

# Attenuation of *Pseudomonas aeruginosa* virulence by medicinal plants in a *Caenorhabditis elegans* model system

Allison Adonizio,<sup>1</sup> Sixto M. Leal, Jr,<sup>1</sup> Frederick M. Ausubel<sup>3</sup>  
and Kalai Mathee<sup>1,2</sup>

Correspondence  
Kalai Mathee  
Kalai.Mathee@fiu.edu

<sup>1</sup>Department of Biological Sciences, College of Arts and Sciences, Florida International University, Miami, FL 33199, USA

<sup>2</sup>Department of Molecular Microbiology and Immunology, College of Medicine, Florida International University, Miami, FL 33199, USA

<sup>3</sup>Department of Genetics, Harvard Medical School, and Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114, USA

Expression of a myriad of virulence factors and innate antibiotic resistance enables the opportunistic human pathogen *Pseudomonas aeruginosa* to create intractable infections. Using a nematode model, we screened for novel inhibitors of this pathogen. Aqueous extracts of three plants, *Conocarpus erectus*, *Callistemon viminalis* and *Bucida buceras*, were examined for their effects on *P. aeruginosa* killing of the nematode *Caenorhabditis elegans*. The results were evaluated in toxin-based and infection-based assays using *P. aeruginosa* strains PAO1 and PA14. The tested plant extracts prevented mortality via gut infection in approximately 60% of the worms and caused a 50–90% reduction in death from toxin production. All extracts inhibited nematode death by *P. aeruginosa* without host toxicity, indicating their potential for further development as anti-infectives.

Received 7 December 2007

Accepted 19 March 2008

## INTRODUCTION

*Pseudomonas aeruginosa* is one of the leading pathogens among patients suffering from cystic fibrosis, diffused pan-bronchitis and chronic obstructive pulmonary disease (Hoiby, 1994; Lieberman, 2003; Registry, 2005). In addition, it remains one of the major causes of nosocomial infections (National Nosocomial Infections Surveillance System, 2004). The success of this organism is attributed to numerous virulence factors (Smith & Iglewski, 2003b; Tang *et al.*, 1996), its ability to form biofilms (Costerton *et al.*, 1995) and innate antibiotic resistance (De Kievit *et al.*, 2001; Fisher *et al.*, 2005).

Conventional anti-pseudomonal treatment includes elevated doses of  $\beta$ -lactam, fluoroquinolone or aminoglycoside antibiotics (Hauser & Sriram, 2005). However, these drugs possess a high degree of toxicity, and mucoid strains of *P. aeruginosa* are rarely eradicated by these treatments (Hauser & Sriram, 2005; Pedersen, 1992). The failure of existing antibiotics to control infection makes it crucial to find alternatives to currently available drugs. Since pathogenicity in many bacteria is regulated by quorum sensing (QS), or cell-to-cell communication, inhibition of this system can cause attenuation of virulence and protect

against infection (Hentzer & Givskov, 2003; Juhas *et al.*, 2005; Smith & Iglewski, 2003a).

Plants have evolved numerous chemical strategies for deterring pathogen attack, including the production of bactericidal and anti-infective compounds, leading to their use as medicines (reviewed by Lewis & Ausubel, 2006). In our previous work, we demonstrated that a number of medicinal plants exhibit anti-QS activity (Adonizio *et al.*, 2006). Extracts of these plants were later shown to have an effect on virulence factor production, biofilm formation, QS gene expression and autoinducer production in *P. aeruginosa* (Adonizio *et al.*, 2008). In this study, we assessed the ability of three plant extracts to attenuate *P. aeruginosa* killing of the nematode *Caenorhabditis elegans*.

*Caenorhabditis elegans* is well established as a pertinent and practical model for studying bacterial virulence (Darby *et al.*, 1999; Tan & Ausubel, 2000), as a number of *P. aeruginosa* factors important in the killing of *Caenorhabditis elegans* are also relevant to mammalian systems (Rahme *et al.*, 1995; Tan *et al.*, 1999a). ‘Fast killing’ of *Caenorhabditis elegans* by *P. aeruginosa* strain PA14 (on rich media) is mediated through the production of virulence factors such as phenazines, whereas ‘slow killing’ (on minimal media) occurs via ingestion of the bacteria and subsequent infection (Mahajan-Miklos *et al.*, 1999;

Abbreviation: QS, quorum sensing.

Tan *et al.*, 1999a, b). The related strain PAO1 causes death through cyanide poisoning and neuromuscular paralysis (Gallagher & Manoil, 2001). Importantly, *P. aeruginosa*-mediated killing of *Caenorhabditis elegans* in all three of these cases is dependent in part on the QS system. Thus addition of plant compounds that have an effect on *P. aeruginosa* QS should attenuate virulence factor production and the subsequent death of *Caenorhabditis elegans*.

The advantage of using a live animal model when screening for anti-infective compounds is that both the efficacy and the host toxicity of a plant extract can be tested concurrently. In this report, we show that extracts from three different plant species caused a marked decrease in *P. aeruginosa*-mediated killing of *Caenorhabditis elegans* without affecting worm fitness on *Escherichia coli*. This approach can be expanded to the screening of natural product libraries or native extract sources.

## METHODS

**Preparation of plant extracts.** Samples of *Conocarpus erectus* (Combretaceae), *Callistemon viminalis* (Myrtaceae) and *Bucida buceras* (Combretaceae) were collected and processed according to methods described previously (Adonizio *et al.*, 2006). Briefly, pulverized plant material was extracted in boiling water, freeze-dried using a lyophilizer and stored at  $-20^{\circ}\text{C}$  until needed. Lyophilized extracts were reconstituted in a small volume of sterile water and added to molten agar at a final concentration of  $1\text{ mg ml}^{-1}$ . This concentration was chosen based on original dose-dependence studies and subsequent analysis of the extracts against *P. aeruginosa* (Adonizio *et al.*, 2006, 2008). This also allowed comparison of experiments in this paper with those in previous studies.

**Bacterial strains and growth conditions.** Because the tested extracts have been shown to affect the bacterial QS system (Adonizio *et al.*, 2006, 2008), the QS mutant  $\Delta lasR$  was used as a reference strain. Wild-type *P. aeruginosa* PAO1 (Holloway & Morgan, 1986) and its isogenic mutant PAO1 $\Delta lasR$  (Gambello & Iglewski, 1991) were used in the paralytic assay. Wild-type *P. aeruginosa* PA14 (Rahme *et al.*, 1995) and its PA14 $\Delta lasR$  (Liberati *et al.*, 2006) were used in the slow-killing and fast-killing assays. *E. coli* OP50 was used as the control in all assays, as this strain is the standard laboratory food of *Caenorhabditis elegans* (Brenner, 1974). All bacterial strains were grown overnight in Luria–Bertani broth at  $37^{\circ}\text{C}$  and transferred to plates, depending on the required conditions.

**Nematode culture.** The wt *Caenorhabditis elegans* (Bristol) N2 hermaphrodite strain was used in this study (Brenner, 1974). Worms were synchronized by hypochlorite treatment of gravid adults, hatching of the eggs overnight in M9 minimum buffer (Brenner, 1974) and plating L1-stage worms onto lawns of *E. coli* on nematode growth medium plates (Brenner, 1974). Synchronized worms were grown to the L4 or young adult stage at  $25^{\circ}\text{C}$  for use in the killing assays.

***Caenorhabditis elegans* paralytic assay.** Brain heart infusion agar plates with or without plant extract were seeded with  $10\ \mu\text{l}$  of an overnight culture of *E. coli* OP50 or *P. aeruginosa* PAO1 or PAO1 $\Delta lasR$  and incubated at  $37^{\circ}\text{C}$  for 24 h to form lawns of bacteria (Darby *et al.*, 1999). Nematodes were washed off stock plates and suspended in a minimal volume of M9 buffer (pH 6.5). Droplets containing 20–40 adult nematodes were placed onto the bacterial lawns and the plates were incubated at room temperature ( $21\text{--}23^{\circ}\text{C}$ ).

Worms were evaluated for viability every hour for a total of 4 h. Worms were scored as dead when they no longer responded to physical stimuli.

***Caenorhabditis elegans* fast-killing assay.** Fast-killing plates (peptone/glucose medium with 0.15 M sorbitol; Mahajan-Miklos *et al.*, 1999) with or without plant extract were seeded with  $10\ \mu\text{l}$  of an overnight culture of OP50, PA14 or PA14 $\Delta lasR$ . Plates were incubated for 24 h at  $37^{\circ}\text{C}$  and then at room temperature ( $21\text{--}23^{\circ}\text{C}$ ) for another 12 h. Approximately 20 L4-stage *Caenorhabditis elegans* were transferred with a wire pick onto plates at this time. Worms were evaluated for viability every hour for a total of 4 h. As in the previous assay, worms were considered dead when they no longer responded to physical stimuli.

***Caenorhabditis elegans* slow-killing assay.** Slow-killing plates (modified nematode growth medium; Tan *et al.*, 1999b) with or without plant extract were seeded with  $10\ \mu\text{l}$  of an overnight culture of OP50, PA14 or PA14 $\Delta lasR$ . Plates were incubated for 24 h at  $37^{\circ}\text{C}$  and then at room temperature ( $21\text{--}23^{\circ}\text{C}$ ) for another 24 h. Approximately 20 L4-stage *Caenorhabditis elegans* were transferred onto plates at this time. Worms were evaluated for viability every 2–4 h for a total of 58 h. As in the previous assays, worms were considered dead when they no longer responded to physical stimuli.

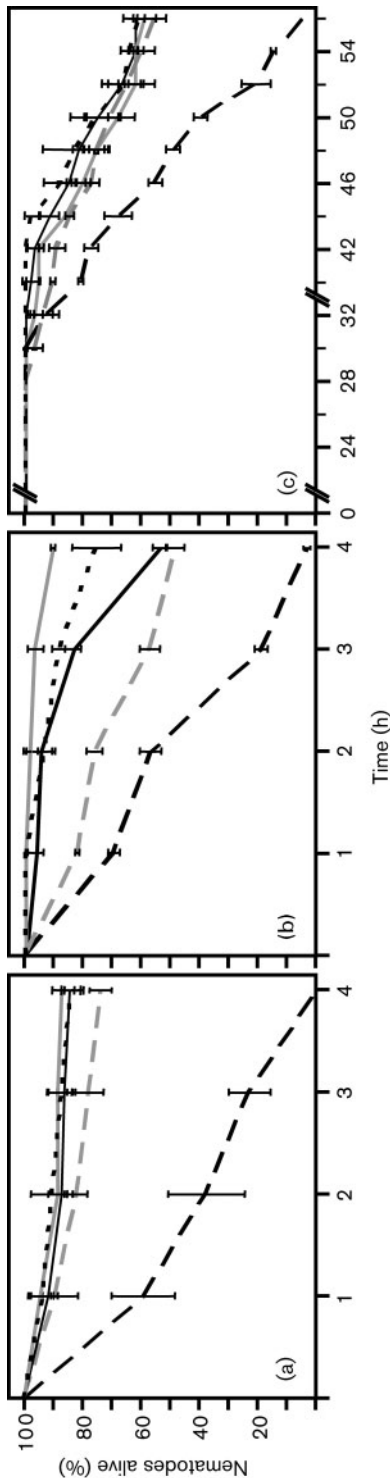
**Statistics and reproducibility.** All experiments were performed in triplicate. Killing curves represent the mean of three separate experiments. Data were analysed using one-way analysis of variance with a *P* value of 0.05 using the statistical software package SPSS. As the starting number of worms was different in each case, the percentage of worms still alive rather than the actual number of worms was used for comparison.

## RESULTS AND DISCUSSION

In this study, we assess the potential of extracts from *Conocarpus erectus*, *Callistemon viminalis* and *B. buceras* to reduce nematode death resulting from *P. aeruginosa* infection. Prior work on these plants revealed an effect on the bacterial QS system (Adonizio *et al.*, 2006, 2008); thus the QS mutant  $\Delta lasR$  was used as a reference strain. Although there is some precedence for testing plant extracts in a nematode model system (Rasmussen *et al.*, 2005), this is the first study to evaluate a statistically significant number of worms in both toxin and infection-based assays.

### Medicinal plants rescue paralytic killing of *Caenorhabditis elegans* by *P. aeruginosa*

Previous studies have shown that QS signalling is required for maximum levels of worm killing (Mahajan-Miklos *et al.*, 1999; Tan *et al.*, 1999b). Thus the success of these plant extracts against *P. aeruginosa* PAO1 in terms of blocking QS signalling (Adonizio *et al.*, 2008) suggested that they might also reduce death in a PAO1–nematode model. Approximately 50% of the worms died between 1 and 2 h after transfer to PAO1, with all nematodes dead after 4 h (Fig. 1a). In contrast, all of the worms on *E. coli* OP50 remained alive throughout the assay (not shown). As expected, the QS mutant PAO1 $\Delta lasR$  showed reduced nematode death, with 85% alive between 1 and 2 h and



**Fig. 1.** Nematode killing curves. The killing curves are compared between wild-type (PAO1 or PA14), QS mutant ( $\Delta lasR$ ) and wild-type with plant extract. (a) Paralytic (PAO1) and (b) fast-killing (PA14) assays for *Caenorhabditis elegans*. Black dashed line, *P. aeruginosa* wild-type; grey dashed line,  $\Delta lasR$  mutant; black dots, wt + *Callistemon viminalis*; black line, wt + *Conocarpus erectus*; grey line, wt + *B. buceras*. In all cases, the addition of plant extract rescued the worms to a level at or above a  $\Delta lasR$  QS mutant.

74 % still alive at 4 h (Fig. 1a). After 4 h, approximately 85, 84 and 87 % of the worms were alive on PAO1 plates that contained *Conocarpus erectus*, *Callistemon viminalis* and *B. buceras* extracts, respectively (Fig. 1a). In this and all other assays in this study, there was no observable effect of the added plant extracts on worm survival, lifespan or brood size on *E. coli* OP50 (not shown), indicating a lack of toxicity of the compounds.

All three of the plant extracts, when added to plates containing PAO1, suppressed killing to a level greater than the QS mutant (Fig. 1a), i.e. there was a significant difference compared with PAO1 $\Delta lasR$  and the extract-containing plates ( $P < 0.05$  in all cases), but not among individual extracts at the end of the assay. All extract plates and the  $\Delta lasR$  mutant were significantly different from wild-type PAO1 without treatment.

Death via PAO1 is due to cyanide asphyxiation and paralysis of *Caenorhabditis elegans* (Gallagher & Manoil, 2001). The *hcn* operon in *P. aeruginosa* mediates cyanide production and is controlled by the QS regulators LasR and RhlR (Pessi & Haas, 2000). Attenuation of virulence and nematode mortality has been shown with both  $\Delta lasR$  (Darby *et al.*, 1999) and  $\Delta hcn$  (Gallagher & Manoil, 2001) strains. Thus the results from the paralytic assay suggested that the addition of these extracts was affecting the production of cyanide either through *hcn* directly or indirectly via the QS genes. The latter hypothesis agrees with our previous *in vitro* analysis in PAO1 (Adonizio *et al.*, 2008), which showed a significant reduction in *lasR* and *rhlR* gene activity by these extracts.

### Medicinal plants prevent fast killing of *Caenorhabditis elegans* by *P. aeruginosa*

The results of the fast-killing assay are shown in Fig. 1(b). On PA14, approximately 50 % of the worms were dead within 2 h, with all worms dead by 4 h. At this point, all of the worms on OP50 were still alive (not shown). Again, the QS mutant (PA14 $\Delta lasR$ ) reduced nematode death, with 75 % alive between 1 and 2 h and 47 % alive at 4 h. At the end of the assay, approximately 53, 75 or 90 % of the worms were alive on PAO1 plates with added *Conocarpus erectus*, *Callistemon viminalis* and *B. buceras* extract, respectively (Fig. 1b). As with PAO1, all three of the plant extracts suppressed killing at or above the level of the QS mutant. There was no significant difference between PA14 $\Delta lasR$  and the *Conocarpus erectus* extract plates at 4 h ( $P > 0.05$ ); however, the *Callistemon viminalis* and *B. buceras* extracts were significantly better at preventing worm death ( $P < 0.01$  in both cases). Extract-containing plates were all significantly different from each other, but still showed a large reduction in nematode death when compared with wild-type PA14 without treatment. Overall, there was a pronounced inhibitory effect of the plant extracts on *P. aeruginosa* PA14 fast killing of *Caenorhabditis elegans*.

Fast killing of *Caenorhabditis elegans* is mediated by the production of virulence factors such as phenazines

(Mahajan-Miklos *et al.*, 1999). A  $\Delta phnAphnB$  deletion mutant has been shown to abolish nematode death completely, whilst a  $TnphoA$  mutation of the related gene *phzB* has been shown to greatly reduce mortality in mice and *Arabidopsis* (Mahajan-Miklos *et al.*, 1999). Like many virulence factors, phenazines are partially under the control of the QS gene *rhlR* (Brint & Ohman, 1995; Latifi *et al.*, 1995). The results from the PA14 fast-killing assay suggested that the addition of extracts affected phenazine production, either directly through the *phz* and *phn* genes or indirectly through the QS system via *rhlR*. All three extracts were shown previously to significantly affect the *rhlI/R* system; however, *Conocarpus erectus* has less of an effect on N-acylhomoserine lactone production and biofilm formation (strain PAO1; Adonizio *et al.*, 2008) than either *B. buceras* or *Callistemon viminalis*. Although still successful, *Conocarpus erectus* was less efficient in preventing nematode death in the fast-killing assay than the other extracts.

### Plant extracts reduce the mortality of *Caenorhabditis elegans* due to slow killing by *P. aeruginosa*

The slow-killing assay left 50% of nematodes dead on PA14 between 48 and 50 h, with all worms dead by 58 h (Fig. 1c). The control worms on *E. coli* OP50 remained alive throughout the assay (not shown). The QS mutant (PA14 $\Delta lasR$ ) reduced nematode death, with 75% alive between 48 and 50 h and 53% alive at 58 h. At this time, approximately 60, 59 and 57% of worms were alive on PA14 plates with added *Conocarpus erectus*, *Callistemon viminalis* and *B. buceras* extract, respectively (Fig. 1c). All three of the plant extracts, when added to plates containing wild-type PA14, suppressed killing to the level of the QS mutant. There was no significant difference between PA14 $\Delta lasR$  and the extract plates or between individual extracts at 58 h ( $P > 0.05$  in all cases); however, all extracts were significantly different from PA14 without treatment, suggesting a marked effect of the plant extracts on *P. aeruginosa* infection of *Caenorhabditis elegans*.

Slow killing of *Caenorhabditis elegans* occurs over approximately 60 h due to ingestion of and subsequent infection by *P. aeruginosa* (Tan *et al.*, 1999a). Nematode mortality is attenuated by  $TnphoA$  mutations of *lasR* and *gacA* (Tan *et al.*, 1999b), suggesting that QS is required for the infection process. The addition of plant extracts in this assay drastically reduced nematode death, suggesting an effect on *lasR* or *gacA*. Previous work on these extracts corroborated the inhibitory effect on *lasR*; however, the effect on *gacA* was not tested directly (Adonizio *et al.*, 2008). An effect on either of these factors remains a plausible hypothesis.

### Conclusions

The three plant extracts from *Conocarpus erectus* (Combretaceae), *Callistemon viminalis* (Myrtaceae) and *B. buceras* (Combretaceae), in all three assays, showed a highly

significant reduction in virulence when compared with wild-type PAO1 and PA14 without treatment. Overall, the tested plant extracts reduced nematode death by approximately 60–90% on wild-type *P. aeruginosa*. In each case, this reduction was equal to or greater than that of the corresponding QS mutant strain. The fact that the plant extracts reduced virulence across the board suggests that they are possibly affecting an upstream QS gene such as *las* or *rhl*, or perhaps a global regulator such as GacA. This further corroborates our previous data on the anti-QS effect of these plant extracts (Adonizio *et al.*, 2008). All extracts inhibited nematode death without any significant bactericidal effect, leaving QS inhibition as a plausible hypothesis. In addition, none of the tested plants showed any toxicity in the nematode model, making them reasonable candidates for purification and drug development.

*Conocarpus erectus*, *B. buceras* and *Callistemon viminalis* (and other closely related species) have been used medicinally to treat bacterial infections either as teas or as poultices (Burkhill, 1985; Irvine, 1961; Melendez, 1982; Morton, 1981; Stewart & Percival, 1997). Thus the plants were extracted with hot water to provide greater congruity with traditional preparation methods. Although teas and poultices are many steps removed from modern formulae, traditional use suggests the potential success of topical or enteral routes of administration.

With the increase in bacterial resistance to antibiotics, we should look to the past in the hope of finding solutions for the future. Plants have been used medicinally for thousands of years and, even without marked antibiotic activity, these three plants are still efficacious in ameliorating disease. We have previously shown the activity of these plants on *P. aeruginosa* alone and, although the exact mechanism of action is not yet known, the nematode experiments described in this paper are consistent with their previous and potential further use as anti-infectives.

### ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support of the National Institute of Health, National Center for Alternative and Complementary Medicine (NCCAM) NRSA #1-T32-AT01060-01 (A. A. and K. M.), NCCAM 1-R15-AT002626-01 (K. M.), National Institute of General Medical Sciences (NIGMS) Research Initiative for Scientific Enhancement (RISE) program grant R25 GM61347 (A. A.), National Institute of Allergy and Infectious Diseases (NIAID) R01 AI072508 (F. M. A.), NIAID R01 AI064332 (F. M. A.) and the Cystic Fibrosis Foundation #ADONIZO6H0 (A. A.). We also thank the members of the Mathee and Ausubel laboratories for assistance and support, especially Terry Moy and Rhonda Feinbaum.

### REFERENCES

- Adonizio, A. L., Downum, K., Bennett, B. C. & Mathee, K. (2006). Anti-quorum sensing activity of medicinal plants in southern Florida. *J Ethnopharmacol* **105**, 427–435.

- Adonizio, A., Kong, K.-F. & Mathee, K. (2008). Inhibition of quorum sensing-controlled virulence factor production in *Pseudomonas aeruginosa* by South Florida plant extracts. *Antimicrob Agents Chemother* **52**, 198–203.
- Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71–94.
- Brint, J. M. & Ohman, D. E. (1995). Synthesis of multiple exoproducts in *Pseudomonas aeruginosa* is under the control of RhlR–RhlI, another set of regulators in strain PAO1 with homology to the autoinducer-responsive LuxR–LuxI family. *J Bacteriol* **177**, 7155–7163.
- Burkhill, H. M. (1985). *The Useful Plants of West Tropical Africa*. London: Kew Royal Botanic Gardens.
- Costerton, J. W., Lewandowski, Z., Caldwell, D. E., Korber, D. R. & Lappin-Scott, H. M. (1995). Microbial biofilms. *Annu Rev Microbiol* **49**, 711–745.
- Darby, C., Cosma, C. L., Thomas, J. H. & Manoil, C. (1999). Lethal paralysis of *Caenorhabditis elegans* by *Pseudomonas aeruginosa*. *Proc Natl Acad Sci U S A* **96**, 15202–15207.
- De Kievit, T. R., Parkins, M. D., Gillis, R. J., Srikumar, R., Ceri, H., Poole, K., Iglewski, B. H. & Storey, D. G. (2001). Multidrug efflux pumps: expression patterns and contribution to antibiotic resistance in *Pseudomonas aeruginosa* biofilms. *Antimicrob Agents Chemother* **45**, 1761–1770.
- Fisher, J. F., Meroueh, S. O. & Mobashery, S. (2005). Bacterial resistance to  $\beta$ -lactam antibiotics: compelling opportunism, compelling opportunity. *Chem Rev* **105**, 395–424.
- Gallagher, L. A. & Manoil, C. (2001). *Pseudomonas aeruginosa* PAO1 kills *Caenorhabditis elegans* by cyanide poisoning. *J Bacteriol* **183**, 6207–6214.
- Gambello, M. J. & Iglewski, B. H. (1991). Cloning and characterization of the *Pseudomonas aeruginosa lasR* gene, a transcriptional activator of elastase expression. *J Bacteriol* **173**, 3000–3009.
- Hauser, A. R. & Sriram, P. (2005). Severe *Pseudomonas aeruginosa* infections. Tackling the conundrum of drug resistance. *Postgrad Med* **117**, 41–48.
- Hentzer, M. & Givskov, M. (2003). Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections. *J Clin Invest* **112**, 1300–1307.
- Hoiby, N. (1994). Diffuse panbronchiolitis and cystic fibrosis: East meets West. *Thorax* **49**, 531–532.
- Holloway, B. W. & Morgan, A. F. (1986). Genome organization in *Pseudomonas*. *Annu Rev Microbiol* **40**, 79–105.
- Irvine, F. R. (1961). *Woody Plants of Ghana*. London: Oxford University Press.
- Juhas, M., Eberl, L. & Tumber, B. (2005). Quorum sensing: the power of cooperation in the world of *Pseudomonas*. *Environ Microbiol* **7**, 459–471.
- Latifi, A., Winson, M. K., Foglino, M., Bycroft, B. W., Stewart, G. S., Lazdunski, A. & Williams, P. (1995). Multiple homologues of LuxR and LuxI control expression of virulence determinants and secondary metabolites through quorum sensing in *Pseudomonas aeruginosa* PAO1. *Mol Microbiol* **17**, 333–343.
- Lewis, K. & Ausubel, F. M. (2006). Prospects for plant-derived antibacterials. *Nat Biotechnol* **24**, 1504–1507.
- Liberati, N. T., Urbach, J. M., Miyata, S., Lee, D. G., Drenkard, E., Wu, G., Villanueva, J., Wei, T. & Ausubel, F. M. (2006). An ordered, nonredundant library of *Pseudomonas aeruginosa* strain PA14 transposon insertion mutants. *Proc Natl Acad Sci U S A* **103**, 2833–2838.
- Lieberman, D. (2003). Pseudomonal infections in patients with COPD: epidemiology and management. *Am J Respir Med* **2**, 459–468.
- Mahajan-Miklos, S., Tan, M. W., Rahme, L. G. & Ausubel, F. M. (1999). Molecular mechanisms of bacterial virulence elucidated using a *Pseudomonas aeruginosa*–*Caenorhabditis elegans* pathogenesis model. *Cell* **96**, 47–56.
- Melendez, E. N. (1982). *Plantas Medicinales de Puerto Rico: Folklore y Fundamentos Científicos*. Río Piedras, PR: Editorial de la Universidad de Puerto Rico.
- Morton, J. F. (1981). *Atlas of Medicinal Plants of Middle America: Bahamas to Yucatan*. Springfield, IL: Charles C. Thomas.
- National Nosocomial Infections Surveillance System (2004). National Nosocomial Infections Surveillance (NNIS) system report, data summary from January 1992 through June 2004: a report from the NNIS System. *Am J Infect Control* **32**, 470–485.
- Pedersen, S. S. (1992). Lung infection with alginate-producing, mucoid *Pseudomonas aeruginosa* in cystic fibrosis. *APMIS Suppl* **28**, 1–79.
- Pessi, G. & Haas, D. (2000). Transcriptional control of the hydrogen cyanide biosynthetic genes *hcnABC* by the anaerobic regulator ANR and the quorum-sensing regulators LasR and RhlR in *Pseudomonas aeruginosa*. *J Bacteriol* **182**, 6940–6949.
- Rahme, L. G., Stevens, E. J., Wolfort, S. F., Shao, J., Tompkins, R. G. & Ausubel, F. M. (1995). Common virulence factors for bacterial pathogenicity in plants and animals. *Science* **268**, 1899–1902.
- Rasmussen, T. B., Bjarnsholt, T., Skindersoe, M. E., Hentzer, M., Kristoffersen, P., Kote, M., Nielsen, J., Eberl, L. & Givskov, M. (2005). Screening for quorum-sensing inhibitors (QSI) by use of a novel genetic system, the QSI Selector. *J Bacteriol* **187**, 1799–1814.
- Registry (2005). *Patient Registry 2004 Annual Report*. Bethesda, MD: Cystic Fibrosis Foundation.
- Smith, R. S. & Iglewski, B. H. (2003a). *Pseudomonas aeruginosa* quorum sensing as a potential antimicrobial target. *J Clin Invest* **112**, 1460–1465.
- Smith, R. S. & Iglewski, B. H. (2003b). *Pseudomonas aeruginosa* quorum-sensing systems and virulence. *Curr Opin Microbiol* **6**, 56–60.
- Stewart, K. & Percival, B. (1997). *Bush Foods of New South Wales: a Botanic Record and an Aboriginal Oral History*. Sydney: Royal Botanic Gardens.
- Tan, M. W. & Ausubel, F. M. (2000). *Caenorhabditis elegans*: a model genetic host to study *Pseudomonas aeruginosa* pathogenesis. *Curr Opin Microbiol* **3**, 29–34.
- Tan, M. W., Mahajan-Miklos, S. & Ausubel, F. M. (1999a). Killing of *Caenorhabditis elegans* by *Pseudomonas aeruginosa* used to model mammalian bacterial pathogenesis. *Proc Natl Acad Sci U S A* **96**, 715–720.
- Tan, M. W., Rahme, L. G., Sternberg, J. A., Tompkins, R. G. & Ausubel, F. M. (1999b). *Pseudomonas aeruginosa* killing of *Caenorhabditis elegans* used to identify *P. aeruginosa* virulence factors. *Proc Natl Acad Sci U S A* **96**, 2408–2413.
- Tang, H. B., DiMango, E., Bryan, R., Gambello, M., Iglewski, B. H., Goldberg, J. B. & Prince, A. (1996). Contribution of specific *Pseudomonas aeruginosa* virulence factors to pathogenesis of pneumonia in a neonatal mouse model of infection. *Infect Immun* **64**, 37–43.