

Inhibition of swarming and virulence factor expression in *Proteus mirabilis* by resveratrol

Won-Bo Wang,¹ Hsin-Chih Lai,^{2,3} Po-Ren Hsueh,³ Robin Y.-Y. Chiou,⁴ Shwu-Bin Lin^{2,3} and Shwu-Jen Liaw^{2,3}

Correspondence

Shwu-Jen Liaw
s-jliaw@ha.mc.ntu.edu.tw

^{1,2}Graduate Institute of Microbiology¹ and Department and Graduate Institute of Clinical Laboratory Sciences and Medical Biotechnology², College of Medicine, National Taiwan University, 10016 no. 1 Chang-Te Street, Taipei, Taiwan, Republic of China

³Department of Laboratory Medicine, National Taiwan University Hospital, Taipei, Taiwan, Republic of China

⁴Department of Food Science, National Chiayi University, Chiayi, Taiwan, Republic of China

Resveratrol (3,5,4-trihydroxy-*trans*-stilbene) is a phytoalexin compound with anti-inflammatory and antioxidant activities. The effect of resveratrol on swarming and virulence factor expression of *Proteus mirabilis*, an important pathogen infecting the urinary tract, was determined on swarming agar plates with and without the compound. Bacteria harvested at different times were assayed for cell length and the production of flagella, haemolysin and urease. Resveratrol inhibited *P. mirabilis* swarming and virulence factor expression in a dose-dependent manner. Resveratrol significantly inhibited swarming at 15 µg ml⁻¹, and completely inhibited swarming at 60 µg ml⁻¹. Inhibition of swarming and virulence factor expression was mediated through RsbA, a His-containing phosphotransmitter of the bacterial two-component signalling system possibly involved in quorum sensing. Complementation of an *rsbA*-defective mutant with the *rsbA* gene restored its responsiveness to resveratrol. The compound also inhibited the ability of *P. mirabilis* to invade human urothelial cells. These findings suggest that resveratrol has potential to be developed as an antimicrobial agent against *P. mirabilis* infection.

Received 4 April 2006

Accepted 3 July 2006

INTRODUCTION

Proteus mirabilis, a motile Gram-negative enteric bacterium, is an important pathogen of the urinary tract, and is the primary infectious agent in patients with indwelling urinary catheters (Warren *et al.*, 1982). Individuals suffering from urinary tract infections caused by *P. mirabilis* often develop bacteriuria, cystitis, kidney and bladder stones, catheter obstruction due to stone encrustation, acute pyelonephritis, and fever (Burall *et al.*, 2004; Johnson *et al.*, 1993; Mobley & Warren, 1987).

The ability of *P. mirabilis* to colonize the surfaces of catheters and the urinary tract is believed to be aided by a characteristic known as swarming differentiation and migration (Allison *et al.*, 1994). *P. mirabilis* swarming involves the coordinate differentiation of short, motile, vegetative cells with a few peritrichous flagella into multinucleate, aseptate swarm cells of up to 40 times the vegetative cell length, and with more than a 50-fold greater surface density of flagella. The swarm cells migrate coordinately and rapidly away from the colony as multicellular rafts until they pause (consolidation) and

undergo some de-differentiation (Allison & Hughes, 1991b; Rauprich *et al.*, 1996). Regular cycles of migration and consolidation generate a colony on the agar surface with a characteristic pattern of concentric rings (Allison & Hughes, 1991b; Rauprich *et al.*, 1996).

Several potential virulence factors may be responsible for the pathogenicity of *P. mirabilis*. Among them, flagella, necessary for motility, are involved in establishing infection (Harmon *et al.*, 1989). Urease, which is responsible for the formation of bladder and kidney stones at later stages of infection (Mobley & Hausinger, 1989), can facilitate the colonization of the urinary tract in a mouse model (Jones *et al.*, 1990). Haemolysin, which is cytotoxic for cultured urinary tract epithelial cells (Mobley *et al.*, 1991), has been shown to be correlated with the ability of bacteria to invade cells (Peerbooms *et al.*, 1984). The ability of *P. mirabilis* to express virulence factors, including urease and haemolysin, and to invade human urothelial cells, is coordinately regulated with swarming differentiation (Allison & Hughes, 1991a; Allison *et al.*, 1992b; Liaw *et al.*, 2000, 2001, 2004). Characterization of *Proteus* mutants has indicated that a substantial number of proteins, including FlhD₂C₂, FlhA, Umo, Lrp, RsbA, RsmA, SpeB and others, are involved in regulation of swarming and virulence factor

Abbreviations: QS, quorum sensing; resveratrol, 3,5,4-trihydroxy-*trans*-stilbene; ROS, reactive oxygen species.

expression (Dufour *et al.*, 1998; Fraser & Hughes, 1999; Gygi *et al.*, 1995a; Hay *et al.*, 1997). Among these regulatory proteins, RsbA, which has been suggested to be a His-containing phosphotransmitter of the bacterial two-component signalling system (Belas *et al.*, 1998; Liaw *et al.*, 2001; Takeda *et al.*, 2001), has been shown by us to act as a negative regulator of swarming differentiation and virulence factor expression in *P. mirabilis* (Liaw *et al.*, 2001, 2004).

Resveratrol (3,5,4-trihydroxy-*trans*-stilbene) is a naturally occurring phytoalexin, one of a group of compounds that are produced in plants during the stress of attack by pathogens (Dong, 2003; Jeandet *et al.*, 2002). Invasion of grapevines by fungi induces the production of resveratrol to ward off the damaging microbes (Jeandet *et al.*, 2002). The compound is found in some foods and drinks, including red wine, grapes and peanuts. Resveratrol has been shown to have anti-inflammatory, anti-proliferative, antiviral and antioxidant activities (Jeandet *et al.*, 2002). Intensive research has been directed to the role of resveratrol in human health, primarily because of its protective effects against cardiac ailments and cancer (Jeandet *et al.*, 2002). Recent studies have indicated that resveratrol has growth-inhibitory effects on some bacterial pathogens (Chan, 2002; Docherty *et al.*, 2001; Mahady & Pendland, 2000; Tegos *et al.*, 2002). In the course of studying the effect of resveratrol on human pathogens, we found that resveratrol could inhibit swarming and virulence factor expression in *P. mirabilis*. This finding suggests that resveratrol has the potential to be developed as an antimicrobial agent against *P. mirabilis*.

METHODS

Chemicals. All chemicals were purchased from Sigma.

Bacterial strains and growth conditions. The bacterial strains used in this study were the wild-type *P. mirabilis* P19, a *P. mirabilis* P19 *rsbA*-defective mutant (P1100), and a RsbA-complemented strain (Pc) (Liaw *et al.*, 2001, 2004). Bacteria were routinely cultured at 37 °C in Luria-Bertani (LB) medium.

Swarming behaviour assay. The swarming migration distance assay was performed as described previously (Gygi *et al.*, 1995b; Liaw *et al.*, 2001). Briefly, an overnight bacterial culture (5 µl) was inoculated centrally onto the surface of dry LB swarming agar (2%, w/v) plates with or without resveratrol, which were then incubated at 37 °C. The swarming migration distance was assayed by following swarm fronts of the bacterial cells and recording progress at 30 or 60 min intervals.

Measurement of cell length, haemolysin and urease activities, and flagellin level. Cell length, cell membrane-associated haemolysin activity, urease activity in whole-cell suspensions, and flagellin, were assayed as described previously (Liaw *et al.*, 2001, 2004).

Cell invasion assay. Wild-type or *rsbA*-defective *P. mirabilis* grown overnight in the presence or absence of resveratrol (30 µg ml⁻¹) were used to infect a human urothelial NTUB1 cell line, which was originally derived from a urinary bladder carcinoma, and was obtained from the National Taiwan University Hospital. The cell-invasion ability of the bacteria was then assayed as described previously (Liaw *et al.*, 2000, 2001).

RESULTS

Inhibition of *P. mirabilis* swarming by resveratrol

To test the effect of resveratrol on *P. mirabilis* swarming, the bacteria were inoculated onto the centre of LB swarming agar plates containing various concentrations of resveratrol, and the migration distance of the bacteria was measured. Resveratrol significantly inhibited swarming migration of *P. mirabilis* at concentrations as low as 15 µg ml⁻¹ (Fig. 1b, lower panel); swarming was inhibited in a dose-dependent manner. At a concentration of 60 µg ml⁻¹, resveratrol completely blocked the swarming ability of *P. mirabilis* (Fig. 1a, b). Previously, we have reported that *rsbA*-defective *P. mirabilis* mutants have a higher ability to swarm and to express virulence factors, and have suggested that RsbA acts as a repressor of swarming and virulence factor expression (Liaw *et al.*, 2001). To investigate whether RsbA plays a role in resveratrol-induced swarming inhibition, an *rsbA*-defective mutant, P1100, was tested for its response to resveratrol. Consistent with our previous report (Liaw *et al.*, 2001), the *rsbA*-defective mutant migrated further in a given time than the wild-type cells. Whereas swarming of wild-type *P. mirabilis* was inhibited by resveratrol, that of the *rsbA*-defective mutant was not (Fig. 1a, b). These data indicate that resveratrol inhibited *P. mirabilis* swarming through an RsbA-dependent pathway. The inhibitory effect of resveratrol on swarming might arise from a toxic effect on the bacteria. We therefore tested whether resveratrol affected the growth rate of *P. mirabilis*. As shown in Fig. 1(c), the growth rate of both wild-type and *rsbA*-defective *P. mirabilis* was inhibited slightly by resveratrol at 30 and 60 µg ml⁻¹. At 16 h post-inoculation, the bacteria grew to similar densities, regardless of the presence of resveratrol. Considering the fact that resveratrol could inhibit swarming but only marginally inhibit growth in wild-type *P. mirabilis*, and that resveratrol slightly inhibited growth but not swarming in mutant *P. mirabilis*, we concluded that the inhibitory effect of resveratrol on swarming was unlikely to be due to inhibition of cell growth.

Inhibition of swarming differentiation of *P. mirabilis* by resveratrol

Knowing that the swarming behaviour of *P. mirabilis* was inhibited by resveratrol, experiments were performed to determine whether swarming differentiation of *P. mirabilis* was also affected by resveratrol. Wild-type and *rsbA*-defective mutant cells were spread on LB swarming agar plates in the presence or absence of resveratrol. Cell length, a marker of cell differentiation, was measured 2 h after seeding and hourly thereafter. As shown in Fig. 2(a), in the absence of resveratrol, the *rsbA*-defective mutant formed longer cells than the wild-type strain during the 7 h incubation period, and became longest about 1 h earlier than wild-type cells. Moreover, the *rsbA*-defective mutant retained its elongated cell shape for longer than the wild-type strain. These data, consistent with our previous report

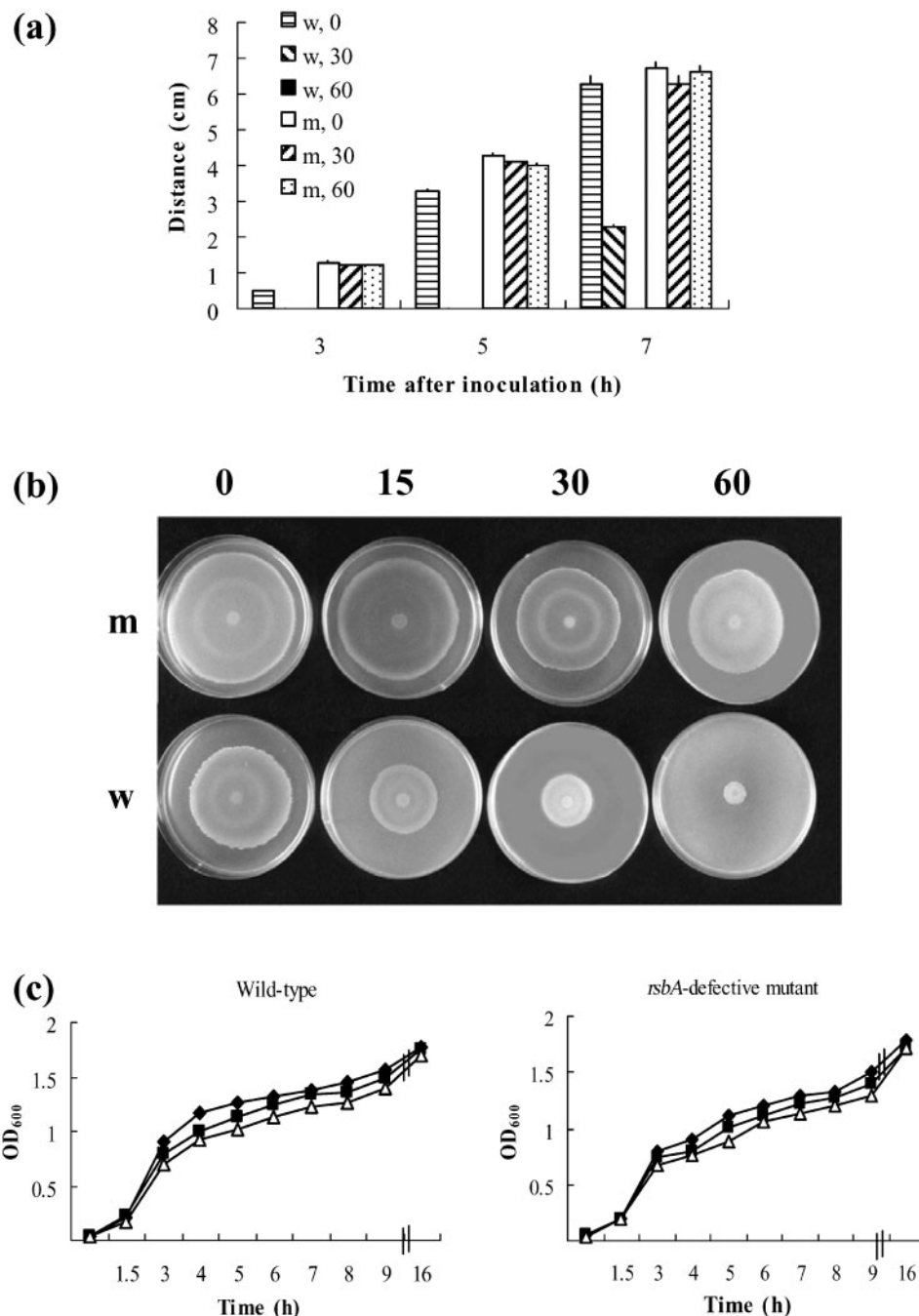


Fig. 1. (a) Effect of resveratrol on the swarming of wild-type and *rsbA*-defective *P. mirabilis*. The histogram shows the swarming migration distance of wild-type and *rsbA*-defective *P. mirabilis* in the presence of various concentrations of resveratrol (0, 30 or 60 $\mu\text{g ml}^{-1}$). The data represent the mean and SD of four independent experiments. w, wild-type; m, *rsbA*-defective mutant. (b) Halo images of swarming plates containing different concentrations of resveratrol (0, 15, 30 or 60 $\mu\text{g ml}^{-1}$) at 8 h after inoculation. (c) Effect of resveratrol on the growth of wild-type and *rsbA*-defective *P. mirabilis*. Overnight bacterial culture was diluted 1 : 100 to fresh LB broth containing different concentrations of resveratrol: 0 $\mu\text{g ml}^{-1}$ (\blacklozenge), 30 $\mu\text{g ml}^{-1}$ (\blacksquare) or 60 $\mu\text{g ml}^{-1}$ (\triangle). The bacterial growth was monitored thereafter as OD₆₀₀. Data are the mean of three determinations.

(Liaw *et al.*, 2001), indicate that the *rsbA*-defective mutant differentiated earlier and maintained a differentiated state for longer than the wild-type strain.

When resveratrol was added to the LB swarming agar plates, the length of the wild-type cells was reduced, but that of the *rsbA*-defective mutant cells was not (Fig. 2a, b). These data

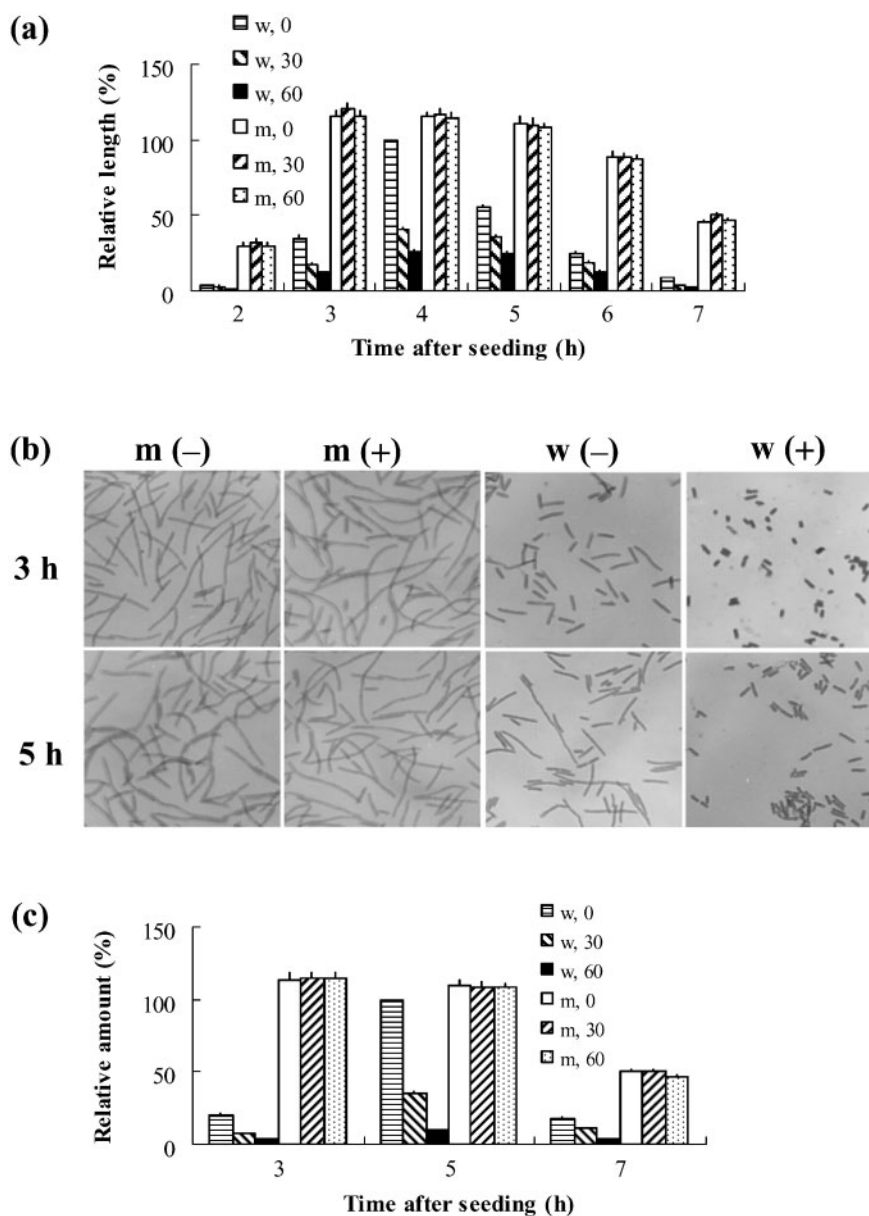


Fig. 2. (a) Effect of resveratrol on the cell length of wild-type and *rsbA*-defective *P. mirabilis*. The histogram shows the relative cell length of the wild-type *P. mirabilis* and its *rsbA* mutant in different concentrations of resveratrol (0, 30 or 60 $\mu\text{g ml}^{-1}$). The values obtained with wild-type cells in the absence of resveratrol at 4 h post-seeding were set at 100%, and all other values were expressed relative to this value. The data represent the means of three independent experiments. w, wild-type; m, *rsbA*-defective mutant. (b) Microscopic observation of wild-type and *rsbA*-defective *P. mirabilis* in the presence or absence of resveratrol at 3 and 5 h post-seeding. –, No resveratrol; +, 60 $\mu\text{g resveratrol ml}^{-1}$. (c) Effect of resveratrol on flagellin production by wild-type and *rsbA*-defective *P. mirabilis*. The amount of flagellin produced by the wild-type cells in the absence of resveratrol at 5 h post-seeding was set at 100%. The data represent the means of three independent experiments.

indicate that resveratrol inhibited swarming differentiation of *P. mirabilis* and that this inhibition was mediated through an RsbA-dependent pathway.

This conclusion was also confirmed by the finding that resveratrol could inhibit flagellin production in wild-type, but not in mutant, *P. mirabilis* (Fig. 2c).

Inhibition of virulence factor expression in *P. mirabilis* by resveratrol

In *P. mirabilis*, expression of virulence factors is regulated by RsbA coordinately with swarming differentiation (Liaw *et al.*, 2001). We next tested whether expression of virulence factors, including haemolysin and urease, was also affected

by resveratrol in *P. mirabilis*. As shown in Fig. 3, in the absence of resveratrol, the *rsbA*-defective mutant expressed higher levels of haemolysin and urease activity than the wild-type strain during the 7 h incubation period, consistent with our previous report (Liaw *et al.*, 2001). Resveratrol inhibited the haemolysin and urease activity of wild-type *P. mirabilis*, but not that of the *rsbA*-defective mutant, indicating that resveratrol could inhibit the expression of haemolysin and urease in *P. mirabilis* through an RsbA-dependent pathway.

Inhibition of the cell-invasion ability of *P. mirabilis* by resveratrol

Swarming differentiation and expression of virulence factors are correlated with the ability of *P. mirabilis* to invade cells (Allison *et al.*, 1992a, b). Knowing that swarming differentiation and virulence factor expression were inhibited by resveratrol, we next tested whether resveratrol could

inhibit the cell-invasion ability of wild-type and *rsbA*-defective *P. mirabilis*. As shown in Table 1, the *rsbA*-defective mutant exhibited higher cell-invasion ability than wild-type cells, consistent with our previous report (Liaw *et al.*, 2001). Resveratrol inhibited the cell-invasion ability of wild-type *P. mirabilis*, but not that of the *rsbA*-defective mutant, indicating that resveratrol could inhibit the cell-invasion ability of *P. mirabilis* through an RsbA-dependent pathway.

Complementation of the *rsbA*-defective mutant by wild-type *rsbA* restores wild-type responsiveness to resveratrol

To further confirm that loss of responsiveness to resveratrol in the *rsbA*-defective mutant is indeed due to the defective *rsbA* gene, an RsbA-complemented strain (Pc), which was generated by transforming the wild-type *rsbA* gene into the *rsbA*-defective mutant (Liaw *et al.*, 2001), was tested for its responsiveness to resveratrol. As shown in Fig. 4(a), in the absence of resveratrol, while the *rsbA*-defective mutant exhibited the superswarming phenotype, the RsbA-complemented strain exhibited swarming behaviour similar to that of wild-type *P. mirabilis*. In the presence of resveratrol, swarming of the *rsbA*-defective mutant was not inhibited, but that of the RsbA-complemented strain was inhibited to a level similar to that of the wild-type strain. Together, these data indicate that expression of RsbA in the *rsbA*-defective mutant led to the restoration of responsiveness to resveratrol, and suggest that inhibition of swarming by resveratrol is mediated through RsbA and not through other proteins in *P. mirabilis*. We also tested whether resveratrol could inhibit haemolysin expression in the RsbA-complemented strain as it could in the wild-type strain. As shown in Fig. 4(b), while the haemolysin activity of the *rsbA*-defective mutant was not inhibited by resveratrol, the activity of the RsbA-complemented strain was inhibited to a level similar to that of the wild-type strain. These data further confirm that complementation of the *rsbA*-defective mutant with the *rsbA* gene restored its responsiveness to resveratrol, and that inhibition of haemolysin expression by resveratrol was mediated mainly through RsbA in *P. mirabilis*.

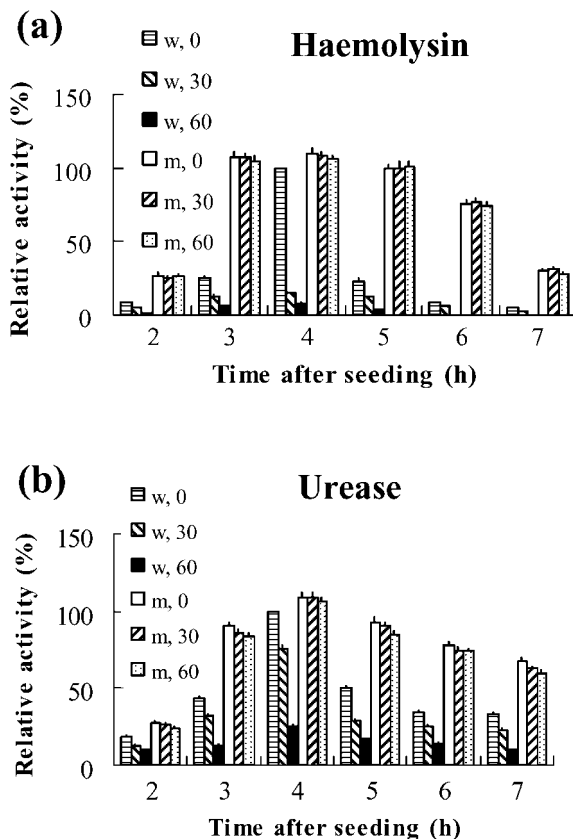


Fig. 3. Effect of resveratrol on the haemolysin activity (a) and urease activity (b) of wild-type and *rsbA*-defective *P. mirabilis*. The histogram shows the relative haemolysin or urease activity of the wild-type *P. mirabilis* and its *rsbA* mutant at different concentrations of resveratrol (0, 30 or 60 $\mu\text{g ml}^{-1}$). The values obtained with wild-type cells in the absence of resveratrol at 4 h post-seeding were set at 100%, and all other values were expressed relative to this value. The data represent the means of three independent experiments. w, wild-type; m, *rsbA*-defective mutant.

Table 1. Effect of resveratrol on the cell-invasion ability of *P. mirabilis* strains

Strain	Cell invasion (%)*
Wild-type	0.09 \pm 0.01
Mutant†	0.21 \pm 0.013
Wild-type + RV‡	0.02 \pm 0.01
Mutant + RV	0.21 \pm 0.012

*Values represent the mean \pm SD of three experiments.

†Mutant, *rsbA*-defective mutant.

‡RV, 30 μg resveratrol ml^{-1} .

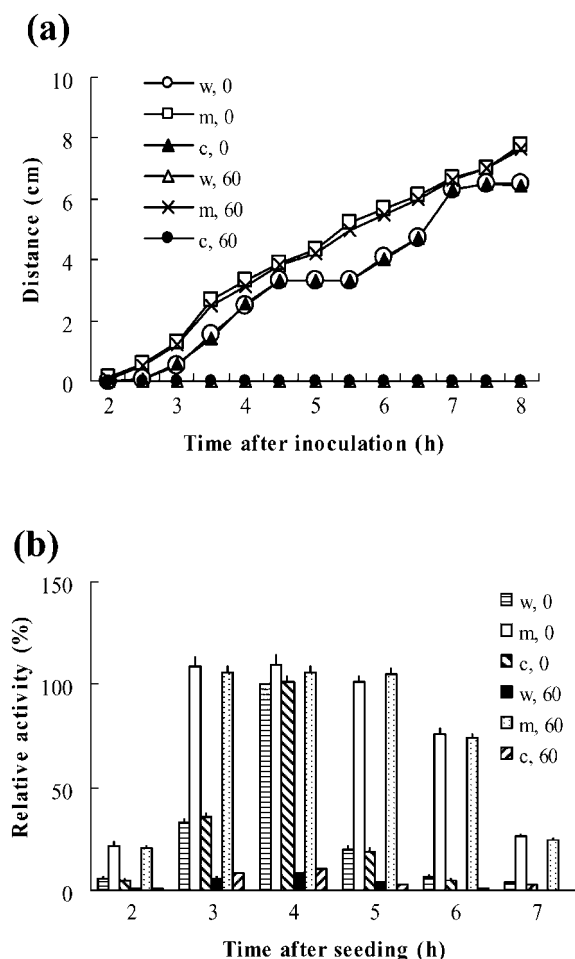


Fig. 4. Complementmentation of the *rsbA*-defective mutant of *P. mirabilis* by the wild-type *rsbA* gene restored wild-type responsiveness to resveratrol. The swarming migration distance (a) and the haemolysin activity (b) of different strains of *P. mirabilis* in the presence or absence of $60 \mu\text{g ml}^{-1}$ at various times after inoculation were determined. For haemolysin activity measurements, the value obtained with the wild-type cells in the absence of resveratrol at 4 h post-seeding was set at 100%. The data represent the means of three independent experiments. w, wild-type; m, *rsbA*-defective mutant; c, RsbA-complemented strain.

DISCUSSION

A wide variety of natural products has been under scrutiny for their clinical potential, both in terms of disease prevention and treatment. Here we report that resveratrol, a naturally occurring phytoalexin, can inhibit swarming and the expression of virulence factors in *P. mirabilis*. To our knowledge, this is the first report describing the inhibitory effect of resveratrol on bacterial swarming and virulence factor expression.

Resveratrol inhibits the growth of some bacterial species, but has little effect on others (Docherty *et al.*, 2001; Mahady & Pendland, 2000; Tegos *et al.*, 2002). Several lines of evidence

indicate that the inhibitory effect of resveratrol on *P. mirabilis* swarming and virulence factor expression is unlikely to be due to its inhibitory effect on bacterial growth. Firstly, resveratrol inhibited *P. mirabilis* swarming and virulence factor expression at a concentration as low as $30 \mu\text{g ml}^{-1}$ (Figs 1 and 3), but did not significantly affect the growth rate of the bacteria at concentrations up to $60 \mu\text{g ml}^{-1}$ (Fig. 1c). Secondly, resveratrol did not affect the viability of *P. mirabilis* at a concentration of $256 \mu\text{g ml}^{-1}$ (data not shown). Thirdly, while resveratrol could slightly inhibit the growth rate of both wild-type and *rsbA*-defective *P. mirabilis*, it could only inhibit swarming and virulence factor expression in the wild-type strain, and not in the mutant strain of *P. mirabilis* (Figs 1 and 3).

If resveratrol is to be used to treat or prevent *P. mirabilis* infection, its effect on human cells needs to be addressed. Our study showed that resveratrol inhibited *P. mirabilis* swarming and virulence factor expression at $30\text{--}60 \mu\text{g ml}^{-1}$. At this concentration, resveratrol is expected to have little effect on normal human cells. For instance, the midpoint cytotoxicity values for a 24 h exposure to resveratrol are $98.6\text{--}105.4 \mu\text{g ml}^{-1}$ for normal fibroblasts isolated from the oral cavity (Babich *et al.*, 2000). Our study also indicated that human embryonal rhabdomyosarcoma cells treated with $30 \mu\text{g resveratrol ml}^{-1}$ for 3 days did not show signs of cell death (data not shown).

Quorum sensing (QS) is a cell to cell communication mechanism that enables bacteria to monitor their own population density and synchronize the expression of virulence factors, and thus to mount effective attacks to overwhelm the host's defence responses (Camara *et al.*, 2002; de Kievit & Iglewski, 2000; Zhang, 2003). Although the regulation of virulence factor expression by QS is generally believed to be dependent on cell density, there is increasing evidence to suggest that the cell-to-cell signalling mediated by the QS system can also be strongly affected by environmental factors other than cell density. For example, bioluminescence, which is regulated by QS in *Vibrio harveyi*, has been found to be sensitive to the availability of iron, oxygen and carbohydrate (Nealson & Hastings, 1991). *P. mirabilis* swarming and virulence factor expression are generally believed to be regulated through a QS system (Holden *et al.*, 1999; Schneider *et al.*, 2002), and require the sensing and integration of a variety of environmental, cell-to-cell and intracellular signals. These signals may include those transmitted by high population density, autoinducer-2 (AI-2), cyclic dipeptides, putrescine, glutamine, intracellular cations and fatty acids (Allison *et al.*, 1993; Holden *et al.*, 1999; Lai *et al.*, 1998; Liaw *et al.*, 2004; Rather, 2005; Schneider *et al.*, 2002; Sturgill & Rather, 2004). Recently, oxidative stress has been found to upregulate the expression of QS-controlled genes in *Pseudomonas aeruginosa* (Juhas *et al.*, 2004; Kim *et al.*, 2005). It is possible that oxidative stress also serves as an environmental signal to regulate QS in *P. mirabilis*. We propose that oxidative stress, as in *Ps. aeruginosa*, may also upregulate QS-regulated genes in *P.*

mirabilis. Resveratrol, a powerful antioxidant, may relieve oxidative stress, and thus lead to the inhibition of swarming and virulence factor expression. This proposition is supported by our preliminary results that soy-derived isoflavones, also potent antioxidants, can inhibit swarming, but not growth, in *P. mirabilis* (data not shown).

The hypothesis that oxidative stress regulates *P. mirabilis* swarming is attractive, because it can explain the phenomenon by which swarming differentiation and virulence factor expression are dependent on cell density. Reactive oxygen species (ROS) are known to be generated physiologically during normal cell metabolism, and high cell density may lead to the accumulation of excessive ROS. At this stage, *P. mirabilis* starts to differentiate and migrate. As cells move out in swarming rafts, the concentration of ROS decreases, and eventually cells are unable to maintain the differentiated state and de-differentiate back to non-motile vegetative cells. During growth, ROS build up again, and differentiation/swarming proceeds for a second cycle. Regular cycles of differentiation/migration and de-differentiation/non-migration generate a colony on the agar surface with a characteristic pattern of concentric rings. That oxidative stress may cause bacteria to migrate has been reported previously (Nachin *et al.*, 2005; Tang *et al.*, 2004). It is also of interest to note that bacterial infections of the genital tract can lead to the production of excess ROS from neutrophils/macrophages as a first-line defence mechanism (Ochsendorf, 1999). *P. mirabilis* may sense this increase in ROS and migrate away from the attack by immune cells.

RsbA, a His-containing phosphotransmitter of the bacterial two-component signalling system (Belas *et al.*, 1998; Liaw *et al.*, 2001; Takeda *et al.*, 2001), has previously been shown by us to act as a negative regulator of swarming differentiation and virulence factor expression in *P. mirabilis* (Liaw *et al.*, 2001, 2004). Here, we have shown that resveratrol inhibits *P. mirabilis* swarming and virulence factor expression through an RsbA-dependent pathway. At least two models can explain the role of RsbA in resveratrol-mediated suppression of swarming and virulence factor expression. First, *P. mirabilis* may contain a two-component membrane sensor kinase which can sense the low-ROS condition that results from resveratrol treatment. Upon sensing low ROS, this kinase may transduce negative signals via the phosphotransmitter RsbA to inhibit swarming and virulence factor expression. Alternatively, *P. mirabilis* may contain a two-component sensor kinase which can sense the high-ROS condition and transduce signals to down-regulate the expression of RsbA, a negative regulator of swarming and virulence factor expression in *P. mirabilis*. Upon resveratrol treatment (which results in low ROS), the presumed sensor kinase can no longer transduce signals to inhibit RsbA expression, leading to accumulation of RsbA, and inhibition of swarming and virulence factor expression. To distinguish these two possibilities, we measured the expression of RsbA upon resveratrol treatment. Our preliminary data indicated that the production of RsbA mRNA in *P. mirabilis* was

increased six- to sevenfold after resveratrol treatment. This result is more consistent with the second model.

Another possible mechanism by which resveratrol could inhibit *P. mirabilis* swarming and virulence factor expression is by acting as a QS signal mimic that disrupts the bacterial QS system. There is increasing evidence to suggest that plants can produce a variety of signal mimics that inhibit QS-regulated behaviours (Bauer & Robinson, 2002; Teplitski *et al.*, 2000). For example, the halogenated furanones produced by the seaweed *Delisea pulchra* can specifically inhibit QS-regulated biological activities in various Gram-negative bacteria (Givskov *et al.*, 1996; Hentzer *et al.*, 2002; Teplitski *et al.*, 2000; Zhang, 2003). The crude exudates from pea (*Pisum sativum*) and crown vetch (*Coronilla varia*) strongly inhibit the QS-regulated synthesis of violacein in *Chromobacterium violaceum* (Teplitski *et al.*, 2000). That some plants can produce QS-inhibitory molecules after their long association with bacterial pathogens is not surprising, because in this way, hosts can evade the pathogenic effects of the bacteria. It is possible that resveratrol, a natural product of many plants (about 31 genera), also acts as a QS-inhibitory molecule to protect plants from bacterial infection. Since different bacterial species may use similar QS signals to communicate with each other, it is possible that resveratrol, although produced by plants, also acts on human pathogens to disrupt their QS systems.

The emergence of bacterial strains that exhibit resistance to various antibiotics poses a major threat to medicine and public health. As a consequence, there is renewed interest in antibacterial targets which, by attenuating virulence, disrupt the capacity of pathogenic bacteria to cause infection. In this context, the QS system is considered to be a potential therapeutic target, and the inhibition of QS may lead to control of infection.

In this paper, we demonstrate that resveratrol, an antioxidant found in some foods and drinks, has an inhibitory effect on *P. mirabilis* swarming and virulence factor expression. Although the molecular mechanisms underlying resveratrol inhibition of swarming and virulence factor expression are not clear, this finding opens up the opportunity to develop drugs that slow down *P. mirabilis* infection, allowing the host to gain valuable time to activate defence mechanisms, and to stop and eliminate pathogenic invaders.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Science Council and the National Taiwan University Hospital, Taipei, Taiwan. We are grateful to Yeong-Shiau Pu for providing the NTUB1 cell line.

REFERENCES

- Allison, C. & Hughes, C. (1991a). Bacterial swarming: an example of prokaryotic differentiation and multicellular behaviour. *Sci Prog* 75, 403–422.

- Allison, C. & Hughes, C. (1991b). Closely linked genetic loci required for swarm cell differentiation and multicellular migration by *Proteus mirabilis*. *Mol Microbiol* 5, 1975–1982.
- Allison, C., Coleman, N., Jones, P. L. & Hughes, C. (1992a). Ability of *Proteus mirabilis* to invade human urothelial cells is coupled to motility and swarming differentiation. *Infect Immun* 60, 4740–4746.
- Allison, C., Lai, H. C. & Hughes, C. (1992b). Co-ordinate expression of virulence genes during swarm-cell differentiation and population migration of *Proteus mirabilis*. *Mol Microbiol* 6, 1583–1591.
- Allison, C., Lai, H. C., Gygi, D. & Hughes, C. (1993). Cell differentiation of *Proteus mirabilis* is initiated by glutamine, a specific chemoattractant for swarming cells. *Mol Microbiol* 8, 53–60.
- Allison, C., Emody, L., Coleman, N. & Hughes, C. (1994). The role of swarm cell differentiation and multicellular migration in the uropathogenicity of *Proteus mirabilis*. *J Infect Dis* 169, 1155–1158.
- Babich, H., Reisbaum, A. G. & Zuckerbraun, H. L. (2000). In vitro response of human gingival epithelial S-G cells to resveratrol. *Toxicol Lett* 114, 143–153.
- Bauer, W. D. & Robinson, J. B. (2002). Disruption of bacterial quorum sensing by other organisms. *Curr Opin Biotechnol* 13, 234–237.
- Belas, R., Schneider, R. & Melch, M. (1998). Characterization of *Proteus mirabilis* precocious swarming mutants: identification of *rsbA*, encoding a regulator of swarming behavior. *J Bacteriol* 180, 6126–6139.
- Burall, L. S., Harro, J. M., Li, X., Lockatell, C. V., Himpel, S. D., Hebel, J. R., Johnson, D. E. & Mobley, H. L. (2004). *Proteus mirabilis* genes that contribute to pathogenesis of urinary tract infection: identification of 25 signature-tagged mutants attenuated at least 100-fold. *Infect Immun* 72, 2922–2938.
- Camara, M., Williams, P. & Hardman, A. (2002). Controlling infection by tuning in and turning down the volume of bacterial small-talk. *Lancet Infect Dis* 2, 667–676.
- Chan, M. M. (2002). Antimicrobial effect of resveratrol on dermatophytes and bacterial pathogens of the skin. *Biochem Pharmacol* 63, 99–104.
- de Kievit, T. R. & Iglewski, B. H. (2000). Bacterial quorum sensing in pathogenic relationships. *Infect Immun* 68, 4839–4849.
- Docherty, J. J., Fu, M. M. & Tsai, M. (2001). Resveratrol selectively inhibits *Neisseria gonorrhoeae* and *Neisseria meningitidis*. *J Antimicrob Chemother* 47, 243–244.
- Dong, Z. (2003). Molecular mechanism of the chemopreventive effect of resveratrol. *Mutat Res* 523–524, 145–150.
- Dufour, A., Furness, R. B. & Hughes, C. (1998). Novel genes that upregulate the *Proteus mirabilis* *flhDC* master operon controlling flagellar biogenesis and swarming. *Mol Microbiol* 29, 741–751.
- Fraser, G. M. & Hughes, C. (1999). Swarming motility. *Curr Opin Microbiol* 2, 630–635.
- Givskov, M., de Nys, R., Manefield, M., Gram, L., Maximilien, R., Eberl, L., Molin, S., Steinberg, P. D. & Kjelleberg, S. (1996). Eukaryotic interference with homoserine lactone-mediated prokaryotic signalling. *J Bacteriol* 178, 6618–6622.
- Gygi, D., Bailey, M. J., Allison, C. & Hughes, C. (1995a). Requirement for FlhA in flagella assembly and swarm-cell differentiation by *Proteus mirabilis*. *Mol Microbiol* 15, 761–769.
- Gygi, D., Rahman, M. M., Lai, H. C., Carlson, R., Guard-Petter, J. & Hughes, C. (1995b). A cell-surface polysaccharide that facilitates rapid population migration by differentiated swarm cells of *Proteus mirabilis*. *Mol Microbiol* 17, 1167–1175.
- Harmon, R. C., Rutherford, R. L., Wu, H. M. & Collins, M. S. (1989). Monoclonal antibody-mediated protection and neutralization of motility in experimental *Proteus mirabilis* infection. *Infect Immun* 57, 1936–1941.
- Hay, N. A., Tipper, D. J., Gygi, D. & Hughes, C. (1997). A nonswarming mutant of *Proteus mirabilis* lacks the Lrp global transcriptional regulator. *J Bacteriol* 179, 4741–4746.
- Hentzer, M., Riedel, K., Rasmussen, T. B. & 9 other authors (2002). Inhibition of quorum sensing in *Pseudomonas aeruginosa* biofilm bacteria by a halogenated furanone compound. *Microbiology* 148, 87–102.
- Holden, M. T., Ram Chhabra, S., de Nys, R. & 14 other authors (1999). Quorum-sensing cross talk: isolation and chemical characterization of cyclic dipeptides from *Pseudomonas aeruginosa* and other Gram-negative bacteria. *Mol Microbiol* 33, 1254–1266.
- Jeandet, P., Douillet-Breuil, A. C., Bessis, R., Debord, S., Sbaghi, M. & Adrian, M. (2002). Phytoalexins from the Vitaceae: biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. *J Agric Food Chem* 50, 2731–2741.
- Johnson, D. E., Russell, R. G., Lockatell, C. V., Zulty, J. C., Warren, J. W. & Mobley, H. L. (1993). Contribution of *Proteus mirabilis* urease to persistence, urolithiasis, and acute pyelonephritis in a mouse model of ascending urinary tract infection. *Infect Immun* 61, 2748–2754.
- Jones, B. D., Lockatell, C. V., Johnson, D. E., Warren, J. W. & Mobley, H. L. (1990). Construction of a urease-negative mutant of *Proteus mirabilis*: analysis of virulence in a mouse model of ascending urinary tract infection. *Infect Immun* 58, 1120–1123.
- Juhas, M., Wiehlmann, L., Huber, B. & 8 other authors (2004). Global regulation of quorum sensing and virulence by VqsR in *Pseudomonas aeruginosa*. *Microbiology* 150, 831–841.
- Kim, E. J., Wang, W., Deckwer, W. D. & Zeng, A. P. (2005). Expression of the quorum-sensing regulatory protein LasR is strongly affected by iron and oxygen concentrations in cultures of *Pseudomonas aeruginosa* irrespective of cell density. *Microbiology* 151, 1127–1138.
- Lai, H. C., Gygi, D., Fraser, G. M. & Hughes, C. (1998). A swarming-defective mutant of *Proteus mirabilis* lacking a putative cation-transporting membrane P-type ATPase. *Microbiology* 144, 1957–1961.
- Liaw, S. J., Lai, H. C., Ho, S. W., Luh, K. T. & Wang, W. B. (2000). Inhibition of virulence factor expression and swarming differentiation in *Proteus mirabilis* by *p*-nitrophenylglycerol. *J Med Microbiol* 49, 725–731.
- Liaw, S. J., Lai, H. C., Ho, S. W., Luh, K. T. & Wang, W. B. (2001). Characterisation of *p*-nitrophenylglycerol-resistant *Proteus mirabilis* super-swarming mutants. *J Med Microbiol* 50, 1039–1048.
- Liaw, S. J., Lai, H. C. & Wang, W. B. (2004). Modulation of swarming and virulence by fatty acids through the RsbA protein in *Proteus mirabilis*. *Infect Immun* 72, 6836–6845.
- Mahady, G. B. & Pendland, S. L. (2000). Resveratrol inhibits the growth of *Helicobacter pylori* in vitro. *Am J Gastroenterol* 95, 1849.
- Mobley, H. L. & Warren, J. W. (1987). Urease-positive bacteriuria and obstruction of long-term urinary catheters. *J Clin Microbiol* 25, 2216–2217.
- Mobley, H. L. & Hausinger, R. P. (1989). Microbial ureases: significance, regulation, and molecular characterization. *Microbiol Rev* 53, 85–108.
- Mobley, H. L., Chippendale, G. R., Swihart, K. G. & Welch, R. A. (1991). Cytotoxicity of the HpmA hemolysin and urease of *Proteus mirabilis* and *Proteus vulgaris* against cultured human renal proximal tubular epithelial cells. *Infect Immun* 59, 2036–2042.
- Nachin, L., Nannmark, U. & Nystrom, T. (2005). Differential roles of the universal stress proteins of *Escherichia coli* in oxidative stress resistance, adhesion and motility. *J Bacteriol* 187, 6265–6272.

- Nealson, K. & Hastings, J. (1991).** *The Luminous Bacteria*, 2nd edn. New York: Springer.
- Ochsendorf, F. R. (1999).** Infections in the male genital tract and reactive oxygen species. *Hum Reprod Update* **5**, 399–420.
- Peerbooms, P. G., Verweij, A. M. & MacLaren, D. M. (1984).** Vero cell invasiveness of *Proteus mirabilis*. *Infect Immun* **43**, 1068–1071.
- Rather, P. N. (2005).** Swarmer cell differentiation in *Proteus mirabilis*. *Environ Microbiol* **7**, 1065–1073.
- Rauprich, O., Matsushita, M., Weijer, C. J., Siegert, F., Esipov, S. E. & Shapiro, J. A. (1996).** Periodic phenomena in *Proteus mirabilis* swarm colony development. *J Bacteriol* **178**, 6525–6538.
- Schneider, R., Lockatell, C. V., Johnson, D. & Belas, R. (2002).** Detection and mutation of a *luxS*-encoded autoinducer in *Proteus mirabilis*. *Microbiology* **148**, 773–782.
- Sturgill, G. & Rather, P. N. (2004).** Evidence that putrescine acts as an extracellular signal required for swarming in *Proteus mirabilis*. *Mol Microbiol* **51**, 437–446.
- Takeda, S., Fujisawa, Y., Matsubara, M., Aiba, H. & Mizuno, T. (2001).** A novel feature of the multistep phosphorelay in *Escherichia coli*: a revised model of the RcsC→YojN→RcsB signalling pathway implicated in capsular synthesis and swarming behaviour. *Mol Microbiol* **40**, 440–450.
- Tang, Y., Guest, J. R., Artymiuk, P. J., Read, R. C. & Green, J. (2004).** Post-transcriptional regulation of bacterial motility by aconitase proteins. *Mol Microbiol* **51**, 1817–1826.
- Tegos, G., Stermitz, F. R., Lomovskaya, O. & Lewis, K. (2002).** Multidrug pump inhibitors uncover remarkable activity of plant antimicrobials. *Antimicrob Agents Chemother* **46**, 3133–3141.
- Teplitski, M., Robinson, J. B. & Bauer, W. D. (2000).** Plants secrete substances that mimic bacterial *N*-acyl homoserine lactone signal activities and affect population density-dependent behaviors in associated bacteria. *Mol Plant Microbe Interact* **13**, 637–648.
- Warren, J. W., Tenney, J. H., Hoopes, J. M., Muncie, H. L. & Anthony, W. C. (1982).** A prospective microbiologic study of bacteriuria in patients with chronic indwelling urethral catheters. *J Infect Dis* **146**, 719–723.
- Zhang, L. H. (2003).** Quorum quenching and proactive host defense. *Trends Plant Sci* **8**, 238–244.