). With the exception of perhaps only *Pantoea agglomerans*, no other plant pathogen behaves in such an unconventional manner. An explanation of how *P. ananatis* has become so broadly adapted to these different habitats will probably only be resolved once species-specific genes have been identified.

Pantoea ananatis is not only important in the above-mentioned roles. Its ice nucleation activity has been exploited by both the food industry and biological control specialists of insects. Extracellular ice nucleators from *P. ananatis* have been tested and applied in the freezing of foods in order to obtain the desired texture (Zasytkin and Lee, 1999), and in the freeze-drying of

foods (Watanabe and Arai, 1994). Ice-nucleating strains of *P. ananatis* also markedly reduce the cold hardiness of mulberry pyralid larvae (Watanabe and Sato, 1999), and thus these strains have the potential to act as biological control agents of insect pests. In addition, the accomplishment of researchers in Switzerland to genetically modify rice to produce 'yellow rice' owes its success to *P. ananatis*. In this situation, the phytoene desaturase from *P. ananatis* (a strain identified as *Erwinia uredovora*) was used to introduce the β -carotene biosynthesis pathway into rice (Beyer *et al.*, 2002).

In this review, we focus on the taxonomy, detection and identification of *P. ananatis*. We also discuss its role in different ecological niches with a focus on its association with plants as an epiphyte, endophyte and plant pathogen. The molecular basis for its role in these different niches is, however, not well understood.

TAXONOMY

Until recently all phytopathogenic *Enterobacteriaceae* belonged to a single genus *Erwinia*. The genus was proposed by Winslow *et al.* (1920) for all plant-associated, Gram-negative, non-spore-forming, peritrichous, fermentative, rod-shaped bacteria. Dye (1968, 1969a,b,c) divided the genus into four 'natural' clusters, namely the amylovora, carotovora, herbicola and 'atypical' *Erwinia* groups. The 'herbicola' group, of interest in this review, consisted of *Erwinia* strains that usually produced a yellow pigment in culture and related non-pigmented clinical isolates often named *Enterobacter agglomerans*. This group is referred to in the literature as the *Erwinia herbicola*–*Enterobacter agglomerans* complex. Many researchers have subsequently shown that this complex is heterogeneous and contains bacterial strains with different phenotypes and genotypes (Brenner *et al.*, 1984; Mergaert *et al.*, 1983, 1984; Verdonck *et al.*, 1987).

In 1989, Gavini *et al.* proposed the genus *Pantoea* (Gavini *et al.*, 1989). The type strains of *E. herbicola*, *Ent. agglomerans* and *E. milletiae* were found to belong to the same DNA hybridization group (Beji *et al.*, 1988), and thus the combined species, *P. agglomerans*, was proposed for these bacteria (Gavini *et al.*, 1989). Other species included in this genus are *P. dispersa* (Gavini *et al.*, 1989), *P. punctata*, *P. citrea*, *P. terrea* (Kageyama *et al.*, 1992), *P. stewartii*, *P. stewartii* ssp. *indologenes*, *P. stewartii* ssp. *stewartii* and *P. ananatis* (Mergaert *et al.*, 1993). *Pantoea ananatis* was first described by Serrano (1928) as *Erwinia ananas*. Mergaert *et al.* (1993) proposed the name *P. ananas*, which was corrected to '*ananatis*' by Trüper and De'Clari (1997).

Pantoea ananatis and *P. uredovora* are listed in *Bergey's Manual of Determinative Bacteriology* (Holt, 1977) as differing in their ability to reduce nitrate to nitrite. Both species also differ substantially from each other in host range. *Pantoea ananatis* was initially described as a pathogen that caused fruitlet rot of pineapple (Serrano, 1928), whereas *P. uredovora* was described as a

pathogen of the basidiomycete rust fungus, *Puccinia graminis* (Pon *et al.*, 1954). On the basis of a high level of genotypic relatedness, Mergaert *et al.* (1993) synonymized these two species. They found that seven strains received as either one of these two species, and including their type strains, exhibited between 76% and 100% DNA binding and constituted a single DNA hybridization group. It has previously been shown that the phenotypic differentiation of these strains is extremely difficult. However, this synonymy was supported by numerical analysis (Dye, 1981; Mergaert *et al.*, 1984; Verdonck *et al.*, 1987). Furthermore, Waleron *et al.* (2002) have recently shown that the two species are in the same *recA* polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) group, and Brady *et al.* (2007) have shown that these strains also form part of the same fluorescent amplified fragment length polymorphism (F-AFLP) cluster. Despite this research, the synonymy of these two species is not widely accepted, and the name *E. uredovora* is still commonly used in the literature (Beyer *et al.*, 2002).

ISOLATION, DETECTION AND IDENTIFICATION

Most general media, such as blood agar, nutrient agar, tryptic soy agar or media specifically designed for the isolation of the *Enterobacteriaceae*, for example MacConkey and Hektoen agar, can be used for the isolation of *P. ananatis* (Grimont and Grimont, 2005). From diseased plants, the bacterium is usually isolated using nutrient agar (Bruton *et al.*, 1991; Coutinho *et al.*, 2002; Gitaitis and Gay, 1997; Schaad, 2001), but other media, such as yeast extract–dextrose–calcium carbonate agar (Azad *et al.*, 2000) and King's Medium B (Cothier *et al.*, 2004), have also been employed.

Currently, there are only a few semi-selective media for the isolation of *P. ananatis*. Hasegawa *et al.* (2003) developed NSCV-In medium for the selective isolation of pathogenic *P. ananatis* from diseased rice plants. PA 20 was developed for the isolation of *P. ananatis* from onion seed (Goszczyńska *et al.*, 2006a,b), and was reported to inhibit the growth of most of the common saprophytes associated with the seed. Similar to the use of general isolation media to obtain *P. ananatis*, further tests to confirm the identity and pathogenicity of isolates are still required when using selective media.

The tentative identification of isolates as belonging to the genus *Pantoea* is usually performed using commercial identification systems (Azad *et al.*, 2000; Cortesi and Pizzatti, 2007) or sequencing of the 16S rRNA gene (Coutinho *et al.*, 2002). For this group of bacteria, 16S rRNA is, however, too highly conserved to differentiate reliably between closely related species (Stackebrand and Goebels, 1994). API 20E or Biolog systems also have problems in identifying specific *Pantoea* species accurately as they share many phenotypic characteristics. The production of indole by *P. ananatis* is often used to distinguish between this

species and *P. agglomerans* strains, but this characteristic is shared by strains of *P. stewartii* ssp. *indologenes* (Grimont and Grimont, 2005). The identity of *P. ananatis* strains is therefore often confirmed by means of other techniques, such as whole-cell fatty acid methyl ester profiles (Azad *et al.*, 2000; Cother *et al.*, 2004; Gitaitis and Gay, 1997; Schwartz and Otto, 2000), PCR-RFLP analysis of the *recA* gene (Waleron *et al.*, 2002), DNA–DNA hybridization (Coutinho *et al.*, 2002) or F-AFLP (Brady *et al.*, 2007).

At present, there is no recommended method for the direct detection of *P. ananatis* in plant material. Walcott *et al.* (2002) designed species-specific primers for the 16S–23S rDNA internal transcribed spacer region, but the best primer pair detected both *P. ananatis* and *P. stewartii* ssp. *stewartii*. These researchers also tried to overcome the interference of saprophytes on onion seed by enriching samples for *P. ananatis* by means of polyclonal immunomagnetic bead capturing before proceeding with PCR. Cortesi and Pizzatti (2007) used another primer set targeting the same region, but no data on the assay's specificity are available. An oligonucleotide probe for fluorescent *in situ* hybridization was also not species specific and detected both *P. ananatis* and *P. agglomerans* (Nakanishi *et al.*, 2006).

Currently, the most promising approach for species assignment is the use of multilocus sequence analysis (MLSA) (Gevers *et al.*, 2005). Using sequence data from four housekeeping genes, Brady *et al.* (2008) showed that MLSA could clearly differentiate between all the current *Pantoea* spp. A high level of congruence was observed between the *gyrB* sequence data and DNA–DNA hybridization values, and it was recommended that accurate identification of *P. ananatis* strains could be achieved with comparison of either the *gyrB* (Fig. 1) or *rpoB* gene sequences (Brady *et al.*, 2008).

PANTOEA ANANATIS AS AN EPIPHYTE

Pantoea ananatis is a common epiphyte on host and non-host plants. Gitaitis *et al.* (2002) detected and cultured this bacterium as an epiphyte from 25 asymptomatic weed species, including crabgrass, sicklepod and yellow nutsedge, and from crop plants, such as Bermuda grass, cowpea and soybean. It has also been reported as an epiphyte on numerous economically important plant species, including rice (Watanabe *et al.*, 1996), maize (Paccola-Meirelles *et al.*, 2001), barley, buckwheat, uredospores of *Ustilago* smut of maize (Coplin and Kado, 2001), cotton lint (Chun and Perkins, 1997), mulberry (Takahashi *et al.*, 1995), poplar trees (Zeng *et al.*, 1999) and wheat heads (Legard *et al.*, 1994). In many of these cases, the occurrence of *P. ananatis* on the plant surface has not been linked to a specific disease on the host from whence it was isolated as an epiphyte. However, these asymptomatic non-hosts could be providing a source of inoculum, causing disease outbreaks of susceptible hosts grown in their vicinity.

The occurrence of *P. ananatis* on plant surfaces may be beneficial to these plants. The bacterium has both antibacterial and antifungal activity *in vitro* and *in vivo*, thus protecting host plants against infection by other pathogenic fungi and bacteria. A strain, previously described as *P. uredovora*, was found to have *in vitro* antibacterial activity against *Xanthomonas*, *Agrobacterium* and all species belonging to the 'amylovora' group, including *Erwinia amylovora* (Vantomme *et al.*, 1989). Isolates of *P. ananatis* from buckweed seed have been shown to have strong antifungal activity against *Rhizopus* spp. *in vitro* (Iimura and Hosono, 1998). In a study by Pajk (2004), it was reported that '*P. uredovora*' reduced the infection of pome fruit trees by *E. amylovora*. A strain, CPA-3, of *P. ananatis* was discovered to rapidly colonize wounds of harvested pome fruit, protecting them against *Penicillium expansum* (Torres *et al.*, 2005). Similarly, strains of *P. ananatis* have been found to protect tomato fruit against the grey mould fungus, *Botrytis cinerea*, by producing antifungal compounds (Enya *et al.*, 2007). Rice blast caused by *Pyricularia oryzae* has also been controlled by a strain of *P. ananatis* transformed with a chitinolytic enzyme gene (*ChiA*) from *Serratia marcescens* (Simeya *et al.*, 2003).

Pantoea ananatis is one of only a few species of bacteria shown to contain ice-nucleating strains (Abe *et al.*, 1989; Obata *et al.*, 1990). An ice nucleation active (INA) gene, *inaA*, has been sequenced and found to be similar to that of *Pseudomonas* species (Abe *et al.*, 1989). This implies that frost formation on sensitive plants is induced at temperatures slightly higher than would normally occur when the bacterium is absent. Examples of frost-sensitive plants are *Eucalyptus*, poplar, pea, pome and stone fruit trees (Lindow, 1983). *Pantoea ananatis* has been found to be the dominant INA bacterium on maize leaves in China (Sun *et al.*, 2005), and has also been reported to cause frost injury to mulberry (Takahashi *et al.*, 1995), tea (Goto *et al.*, 1988), strawberry (Obata *et al.*, 1990), apricot (Zhao *et al.*, 2007) and citrus (Wang *et al.*, 2008). The freezing injury caused by *P. ananatis* in poplar has resulted in an increased incidence of infection by the fungal pathogen *Dothiorella gregaria* in China (DePeng *et al.*, 1999; Xiang *et al.*, 2001; Zeng *et al.*, 1999). The exact role of frost injury in outbreaks of many of the diseases caused by *P. ananatis* has not been elucidated clearly.

PANTOEA ANANATIS AS AN ENDOPHYTE

Although the term 'endophyte' is more commonly associated with fungi, bacteria are also capable of colonizing the interior of a plant. In this environment, they may be considered beneficial, neutral or existing as latent pathogens (Lodewyckx *et al.*, 2002). In recent years, *P. ananatis* has been isolated as an endophyte from a number of plants, including, for example, coffee (Nunes and de Melo, 2006) and ginseng (Cho *et al.*, 2007). Its role as an endophyte has mainly been found to be beneficial to the plant.

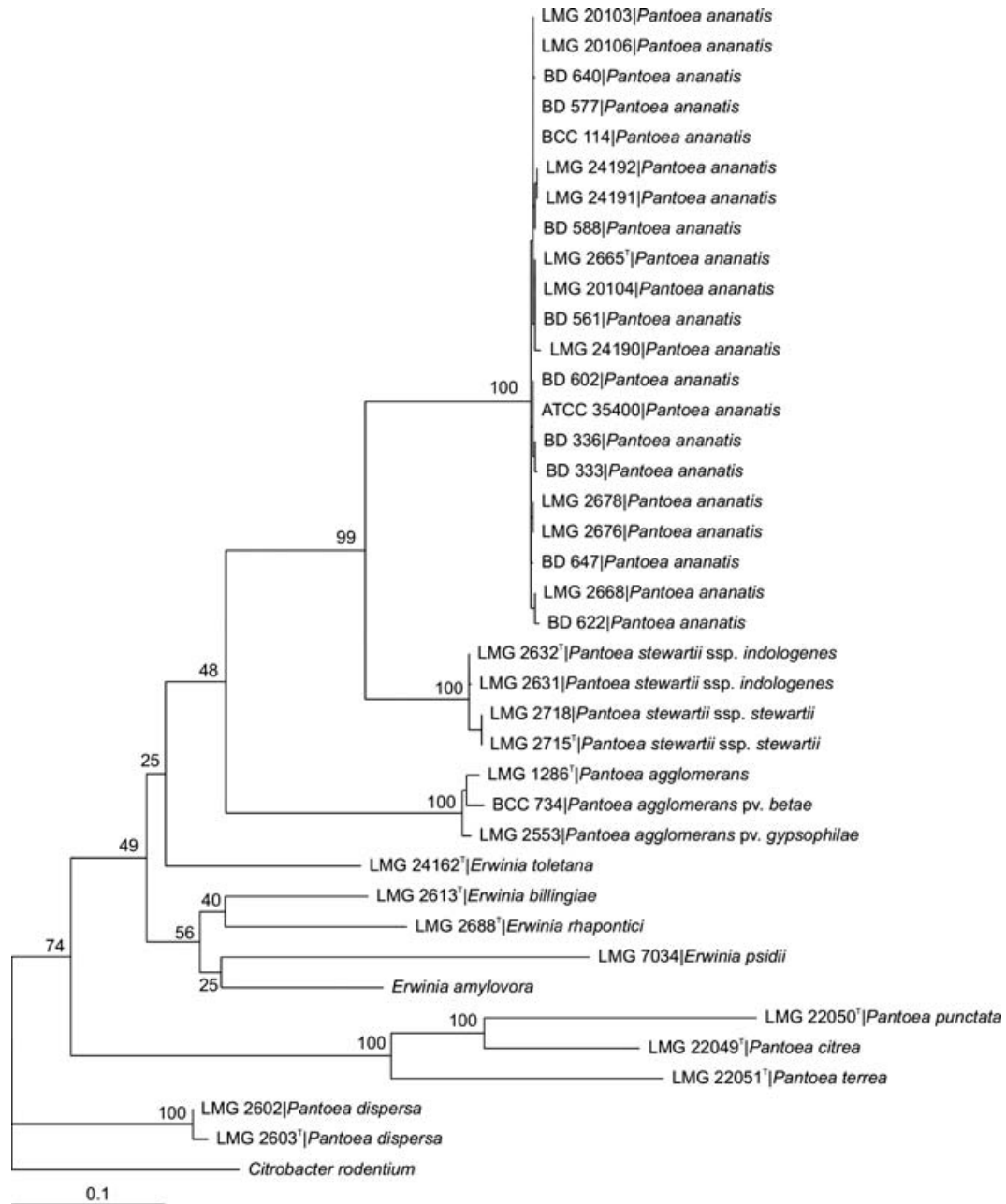


Fig. 1 Maximum likelihood tree based on the *gyrB* sequences of *Pantoea* species and their closest phylogenetic neighbours. Bootstrap values after 1000 replicates are expressed as percentages. *Citrobacter rodentium* was included as an outgroup. BD 333 and 336 were isolated from onion seed in South Africa, ATCC 35400 from honeydew melons in the USA, LMG 2676 and 2678 from *Puccinia graminis* in the USA and Zimbabwe, respectively, LMG 2668 and 2665 from pineapple in Hawaii and Brazil, respectively, LMG 20103, 20104, 20106 and BD 114 from *Eucalyptus* in South Africa, LMG 24190 from onion in the USA, and BD 561, 577, 588, 602, 622, 640, 647 and LMG 24191 and 24192 from maize in South Africa.

In pepper, *P. ananatis* promoted significant growth and induced systemic resistance against *Xanthomonas axonopodis* pv. *vesicatoria* (Kang *et al.*, 2007). Likewise, when shoot tips of papaya were inoculated with *P. ananatis* strains originally acquired as endophytic residents from within papaya tissue, they displayed significantly better root and shoot growth (Thomas *et al.*, 2007).

Strains found to occur endophytically in maize kernels have *in vitro* activity against *Lecanicillium aphanocladii* (Rijavec *et al.*, 2007).

There are few reports of *P. ananatis* behaving as a latent pathogen within susceptible host tissue. It has been reported as an endophyte in rice plants (Mano and Morisaki, 2008), and we

Table 1 Host range of *Pantoea ananatis*

Natural host	After artificial inoculation	Symptoms	Reference
Pineapple		Fruitlet rot	Serrano (1928)
	Sugarcane	Leaf streaks	Serrano (1928)
Sudangrass		Leaf blotches and streaks	Azad <i>et al.</i> (2000)
	Sorghum and oats	Leaf blotches and streaks	Azad <i>et al.</i> (2000)
Cantaloupe fruit		Brown spot	Bruton <i>et al.</i> (1991)
Honeydew melons		Brown spot	Ceponis <i>et al.</i> (1985)
Onions including giant onions		Leaf blight, seed stalk rot, bulb decay	Gitaitis and Gay (1997) Yumiko <i>et al.</i> (2005)
Eucalypts		Leaf blight, shoot tip die-back	Coutinho <i>et al.</i> (2002)
Maize		Necrotic spots and streaks	Paccola-Meirelles <i>et al.</i> (2001)
		Brown stalk rot	Goszczyńska <i>et al.</i> (2007)
Rice		'Palea' browning	Tabei <i>et al.</i> (1988)
		Stalk rot	Cother <i>et al.</i> (2004)
Tomato		'Graywall'	Stall <i>et al.</i> (1969)
<i>Puccinia graminis</i> (causal agent of leaf rust of wheat)		Parasite of the rust	Pon <i>et al.</i> (1954)
King oyster mushroom		Soft rot	Kim <i>et al.</i> (2007)
	Cotton	Internal boll rot	Bell <i>et al.</i> (2007)
Watermelon			Walcott <i>et al.</i> (2003)

have also isolated *P. ananatis* as an endophyte from both susceptible and resistant *Eucalyptus grandis* × *Eu. nitens* (GN) clones in South Africa (T. A. Coutinho and S. N. Venter, unpublished results). In susceptible clones, this pathogen causes leaf blight and die-back (Coutinho *et al.*, 2002). *Pantoea ananatis* also occurs endophytically in rice seed (Mano *et al.*, 2006; Okunishi *et al.*, 2005) and maize kernels (Rijavec *et al.*, 2007). Both are known hosts of this pathogen and its occurrence within seed may be of considerable epidemiological significance.

SAPROPHYTIC NATURE OF *P. ANANATIS*

Pantoea ananatis has been isolated from a diverse range of environments as a saprophyte. These include rivers (Morohoshi *et al.*, 2007), soil (Lai and Hsu, 1974), aviation fuel tanks (Rauch *et al.*, 2006), sorghum fermentation (Mohammed *et al.*, 1991), from the rhizosphere of soft rush (*Juncus effusus*) (Halda-Alija, 2003) and on ticks (Murrell *et al.*, 2003), and is a common inhabitant of the gut microflora of brown plant hoppers (*Nilaparvata lugens*) (Watanabe *et al.*, 1996), mulberry pyralid (*Glyphodes pyloalis*) (Takahashi *et al.*, 1995), cotton fleahoppers (*Pseudatomoscelis seriatus*) (Bell *et al.*, 2007) and tobacco thrips (*Frankliniella fusca*) (Gitaitis *et al.*, 2003; Wells *et al.*, 2002). *Pantoea ananatis* has also been isolated from *Pinus elliottii* roots colonized by ectomycorrhizal fungi in Australia (Izumi *et al.*, 2008). Many of these studies which show that *P. ananatis* is present as a saprophyte involved the analyses of bacterial communities either occurring in or contaminating a specific location. The identification of the inhabitants of these sites was mostly based on partial sequencing of the 16S rRNA gene and determining the similarity of sequences

to those in GENBANK. This introduces an element of caution into the interpretation of these results, and one cannot state with absolute confidence that *P. ananatis* does indeed reside in these habitats.

PANTOEA ANANATIS AS A PATHOGEN

Host range and symptom expression

Pantoea ananatis infects both monocotyledonous and dicotyledonous plants (Table 1). This bacterium has also been deposited in numerous culture collections, where its role, as an epiphyte or pathogen, is not clearly indicated. These hosts include *Cattleya* sp. (LMG 2807), *Musa* sp. (LMG 2628), *Cassia pectuta* (ICMP 12183) and sugarcane (ICMP 10132). The symptoms caused by *P. ananatis* are diverse depending on the host infected, and include blotches and spots (Fig. 2A), die-back, fruit, boll and bulb rot (Fig. 2B), and decay (Fig. 2C). In the case of infection of honeydew melons and cantaloupes, the brown spot symptoms occur only after harvesting (Bruton *et al.*, 1991; Wells *et al.*, 1987). The disease apparently originates in the form of field infections that remain quiescent until the fruit ripens. Following the infection of young *Eucalyptus* trees or seedlings/cuttings with *P. ananatis*, the shoots wilt and die-back occurs (Coutinho *et al.*, 2002). The pathogen appears to spread from the petioles into the main leaf veins, and from these parts into adjacent leaf tissue. In cases in which monocotyledonous plants are infected, symptoms are in the form of blotches and streaks, forming parallel to the main leaf vein (Azad *et al.*, 2000; Paccola-Meirelles *et al.*, 2001). Outbreaks of diseases caused by *P. ananatis* are usually sporadic, possibly as a result of its opportunistic nature.

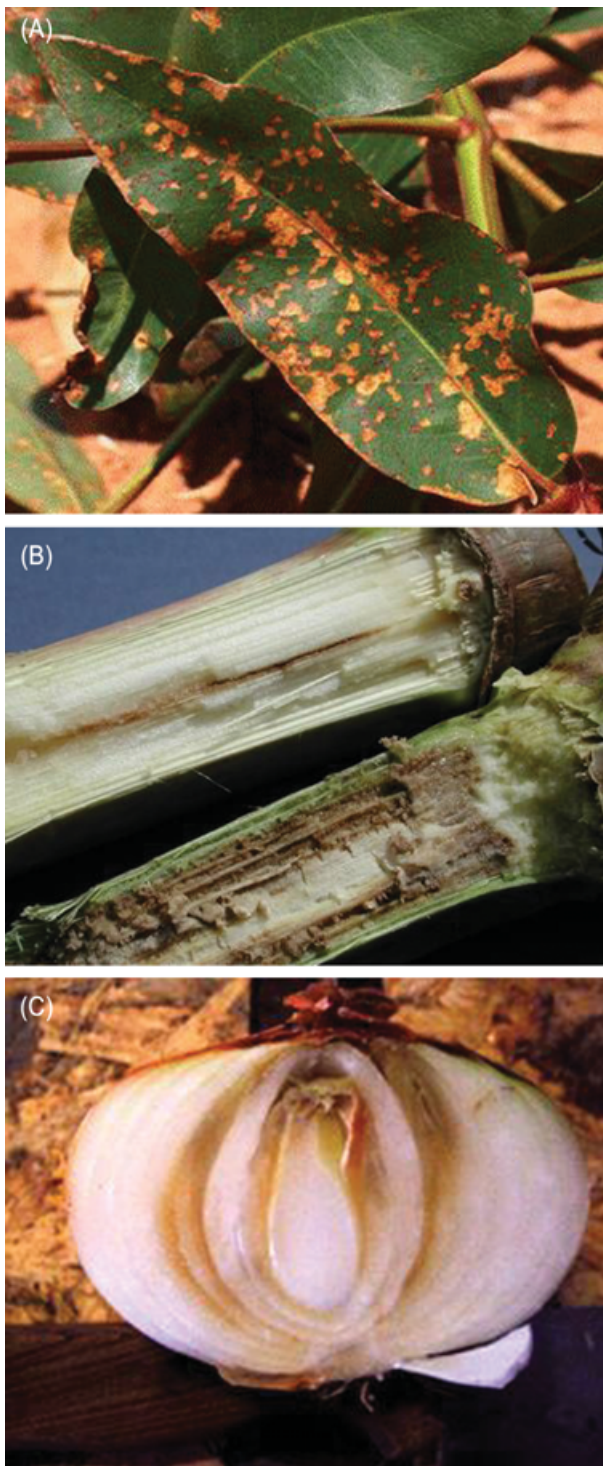


Fig. 2 Disease symptoms caused by *Pantoea ananatis*. (A) Bacterial blight of *Eucalyptus*. (B) Brown stalk rot of maize (photograph courtesy of Dr Teresa Goszczynska, Agricultural Research Council-Plant Protection Research Institute, Pretoria, South Africa). (C) Centre rot of onion (photograph courtesy of Professor Ron Gitaitis, University of Georgia, Tifton, GA, USA).

Of particular interest is that symptoms caused by *P. ananatis* on the same host may differ from country to country. On maize in Brazil, the symptoms described are necrotic or white leaf spots and streaks (Bomfeti *et al.*, 2008; Paccola-Meirelles *et al.*, 2001), whereas, in South Africa, infection results in stalk rot (Goszczynska *et al.*, 2007). Similarly, on rice in Japan and elsewhere in the world, the pathogen infects the developing seed causing palea browning, whereas, in Australia, the symptom caused by *P. ananatis* is stem necrosis (Cother *et al.*, 2004).

Epidemiology

The epidemiology of plant diseases caused by *P. ananatis* on different hosts is relatively unknown. What has been established is that the pathogen enters its host through flowers (Hasegawa *et al.*, 2003; Serrano, 1928) and/or wounds created by feeding insects (Gitaitis *et al.*, 2003; Watanabe *et al.*, 1996; Wells *et al.*, 2002), mechanical injury (Serrano, 1928) and plant to plant contact during high winds (Azad *et al.*, 2000; Cother *et al.*, 2004). The development of brown hopper burn symptoms on rice was found to be accelerated when *P. ananatis* was present on the leaf surfaces. Gitaitis *et al.* (2003) were also able to show that tobacco thrips vector *P. ananatis* in onion fields. Although *P. ananatis* was isolated from Miridae feeding on infected plant tissue in a field outbreak of blight and die-back of eucalypts in South Africa, its exact role in disease outbreaks is currently unknown (J. Roux and T. A. Coutinho, unpublished results).

Pantoea ananatis is both seed-borne and seed-transmitted in onions (Goszczynska *et al.*, 2006a; Walcott *et al.*, 2002), sudangrass (Azad *et al.*, 2000) and rice (Azegami *et al.*, 1983; Tabei *et al.*, 1988). Together with a number of other bacterial species, this bacterium was also found in buckwheat seed (Iimura and Hosono, 1996) and in maize kernels (Rijavec *et al.*, 2007). The recent appearances of bacterial blight and die-back on *Eucalyptus* in countries that have purchased seed from South Africa suggest seed transmission (Dr. G. Nakabonge, FABI, University of Pretoria, Pretoria, South Africa, unpublished data). Similarly, outbreaks of centre rot of onions may be a result of the introduction of infested seed into new environments/countries (Gitaitis *et al.*, 2004; Goszczynska *et al.*, 2006a).

Environmental factors influence the severity of the diseases caused by *P. ananatis* on its different hosts. In the case of maize and *Eucalyptus*, high humidity and moderate temperature conditions (between 20 and 25 °C) increase the incidence and severity of the disease (Coutinho *et al.*, 2002; Paccola-Meirelles *et al.*, 2001). In contrast, on sudangrass, infection was found to be worst at a temperature of 32 °C and a high relative humidity (Azad *et al.*, 2000). This is similar to the situation with onions, where *P. ananatis* was found to be active at bulb formation when moisture was high and temperatures ranged from 28 to 35 °C (Schwartz *et al.*, 2003).

Economic importance

As a plant pathogen, *P. ananatis* can lead to serious economic losses. In the case of sudangrass, blighted foliage can reach 50% or more of the total leaf area (Azad *et al.*, 2000). Losses of up to 100% have been recorded in the case of onions (Gitaitis and Gay, 1997). Severe infections of maize resulted in leaf senescence and a sharp decrease in grain size and weight (Pinto, 1995 cited by Paccola-Meirelles *et al.*, 2001). Where an outbreak of *P. ananatis* occurs in a *Eucalyptus* plantation, trees either fail to survive or are multistemmed (Coutinho *et al.*, 2002). What is of particular concern is the fact that this pathogen is able to infect a wide range of *Eucalyptus* species, hybrids and clones. If left unchecked, the disease on its numerous hosts has the potential to reach epidemic proportions under favourable environmental conditions. In addition to being a primary plant pathogen in the field, *P. ananatis* is also responsible for post-harvest losses of cantaloupe fruit (Bruton *et al.*, 1991), honeydew melons (Wells *et al.*, 1987) and onions (Gitaitis *et al.*, 2003).

Pathogenesis

Pantoea ananatis has been reported to produce indole-3-acetic acid (Enya *et al.*, 2007; Halda-Alija, 2003; Mano and Morisaki, 2008; Sessitsch *et al.*, 2004), which could play a role in pathogenesis. This plant growth promoter affects plants at very low concentrations and promotes cell wall loosening during cell elongation (Brandl and Lindow, 1998). The major virulence factors of *P. ananatis* are, however, currently unknown. The genome of a virulent strain of *P. ananatis* from eucalypts, as well as the type strain from pineapple, have recently been sequenced using 454 pyrosequencing (De Maayer *et al.*, 2008) and solexa technology, respectively, and detailed information will soon become available. Initial comparative genomics revealed the absence of the Type II, Type III and Type IV secretion systems in *P. ananatis*. These secretion systems are located on pathogenicity islands in a broad range of animal- and other plant-associated bacteria. The genome of *P. ananatis* does, however, contain a cluster of genes with high homology to members of the novel Type VI secretion system. This system plays a role in diseases caused by several human and animal- as well as plant-pathogenic Gram-negative bacteria, but whose function has yet to be elucidated fully (Mattinen *et al.*, 2008; Pukatzki *et al.*, 2006).

Pantoea ananatis produces quorum sensing-related signal molecules. Yoshida *et al.* (2006) revealed that, when this bacterium inhabited wheat heads, it produced at least two *N*-acyl-L-homoserine lactones (AHLs): *N*-hexanoyl-L-homoserine lactone (C6-HSL) and *N*-(3-oxohexanoyl)-L-homoserine lactone (3-oxo-C6-HSL). Pomini *et al.* (2006) reported that *P. ananatis* produced three AHLs with the major substance identified being C6-HSL. Morohoshi *et al.* (2007) identified the LuxRI homologue, EanRI,

and C6-HSL and 3-oxo-C6-HSL in a strain isolated from the Shirakwa river in Japan. They were able to show the involvement of the quorum-sensing system in the regulation of exopolysaccharide biosynthesis, biofilm formation and the infection of onion leaves.

Control

The control of diseases caused by *P. ananatis* is usually achieved through the deployment of resistant/tolerant cultivars/clones. Blight and die-back of eucalypts in South Africa are currently controlled by the use of resistant clones (T.A. Coutinho, unpublished data). They are selected in a cutting production nursery where the incidence of disease is high. Similarly, in a study by Paccola-Meirelles *et al.* (2002), it was discovered that, by artificially inoculating maize lines, it was possible to select genotypes resistant to *P. ananatis*. The control of white spot disease of maize in Brazil has also been achieved by applying the fungicide Mancozeb in the initial phases of disease development (Bomfeti *et al.*, 2007). The use of mulch and irrigation systems has been investigated for the control of centre rot of onion (Gitaitis *et al.*, 2004). Irrigation type had no effect on the incidence and severity of disease. However, the use of straw mulch or bare ground was found to delay symptom development by 7–14 days compared with the use of black plastic. Avoidance and eradication of the initial inoculum are probably the most appropriate management strategies that can be recommended against diseases caused by *P. ananatis* and most other phytopathogenic bacteria.

CONCLUSIONS

- The conclusive identification of strains of *P. ananatis* has been difficult in the past. However, the use of gene sequences, such as *gyrB* or *rpoB*, now provides plant pathologists with a tool to rapidly and reliably identify this species. In addition, this will allow bacteriologists to clarify the ecological role of this bacterium in the natural environment.
- The association of *P. ananatis* with plants as a pathogen has been known since 1928. In the past 20 years, new reports of *P. ananatis* on previously unreported hosts have highlighted the re-emergence of this bacterium as a potentially economically important plant pathogen.
- Little is known of how *P. ananatis* induces diseases in its hosts. However, now that the genomes of virulent strains from pineapple and eucalypts have been sequenced, information pertaining to pathogenicity and host specificity will become available. This approach will facilitate the development of novel approaches to pathogen control in the future.

ACKNOWLEDGEMENTS

We wish to thank the National Research Foundation (NRF) and the THRIP initiative of the Department of Trade and Industry for

funding. We would also like to thank Jolanda Roux for an early review of the manuscript and Carrie Brady for providing Fig. 1.

REFERENCES

- Abe, K., Watabe, S., Emori, Y., Watanabe, M. and Arai, S. (1989) An ice nucleation active gene of *Erwinia ananas*. Sequence similarity to those of *Pseudomonas* species and regions required for ice nucleation activity. *FEBS Lett.* **258**, 297–300.
- Anderson, P.K., Cunningham, A.A., Patel, N.G., Morales, F.J., Epstein, P.R. and Daszak, P. (2004) Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. *Trends Ecol. Evol.* **19**, 535–544.
- Azad, H.R., Holmes, G.J. and Cooksey, D.A. (2000) A new leaf blotch disease of sudangrass caused by *Pantoea ananas* and *Pantoea stewartii*. *Plant Dis.* **84**, 973–979.
- Azegami, K., Ozaki, K. and Matsuda, A. (1983) Bacterial palea browning, a new disease of rice caused by *Erwinia herbicola*. *Bull. Nat. Inst. Agric. Sci. Ser. C* **39**, 1–12.
- Bandyopadhyay, R. and Frederiksen, R.A. (1999) Contemporary global movement of emerging pathogens. *Ann. NY Acad. Sci.* **894**, 28–36.
- Beji, A., Mergaert, J., Gavini, F., Izard, D., Kersters, K., Leclec, H. and De Ley, J. (1988) Subjective synonymy of *Erwinia herbicola*, *Erwinia milletiae*, and *Enterobacter agglomerans* and redefinition of the taxon by genotypic and phenotypic data. *Int. J. Syst. Bacteriol.* **38**, 77–88.
- Bell, A.A., Medrano, E.G., Lopez, J.D. and Luff, R.K. (2007) Transmission and importance of *Pantoea ananatis* during feeding on cotton buds (*Gossypium hirsutum* L.) by cotton fleahoppers (*Pseudatomoscelis seriatus* Reuter). *World Cotton Research Conference-4, Lubbock, TX, USA, 10–14 September 2007*. (Abstract.) <http://wrcr.confex.com/wrcr/2007/techprogram/P1835.HTM>
- Beyer, P., Al-Babili, S., Ye, X.-D., Lucca, P., Schaub, P., Welsch, R. and Potrykus, I. (2002) Golden rice: introducing the beta-carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat Vitamin A deficiency. *J. Nutr.* **132**, 506S–510S.
- Bomfeti, C.A., Meirelles, W.F., Souza-Paccola, E.A., Casela, C.R., Ferreira, A.S., Marriel, I.E. and Paccola-Meirelles, L.D. (2007) Evaluation of commercial chemical products *in vitro* and *in vivo* in the control of foliar disease, maize white spot, caused by *Pantoea ananatis*. *Summa Phytopathol.* **33**, 63–67.
- Bomfeti, C.A., Souza-Paccola, A., Massola Júnior, N.S., Marriel, I.E., Meirelles, W.F., Casela, C.R. and Paccola-Meirelles, L.D. (2008) Localization of *Pantoea ananatis* inside lesions of maize white spot disease using transmission electron microscopy and molecular techniques. *Trop. Plant Pathol.* **33**, 1–6.
- Brady, C., Venter, S.N., Cleenwerck, I., Vancanneyt, M., Swings, J. and Coutinho, T.A. (2007) An FAFLP-system for the improved identification of plant-pathogenic and -associated species of the genus *Pantoea*. *Syst. Appl. Microbiol.* **30**, 413–417.
- Brady, C., Cleenwerck, I., Venter, S.N., Vancanneyt, M., Swings, J. and Coutinho, T.A. (2008) Phylogeny and identification of *Pantoea* species associated with plants, humans and the natural environment based on multilocus sequence analysis (MLSA). *Syst. Appl. Microbiol.* **31**, 447–460.
- Brandl, M.T. and Lindow, S.E. (1998) Contribution of indole-3-acetic acid production to the epiphytic fitness of *Erwinia herbicola*. *Appl. Environ. Microbiol.* **64**, 3256–3263.
- Brenner, D.J., Fanning, G.R., Leete Knutson, J.K., Steigerwalt, A.G. and Krichevsky, M.L. (1984) Attempts to classify herbicola group-*Enterobacter agglomerans* strains by deoxyribonucleic acid hybridization and phenotypic tests. *Int. J. Syst. Bacteriol.* **34**, 45–55.
- Bright, C. (1998) *Life Out of Bounds. Bioinvasion in a Borderless World*. New York: W.W. Norton.
- Bruton, B.D., Wells, J.M., Lester, G.E. and Patterson, C.L. (1991) Pathogenicity and characterization of *Erwinia ananas* causing a postharvest disease of cantaloupe fruit. *Plant Dis.* **75**, 180–183.
- Ceponis, M.J., Wells, J.M. and Cappellini, R.A. (1985) Bacterial brown spot of honeydew melons. *HortSci.* **20**, 302–303.
- Cho, K.M., Hong, S.Y., Lee, S.M., Kim, Y.H., Kahng, G.G., Lim, Y.P., Kim, H. and Yun, H.D. (2007) Endophytic bacterial communities in ginseng and their antifungal activity against pathogens. *Microb. Ecol.* **54**, 341–351.
- Chun, D.T.W. and Perkins, H.H. (1997) Profile of bacterial genera associated with cotton from low endotoxin and high endotoxin growing regions. *Ann. Agric. Environ. Med.* **4**, 233–242.
- Coplin, D.L. and Kado, C.I. (2001) *Pantoea*. In: *Laboratory Guide for Identification of Plant Pathogenic Bacteria*, 3rd edn. (Schaad, N.W., Jones, J.B. and Chun, W., eds), pp. 73–83. St. Paul, MN: APS Press.
- Cortesi, P. and Pizzatti, C. (2007) Palea browning, a new disease of rice in Italy caused by *Pantoea ananatis*. *J. Plant Pathol.* **89**, S76.
- Cother, E.J., Reinke, R., McKenzie, C., Lanoiselet, V.M. and Noble, D.H. (2004) An unusual stem necrosis of rice caused by *Pantoea ananas* and the first record of this pathogen on rice in Australia. *Australas. Plant Pathol.* **33**, 495–503.
- Coutinho, T.A., Preisig, O., Mergaert, J., Cnockaert, M.C., Riedel, K.-H., Swings, J. and Wingfield, M.J. (2002) Bacterial blight and die-back of *Eucalyptus* species, hybrids and clones in South Africa. *Plant Dis.* **86**, 20–25.
- De Baere, T., Verhelst, R., Labit, C., Verschraegen, G., Wauters, G., Claeys, G. and Vanechoutte, M. (2004) Bacteremic infection with *Pantoea ananatis*. *J. Clin. Microbiol.* **42**, 4393–4395.
- De Maayer, P., Venter, S.N. and Coutinho, T.A. (2008) The genome sequence of *Pantoea ananatis*—a comparative approach. Proceedings of the 20th Congress of the South African Genetics Society, Pretoria, South Africa, 27–29 March 2008, p. 59. Department of Genetics, University of Pretoria, Pretoria, South Africa. (Abstract.)
- DePeng, Z., LongJun, C., FuZai, S. and TingChang, X. (1999) The ice nucleation active bacteria on poplar trees and their effects on the causes of freezing injury and induction of fungal canker. *Sci. Sil. Sin.* **35**, 53–57.
- Dye, D.W. (1968) A taxonomic study of the genus *Erwinia*. I. The 'Amylovora' Group. *N. Z. J. Sci.* **11**, 590–607.
- Dye, D.W. (1969a) A taxonomic study of the genus *Erwinia*. II. The 'Carotovora' Group. *N. Z. J. Sci.* **12**, 81–97.
- Dye, D.W. (1969b) A taxonomic study of the genus *Erwinia*. III. The 'Herbicola' group. *N. Z. J. Sci.* **12**, 223–236.
- Dye, D.W. (1969c) A taxonomic study of the genus *Erwinia*. IV. 'Atypical' *Erwinias* Group. *N. Z. J. Sci.* **12**, 833–839.
- Dye, D.W. (1981) A numerical taxonomic study of the genus *Erwinia*. *N. Z. J. Agric. Res.* **24**, 223–229.
- Enya, J., Shinohara, H., Yoshida, S., Tsukiboshi, T., Negishi, H., Suyama, K. and Tsushima, S. (2007) Culturable leaf-associated bacteria on tomato plants and their potential as biological control agents. *Microb. Ecol.* **53**, 524–436.
- Gavini, F., Mergaert, J., Beji, A., Mielcarek, C., Izard, D., Kersters, K. and De Ley, J. (1989) Transfer of *Enterobacter agglomerans* (Beijerinck 1888) Ewing and Fife 1972 to *Pantoea* gen. nov. as *Pantoea agglomerans*

- comb. nov. and description of *Pantoea dispersa* sp. nov. *Int. J. Syst. Bacteriol.* **39**, 337–345.
- Gevers, D., Cohan, F.M., Lawrence, J.G., Spratt, B.G., Coenye, T., Feil, E.J., Stakebrandt, E., van de Peer, Y., Vandamme, P., Thompson, L. and Swings, J. (2005) Re-evaluating prokaryotic species. *Nat. Rev. Microbiol.* **3**, 733–739.
- Gitaitis, R.D. and Gay, J.D. (1997) First report of leaf blight, seed stalk rot, and bulb decay of onion by *Pantoea ananatis* in Georgia. *Plant Dis.* **81**, 1096.
- Gitaitis, R.D., Walcott, R., Culpepper, S., Sanders, H., Zolobowska, L. and Langston, D. (2002) Recovery of *Pantoea ananatis*, causal agent of center rot of onion, from weeds and crops in Georgia, USA. *Crop Prot.* **21**, 983–989.
- Gitaitis, R.D., Walcott, R.R., Wells, M.L., Diaz Perez, J.C. and Sanders, F.H. (2003) Transmission of *Pantoea ananatis*, the causal agent of center rot of onion, by tobacco thrips, *Frankliniella fusca*. *Plant Dis.* **87**, 675–678.
- Gitaitis, R.D., Walcott, R.R., Sanders, H.F., Zolobowska, L. and Diaz-Perez, J.C. (2004) Effects of mulch and irrigation system on sweet onion: II. The epidemiology of center rot. *J. Am. Soc. Hortic. Sci.* **129**, 225–230.
- Goszczyńska, T., Moloto, V.M., Venter, S.N. and Coutinho, T.A. (2006a) Isolation and identification of *Pantoea ananatis* from onion seed in South Africa. *Seed Sci. Technol.* **34**, 655–668.
- Goszczyńska, T., Venter, S.N. and Coutinho, T.A. (2006b) PA 20, a semi-selective medium for isolation and enumeration of *Pantoea ananatis*. *J. Microbiol. Methods*, **64**, 225–231.
- Goszczyńska, T., Venter, S.N. and Coutinho, T.A. (2007) Isolation and identification of the causal agent of brown stalk rot, a new disease of corn in South Africa. *Plant Dis.* **91**, 711–718.
- Goto, M., Huang, B.L., Makino, T., Goto, T. and Inaba, T. (1988) A taxonomic study on ice nucleation-active bacteria isolated from gemisphere of tea (*Thea sinensis* L.), phylloplane of vegetables and flowers of *Magnolia denudata* Desr. *Ann. Phytopathol. Soc. Jpn.* **54**, 189–197 (Abstract).
- Grimont, P.A.D. and Grimont, F. (2005) Genus: *Pantoea*. In: *Bergey's Manual of Systematic Bacteriology*, Vol. 2 (Brenner, D.J., Krieg, N.R. and Staley, J.T., eds), pp. 713–720. *The Proteobacteria*, Part B, *The Gammaproteobacteria*, 2nd edn. New York: Springer.
- Halda-Alija, L. (2003) Identification of indole-3-acetic acid producing freshwater wetland rhizosphere bacteria associated with *Juncus effusus* L. *Can. J. Microbiol.* **49**, 781–787.
- Hasegawa, M., Azegami, K., Yoshida, H. and Otani, H. (2003) Behaviour of *Erwinia ananas* transformed with bioluminescence genes on rice plants. *J. Gen. Plant Pathol.* **69**, 267–270.
- Holt, J.G. (1977) *The Shorter Bergey's Manual of Determinative Bacteriology*, 8th edn. Baltimore, MD: Williams & Wilkins Company.
- Iimura, K. and Hosono, A. (1996) Biochemical characteristics of *Enterobacter agglomerans* and related strains found in buckwheat seeds. *Int. J. Food Microbiol.* **30**, 243–253.
- Iimura, K. and Hosono, A. (1998) Antifungal activities of bacteria endemic to buckwheat seeds. *Fagopyrum*, **15**, 42–54 (Abstract).
- Izumi, H., Cairney, J.W.G., Killham, K., Moore, E., Alexander, I.J. and Anderson, I.C. (2008) Bacteria associated with ectomycorrhizas of slash pine (*Pinus elliottii*) in south-eastern Queensland, Australia. *Fems Microbiol. Lett.* **282**, 196–204.
- Kageyama, B., Nakae, M., Yagi, S. and Sonoyama, T. (1992) *Pantoea punctata* sp. nov., *Pantoea citrea* sp. nov., and *Pantoea terrea* sp. nov. isolated from fruit and soil samples. *Int. J. Syst. Bacteriol.* **42**, 203–210.
- Kang, S.H., Cho, H.S., Cheong, H., Ryu, C.M., Kim, J.F. and Park, S.H. (2007) Two bacterial endophytes eliciting both growth promotion and plant defense on pepper (*Capsicum annuum* L.). *J. Microbiol. Biotechnol.* **17**, 96–103.
- Kim, M.K., Ryu, J.S., Lee, Y.H. and Yun, H.D. (2007) First report of *Pantoea* sp. induced soft rot disease of *Pleurotus eryngii* in Korea. *Plant Dis.* **91**, 109.
- Kim, Y.C., Kim, K.C. and Choi, B.H. (1989) Palea browning disease of rice caused by *Erwinia herbicola* and ice nucleation activity of the pathogenic bacterium. *Korean J. Plant Pathol.* **5**, 72–79.
- Lai, S-C. and Hsu, S-T. (1974) Survival of *Erwinia ananas* in soil. *Plant Protect. Bull., Taiwan*, **16**, 12–19 (Abstract).
- Legard, D.E., McQuilken, M.P., Whipps, J.M., Penlon, J.S., Fermor, T.R., Thompson, I.P., Bailey, M.J. and Lynch, J.M. (1994) Studies on the seasonal changes in the microbial populations on the phyllosphere of spring wheat as a prelude to the release of genetically modified microorganisms. *Agric. Ecosys. Environ.* **50**, 87–101.
- Lindow, S.E. (1983) The role of bacterial ice nucleation in frost injury to plants. *Annu. Rev. Phytopathol.* **21**, 363–384.
- Lodewyckx, C., Vangronsveld, J., Porteous, F., Moore, E.R.B., Taghavi, S., Mezgeay, M. and Van der Lelie, D. (2002) Endophytic bacteria and their potential applications. *Crit. Rev. Plant Sci.* **21**, 583–606.
- Mano, J. and Morisaki, H. (2008) Endophytic bacteria in the rice plant. *Microbes Environ.* **23**, 109–117.
- Mano, J., Tanaka, F., Watanabe, A., Kaga, H., Okunishi, S. and Morisaki, H. (2006) Culturable surface and endophytic bacterial flora of the maturing seeds of rice plants (*Oryza sativa*) cultivated in a paddy field. *Microbes Environ.* **21**, 86–100.
- Mattinen, L., Somervuo, P., Nykyri, J., Nissenen, R., Kouvonen, P., Corthals, G., Auvinen, P., Aittamaa, M., Valkonen, J.P.T. and Pirhonen, M. (2008) Microarray profiling of host-extract-induced genes and characterization of the type VI secretion cluster in the potato pathogen *Pectobacterium atrosepticum*. *Microbiology*, **154**, 2387–2396.
- Mergaert, J., Gavini, J., Kersters, K., Leclerc, H. and De Ley, J. (1983) Phenotypic and protein electrophoretic similarities between strains of *Enterobacter agglomerans*, *Erwinia herbicola*, and *Erwinia milletiae* from clinical or plant origin. *Curr. Microbiol.* **8**, 327–331.
- Mergaert, J., Verdonck, L., Kersters, K., Swings, J., Boeufgras, J-M. and De Ley, J. (1984) Numerical taxonomy of *Erwinia* species using API systems. *J. Gen. Microbiol.* **130**, 1893–1810.
- Mergaert, J., Verdonck, L. and Kersters, K. (1993) Transfer of *Erwinia ananas* (synonym, *Erwinia uredovora*) and *Erwinia stewartii* to the Genus *Pantoea* emend. as *Pantoea ananas* (Serrano 1928) comb. nov. and *Pantoea stewartii* (Smith 1898) comb. nov., respectively, and description of *Pantoea stewartii* subsp. *indologenes* subsp. nov. *Int. J. Syst. Bacteriol.* **43**, 162–173.
- Mohammed, S.I., Steenson, L.R. and Kirleis, A.W. (1991) Isolation and characterization of microorganisms associated with traditional sorghum fermentation for production of Sudanese Kisra. *Appl. Environ. Microbiol.* **57**, 2529–2533.
- Morohoshi, T., Nakamura, Y., Yamazaki, G., Ishida, A., Kato, N. and Ikeda, T. (2007) The plant pathogen *Pantoea ananatis* produces N-acylhomoserine lactone and causes center rot disease of onion by quorum sensing. *J. Bacteriol.* **189**, 8333–8338.
- Murrell, A., Dobson, S.J., Yang, X., Lacey, E. and Barker, S.C. (2003) A survey of bacterial diversity in ticks, lice and fleas from Australia. *Parasitol. Res.* **89**, 326–334.

- Nakanishi, Y., Adanonon, A., Okabe, I., Hoshino, Y.T. and Matsumoto, N. (2006) An oligonucleotide probe for the detection of *Erwinia herbicola* and *Erwinia ananas*. *J. Gen. Plant Pathol.* **72**, 328–333.
- Nunes, F.V. and de Melo, I.S. (2006) Isolation and characterisation of endophytic bacteria of coffee plants and their potential in caffeine degradation. *Environ. Toxicol.* **1**, 293–297.
- Obata, H., Takinami, K., Tanishita, J., Hasegawa, Y., Kawate, S., Tokuyama, T. and Ueno, T. (1990) Identification of a new ice-nucleating bacterium and its ice nucleation properties. *Agric. Biol. Chem.* **54**, 725–730.
- Okunishi, S., Sako, K., Mano, H., Imamura, A. and Morisaki, H. (2005) Bacterial flora of endophytes in the maturing seed of cultivated rice (*Oryza sativa*). *Microbes Environ.* **20**, 168–177.
- Paccola-Meirelles, L.D., Ferreira, A.S., Meirelles, W.F., Marriel, I.E. and Casela, C.R. (2001) Detection of a bacterium associated with leaf spot disease of maize in Brazil. *J. Phytopathol.* **149**, 275–279.
- Paccola-Meirelles, L.D., Meirelles, W.F., Parentoni, S.N., Marriel, I.E., Ferreira, A.S. and Casela, C.R. (2002) Reaction of maize inbred lines to the bacterium *Pantoea ananatis* isolated from Phaeosphaeria leaf spot lesions. *Crop Breed. Appl. Biotechnol.* **2**, 587–589.
- Pajk, P. (2004) Possibilities of application of abiotic methods for suppression of bacteria *Erwinia amylovora* (Burr.) Winsl. *et al.* in Slovenia. *Zbornik Referatov 1. Slovenskega Sadjarskega Kongresa z Mednarodno Udeležbo, Krško, Slovenia, 24–26 March 2004, Del 2, 2004* (M. Huaina, ed.) pp. 449–453. Krško, (Abstract).
- Palm, M.E. (1999) Mycology and world trade: a view from the front line. *Mycologia* **91**, 1–12.
- Pomini, A.M., Araújo, W.L. and Marsaioli, A.J. (2006) Structural elucidation and biological activity of acyl-homoserine lactones from the phytopathogen *Pantoea ananatis* Serrano 1928. *J. Chem. Ecol.* **32**, 1769–1778.
- Pon, D.S., Townsend, C.E., Wessman, G.E., Schmitt, C.G. and Kingsolver, C.H. (1954) A *Xanthomonas* parasitic on uredia of cereal rust. *Phytopathology*, **44**, 707–710.
- Pukatzki, S., Ma, M.T., Sturtevant, D., Krastins, B., Sarracino, D., Nelson, W.C., Heidelberg, J.F. and Mekalanos, J.J. (2006) Identification of a conserved bacterial protein secretion system in *Vibrio cholerae* using the *Dictyostelium* host model system. *Proc. Natl. Acad. Sci. USA*, **103**, 1528–1533.
- Rauch, M.E., Graef, H.W., Rozenzhak, S.M., Jones, S.E., Bleckmann, C.A., Kruger, R.L., Naik, R.R. and Stone, M.O. (2006) Characterization of microbial contamination in United States Air Force aviation fuel tanks. *J. Ind. Microbiol. Biotech.* **33**, 29–36.
- Rijavec, T., Lapanje, A., Dermastia, M. and Rupnik, M. (2007) Isolation of bacterial endophytes from germinated maize kernels. *Can. J. Microbiol.* **53**, 802–808.
- Schaad, N.W. (2001) Initial identification of common genera. In: *Laboratory Guide for Identification of Plant Pathogenic Bacteria*, 3rd edn. (Schaad, N.W., Jones, J.B. and Chun, W., eds), pp. 1–16. St. Paul, MN: APS Press.
- Schwartz, H.F. and Otto, K. (2000) First report of a leaf blight and bulb decay of onion by *Pantoea ananatis* in Colorado. *Plant Dis.* **84**, 808.
- Schwartz, H.F., Otto, K.L. and Gent, D.H. (2003) Relation of temperature and rainfall to development of *Xanthomonas* and *Pantoea* leaf blights of onion in Colorado. *Plant Dis.* **87**, 11–14.
- Serrano, F.B. (1928) Bacterial fruitlet brown-rot of pineapple in the Philippines. *Philippine J. Sci.* **36**, 271–324.
- Sessitsch, A., Reiter, B. and Berg, G. (2004) Endophytic bacterial communities of field-grown potato plants and their plant-growth-promoting and antagonistic abilities. *Can. J. Microbiol.* **50**, 239–249.
- Simeya, N., Numata, S., Nakjima, M., Haseba, A., Hibi, T. and Akutsu, K. (2003) Biological control of rice blast by the epiphytic bacterium *Erwinia ananatis* transformed with a chitinolytic enzyme gene from an antagonistic bacterium, *Serratia marcescens*. *J. Gen. Plant Pathol.* **69**, 276–282.
- Stackebrand, E. and Goebels, B.M. (1994) Taxonomic note: a place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.* **44**, 846–849.
- Stall, R.E., Alexander, L.J. and Hall, C.B. (1969) Effect of tobacco mosaic virus and bacterial infections on occurrence of graywall of tomato (*Erwinia ananatis*). *Fla. State Hortic. Soc. Proc.* **81**, 157–161.
- Sun, F.-Z., Zhao, T.-C., Wang, J.-J., Mu, F.-S., An, J.-Y. and Jin, Q.-M. (2005) Dynamic changes of ice nucleation active bacterial populations inhabiting corn in northern China. *Acta Ecol. Sin.* **25**, 785–790.
- Tabei, H., Azegami, K. and Fukuda, T. (1988) Infection site of rice grain with *Erwinia herbicola*, the causal agent of bacterial palea browning of rice. *Ann. Phytopathol. Soc. Jpn.* **54**, 637–639 (Abstract).
- Takahashi, K., Watanabe, K. and Sato, M. (1995) Survival and characteristics of ice nucleation-active bacteria on mulberry trees (*Morus* spp.) and in mulberry pyralid (*Glyphodes pyloalis*). *Ann. Phytopathol. Soc. Jpn.* **61**, 439–443 (Abstract).
- Thomas, P., Kumari, S., Swarna, G.K. and Gowda, T.K.S. (2007) Papaya shoot tip associated endophytic bacteria isolated from *in vitro* cultures and host–endophyte interaction *in vitro* and *in vivo*. *Can. J. Microbiol.* **53**, 380–390.
- Torres, R., Teixidó, N., Usali, J., Abadias, M. and Viñas, I. (2005) Post-harvest control of *Penicillium expansum* on pome fruits by the bacterium *Pantoea ananatis* CPA-3. *J. Hortic. Sci. Biotechnol.* **80**, 75–81.
- Trüper, H.G. and De'Clari, L. (1997) Taxonomic note: necessary correction of specific epithets formed as substantives (nouns) 'in apposition'. *Int. J. Syst. Bacteriol.* **47**, 908–909.
- Vantomme, R., Mergaert, J., Verdonck, L. and De Ley, J. (1989) Antagonistic effect *in vitro* of *Erwinia uredovora* LMG 2678 against some other bacteria. *J. Phytopathol.* **124**, 372–376.
- Verdonck, L., Mergaert, J., Rijckaert, C., Swings, J., Kersters, K. and De Ley, J. (1987) The genus *Erwinia*: a numerical analysis of phenotypic features. *Int. J. Syst. Bacteriol.* **37**, 4–18.
- Walcott, R.R., Gitaitis, R.D., Castro, A.C., Sanders, F.H. Jr. and Diaz-Perez, J.C. (2002) Natural infestation of onion seed by *Pantoea ananatis*, causal agent of center rot. *Plant Dis.* **86**, 106–111.
- Walcott, R.R., Gitaitis, R.D. and Castro, A.C. (2003) Role of blossoms in watermelon seed infestation of *Acidovorax avenae* subsp. *citrulli*. *Phytopathology*, **93**, 528–534.
- Waleron, M., Waleron, K., Podhajska, A.J. and Lojkowska, E. (2002) Genotyping of bacteria belonging to the former *Erwinia* genus by PCR-RFLP analysis of a *recA* gene fragment. *Microbiology*, **148**, 583–595.
- Wang, Z.H., Liao, M., Yang, W.Y., Chen, S.B., Huang, L. and Bian, Q.J. (2008) Isolation and identification of INA bacteria in hybrid 'Skiranui tangerine' citrus cultivars and their indoor control. *Acta Phytophyl. Sin.* **35**, 51–57 (Abstract).
- Watanabe, K. and Sato, M. (1999) Gut colonization of an ice nucleation active bacterium, *Erwinia (Pantoea) ananatis*, reduces the cold hardness of mulberry pyralid larvae. *Cryobiology*, **38**, 281–289.
- Watanabe, K., Kawakita, H. and Sato, M. (1996) Epiphytic bacterium, *Erwinia ananatis*, commonly isolated from rice plants and brown planthoppers (*Nilaparvata lugens*) in hopperburn patches. *Appl. Entomol. Zool.* **31**, 459–462.

- Watanabe, M. and Arai, S.** (1994) Bacterial ice-nucleation activity and its application to freeze concentration of fresh foods for modification of their properties. *J. Food Eng.* **22**, 453–473.
- Wells, J.M., Sheng, W-S., Ceponis, M.J. and Chen, T.A.** (1987) Isolation and characterization of strains of *Erwinia ananas* from honeydew melons. *Phytopathology*, **77**, 511–514.
- Wells, M.L., Gitaitis, R.D. and Sanders, F.H.** (2002) Association of tobacco thrips, *Frankliniella fusca* (Thysanoptera: Thripidae), with two species of bacteria of the genus *Pantoea*. *Ann. Entomol. Soc. Am.* **95**, 719–723.
- Winslow, C.E.A., Broadhurst, J., Buchanan, R.E., Krumwiede, C. Jr., Rogers, L.A. and Smith, G.H.** (1920) The families and genera of the bacteria. Final report of the Committee of the Society of American Bacteriologists on characterization and classification of bacterial types. *J. Bacteriol.* **5**, 191–229.
- Xiang, C.T., Song, F.Q., Liu, J.H., Dong, A.J., Xue, Y., Yuan, S.Z., Zhang, J.H., Jiang, X.D., Shi, X.F., Wang, C.W., Lin, H.B. and Han, W.X.** (2001) The dominant factors caused by INA bacterial canker. *J. N.E. For. Uni.* **29**, 109–113 (Abstract).
- Xie, G.L.** (2001) First report of palea browning in China and characterization of the causal organism by phenotypic tests and Biolog. *Int. Rice Res. Notes* **26**, 25–26.
- Yoshida, S., Kinkei, L.L., Shinohara, H., Numajiri, N., Hiradate, S., Koitabashi, M., Suyama, K., Negishi, H. and Tsushima, S.** (2006) Production of quorum-sensing-related signal molecules by epiphytic bacteria inhabiting wheat heads. *Can. J. Microbiol.* **52**, 411–418.
- Yumiko, T., Toshiyuki, M. and Yoshiaki, C.** (2005) Bacterial rot of *Allium giganteum* caused by *Erwinia ananas* (= *Pantoea ananatis*), and basal rot of *Belamcanda chinensis* caused by *Aphanomyces* sp. *Bull. Toyama Agric. Res. Cent.* **22**, 1–6 (Abstract).
- Zasykin, D.V. and Lee, T-C.** (1999) Extracellular ice nucleators from *Pantoea ananas*: effect of freezing on model foods. *J. Food Sci.* **64**, 473–478.
- Zeng, D.P., Chao, L.J., Sun, S.Z. and Zhou, T.C.** (1999) The ice nucleation active bacteria on poplar trees and their effects on the courses of freezing injury and induction of fungal canker. *Sci. Sil. Sin.* **35**, 53–57 (Abstract).
- Zhao, R.Y., Xu, M., Fu, Z.F., Sun, F.Z., Li, S.T., Yang, J.M. and Li, S.H.** (2007) Ice-nucleation-active bacterial species and their dynamics in Beijing apricot trees. *Sci. Agric. Sin.* **40**, 1174–1180 (Abstract).