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Flagella-mediated motility is required for biofilm formation by *Erwinia carotovora* subsp. *carotovora*

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Abstract To elucidate the role of flagella in biofilm formation by *Erwinia carotovora* subsp. *carotovora* EC1, we used a nonflagellate, nonmotile mutant (Δ*fliC*) and a flagellate, nonmotile mutant (Δ*motA*). A biofilm-inducing medium, which contains the yeast peptone (YP) medium plus the salts of M-63 minimal medium, supported biofilm formation to a greater extent than either the YP or Luria Bertani (LB) medium alone. We demonstrated that both the Δ*fliC* and Δ*motA* mutants greatly reduced their ability to form a biofilm on the surface of the wells of polyvinyl chloride (PVC) microtiter plates. The inability of both mutants to form biofilm on the PVC surface was further confirmed with phase-contrast microscopy. Both aflagellate (Δ*fliC*) and flagellate (Δ*motA*) nonmotile mutants were equally defective in attachment to the PVC surface. The treatment of bacteria with the protonophore carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), which inhibits the motility of this organism, reduced greatly the biofilm formation. Based on these results, flagella-mediated motility may play an important role in biofilm formation of *E. carotovora* subsp. *carotovora* EC1.

Key words *Erwinia carotovora* subsp. *carotovora* EC1 · Biofilm formation · Motility · Carbonyl cyanide *m*-chlorophenylhydrazone · Δ*fliC* · Δ*motA*

Introduction

Soft-rot *Erwinia* spp. such as *Erwinia carotovora* subsp. *carotovora* are economically important because they cause serious damage worldwide on a wide variety of crop species including Brussels sprout, carrot, celery, cucumber, capsicum, turnip, chicory, and potato (Perombelon and Kelman 1980). These species cause soft-rot diseases, mainly through the secretion of cell wall-degrading enzymes such as pectate lyase (Pel), polygalacturonase (Peh), cellulase (Cel), and protease (Prt) (Barras et al. 1994). In addition, several secondary factors such as exopolysaccharides (EPS) (Condemine et al. 1999), lipopolysaccharides (LPS) (Schoonejans et al. 1987), and motility (Mulholland et al. 1993; Hossain et al. 2005) contribute to virulence in soft-rot *Erwinia* spp.

Bacterial populations consisting of single or multiple species that are attached to a surface are commonly referred to as biofilms (Costerton 1995; Costerton et al. 1995, 1999; Geesey et al. 1977). Bacteria in aquatic environments are often found in association with a solid surface rather than in a planktonic or free-swimming phase (Costerton et al. 1987). Attached bacteria may exist as a dispersed monolayer of cells on a surface in a microcolony or in a three-dimensional structure (Costerton et al. 1995).

Bacteria in biofilms are more resistant to adverse environmental conditions such as desiccation and extreme temperature (Zottola and Sasahara 1994; Costerton et al. 1995; Dewanti and Wong 1995). Biofilm-associated bacteria also generally acquire resistance to antimicrobial agents (Hoyle and Costerton 1991; Ichimiya et al. 1994), and are thought to be protected from the host defense responses of animals and plants (Kharazmi 1991; Leid et al. 2002; Walker et al. 2004). In this sense, the bacterial biofilm may be considered as an important virulent factor of several animal and plant pathogenic bacteria (Verhoef 1997).

Biofilm development is a multistep process. In general, its developmental pathway proceeds from the initial attachment and immobilization of bacteria to a solid surface, followed by the formation of the cell cluster or microcolony and ultimately by a three-dimensional architecture. The three-dimensional structure is created by pillars of bacteria surrounded by water channels, that allow the influx of nutrients and the efflux of waste products (Costerton et al. 1995).

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Recent molecular and genetic studies have identified several genes whose products are thought to be important for biofilm formation. Flagellar or twitching-mediated motility has been reported to be important for free swimming cells of several bacterial species to initiate the formation of biofilms (O'Toole and Kolter 1998b; Pratt and Kolter 1998; Watnick et al. 1999). In addition, the production of EPS (Davies et al. 1993; Boyd and Chakrabarty 1995; Davies and Geesey 1995; Yildiz and Schoolnik 1999) and outer membrane proteins (Danese et al. 2000) was also shown to be required for biofilm formation. The catabolite repressor control (Crc) protein, which acts as a positive factor to induce the expression of many genes including those for carbon metabolism, was also reported to be necessary for biofilm formation in *Pseudomonas aeruginosa* (O'Toole et al. 2000).

No information is available about the role of motility in biofilm formation by *E. carotovora* subsp. *carotovora* and in turn the contribution of the biofilm to pathogenicity. To elucidate the role of flagella or motility in biofilm formation by *E. carotovora* subsp. *carotovora* EC1, we tested nonpolar deletion mutants of *fliC* and *motA* homologues denoted as Δ*fliC* and Δ*motA*, respectively, for their ability to form biofilms in microtiter plates. The Δ*fliC* mutant was found to be nonflagellated and nonmotile, whereas the Δ*motA* mutant was flagellated like its parental strain but was nonmotile (Hossain et al. 2005). Using these two mutants, we could demonstrate that motility but not the physical presence of flagella is necessary for the formation of biofilm by *E. carotovora* subsp. *carotovora* EC1. We also raised the possibility that flagella-mediated motility may not be directly involved in pathogenicity but may be involved in the formation of a biofilm, a factor required for pathogenicity.

Materials and methods

Bacterial strains, plasmids, growth media, and chemicals

Bacterial strains and plasmids used in this study are listed in Table 1. The various constructs of *E. carotovora* subsp. *carotovora* EC1 and *Escherichia coli* were grown in YP medium (1% peptone, 0.5% yeast extract, pH 6.8) at 27°C and in Luria-Bertani (LB) medium (1% tryptone, 0.5% yeast extract, 0.5% NaCl, pH 7.0) at 37°C, respectively. M63 minimal medium (Ausubel et al. 1987) contains 2.5 g NaCl, 3.0 g KH_2PO_4, 7.0 g K_2HPO_4, 2.0 g $(NH_4)_2SO_4$, 0.5 mg $FeSO_4$, 1.0 g $MgSO_4$, and 2.0 g thiamine hydrochloride in 1 liter of distilled water. For the biofilm formation study, we used a biofilm-inducing medium, comprised of YP medium plus M63 minimal medium (i.e., M63 minimal medium was supplemented with 1.0% peptone and 0.5% yeast extract). When required, antibiotics were added at the following concentrations: nalidixic acid 50 μg/ml, ampicillin 50 μg/ml, kanamycin 50 μg/ml, streptomycin 25 μg/ml, and spectinomycin 70 μg/ml. The optical density (OD) of the bacterial culture was measured with a Bactomonitor BACT-500 (Intertech, Tokyo, Japan) at 660 nm. A protonophore, carbonyl cyanide *m*-chlorophenylhydrazone

Table 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant chracteristics[a]	Source or reference
Erwinia carotovora subsp. *carotovora*		
EC1	Wild type, isolated from infected radish	Tsuyumu 1977
EC1-N	Spontaneous Nal^r mutant of EC1, Nal^r	Laboratory collection
Δ*fliC*	EC1-NΔ*fliC*, Nal^r	Laboratory collection
Δ*motA*	EC1-NΔ*motA*, Nal^r	Laboratory collection
Escherichia coli		
CC118	Δ(*ara*, *leu*) *araD* Δ*lacX* 74 *galE galK PhoA*20 *thi*-1 *rpsE rpoB argE* (am) *recA*1, Sp^r	Herroro et al. 1990
CC118 (λ*pir*)	CC118 lysogenized with λ*pir* phage, Sp^r	Herroro et al. 1990
HB101	F^- *hsdS* (r_B^- m_B^-) *thi pro leu lacY ara xyl supE recA*13, Stm^r	Sambrook et al. 1989
DH5α	F^-, f80d*lacZ*DM15, *lacZYA-argF*, *recA*1, Nal^r	Takara, Japan
DH10B	F^- *mcrA* Δ(*mrr-hsdRMS-mcrBC*) f80d*lac*ZD *recA*1, Nal^r	Invitrogen, USA
Plasmids		
pGEM-T Easy	A-T cloning vector, Ap^r	Promega, USA
pBluescript II-SK^-	Cloning vector, Ap^r	Nippon Gene, Japan
pKNG101	R6K origin *sacB* marker-exchange vector, *mob*$^+$, Stm^r	Kaniga et al. 1991
pRK2013	Helper plasmid carrying genes for conjugation, Km^r	Figurski and Helinski 1979
pFliC	1.96-kb *Sal*I-*Xba*I *fliC* fragment cloned into the same site of pGEM-T Easy	Laboratory collection
pMotA	1.96-kb *Sal*I-*Spe*I *motA* fragment cloned into the same site of pGEM-T Easy	Laboratory collection
pDEL-FliC	1.30-kb *Sal*I-*Xba*I fragment containing ORF of the *fliC* homologue deleted in frame, cloned into the same site of pKNG101	Laboratory collection
pDEL-MotA	1.24-kb *Sal*I-*Spe*I fragment containing ORF of the *motA* homologue deleted in frame, cloned into the same site of pKNG101	Laboratory collection

[a] Ap, ampicillin; Km, kanamycin; Nal, nalidixic acid; Sp, spectinomycin; Stm, streptomycin; ^{r}, resistance

(CCCP), which acts as an inhibitor of proton-driven force of the flagellar motor was purchased from Sigma (Tokyo, Japan).

Biofilm assay on microtiter plates

Biofilm formation was assayed by the ability of cells to adhere to the wells of 96-well microtiter plates made of polyvinyl chloride (PVC) (Becton Dickinson Labware, Lincoln Park, NJ, USA) with a slight modification of a previously described method (O'Toole and Kolter 1998a). An overnight culture grown in YP medium (approximately OD_{660} at 1.0) was diluted (1:10) in the indicated medium. The culture (100 µl, approximately 10^5 cfu) was added to each well of the microtiter plates and incubated for 12 h at 27°C without shaking. Then the cultures were removed from the wells and rinsed three times with sterile distilled water (SDW) to remove loosely associated bacteria. A 1% solution of crystal violet (CV) solution (125 µl) was added to each well (this dye stains the cells but not the plastic surface). Plates were incubated at room temperature for 15 min and rinsed with SDW. At this point, the biofilm was visible as purple rings formed at the interface between the air and liquid medium.

For quantitative analysis of biofilm production, 200 µl of 95% ethanol was added to each CV-stained well of a microtiter plate to solubilize the CV in the stained cells. The ethanol extracts from five wells were transferred to a 1.5-ml Eppendorf tube for a total volume of 1 ml. The absorbance of 1 ml of CV extracted from biofilm was determined at 600 nm in a spectrophotometer (Spectronic, Rochester, NY, USA). The absorbance thus obtained was divided by five to give an average absorbance for each well. Similarly, to study the effect of protonophore CCCP on biofilm formation, bacteria were grown until stationary phase (OD_{600} at about 1) in biofilm-inducing medium and diluted (1:10) into the same medium, after which 100 µl of the bacterial suspension was added to each well of the plates and biofilm formation was assayed as described.

Phase-contrast microscopy

After CV staining, the attached bacterial cells in the biofilm on the PVC surface at the air–liquid interface were directly visualized using a phase-contrast microscope (Olympus BX51, Tokyo, Japan). Images were captured using Viewfinder (Pixera, version 3.0.1).

Results

Optimum conditions for biofilm formation by *E. carotovora* subsp. *carotovora*

When we examined the ability of *E. carotovora* subsp. *carotovora* EC1 to form a biofilm on different abiotic surfaces such as PVC, polystyrene, and glass, PVC was the best surface (data not shown). YP medium supplemented with the salts of minimal medium (the biofilm-inducing medium) was found to support biofilm formation to a greater extent than either YP or LB medium alone (Fig. 1).

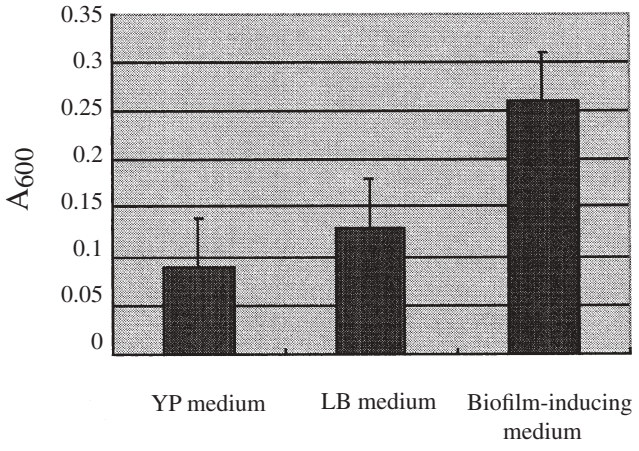

Fig. 1. The effect of different media on biofilm formation by *Erwinia carotovora* subsp. *carotovora* EC1. The extent of biofilm formation after 12 h of incubation in the indicated media is expressed as the absorbance at 600 nm

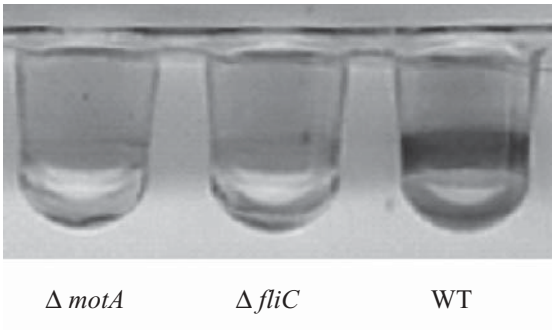

Fig. 2. Biofilm formation on the wells of microtiter plates by wild-type EC1 (*WT*), Δ*fliC*, and Δ*motA* mutants after 12 h of incubation at 27°C without shaking. A purple ring at the interface between the air and liquid medium was visible in the wild-type strain, but no such ring was visible in Δ*fliC* and Δ*motA* mutants

Analysis of Δ*fliC* and Δ*motA* mutants for biofilm formation

The biofilm was visualized as a purple ring of CV-stained cells that formed at the interface between the air and the medium of a static liquid culture in the wild-type strain. Biofilm formation in the wells of the microtiter plates by the nonflagellate (Δ*fliC*) and paralyzed (Δ*motA*) mutants were drastically reduced when compared with the wild-type strain (Fig. 2). When the formation of the biofilm was quantified by absorbance at 600 nm after solubilizing the ethanol-extracted CV from the stained biofilm, both the aflagellated mutant (Δ*fliC)* and the paralyzed mutant (Δ*motA*) had about one fifth of the absorbance of the wild type (Fig. 3). Thus, flagella-mediated motility seems to play an important role in biofilm formation.

Observation of biofilm with phase-contrast microscopy

When the biofilm on the PVC surface was examined with phase-contrast microscopy, the wild-type strain formed large clusters of cells closely adhering to each other over almost the entire surface of the well. In contrast, nonflagellated and paralyzed mutants formed small clusters of cells in a scattered pattern across mostly uncolonized regions (Fig. 4). No obvious difference was observed in the number of attached cells between the Δ*fliC* and Δ*motA* mutants.

Effect of protonophore CCCP on biofilm formation

We observed that in the presence of 10μM of the protonophore CCCP on YP agar medium, motility of *E. carotovora* subsp. *carotovora* EC1 was dramatically reduced without affecting growth (data not shown). The quantity of biofilm formation by wild type EC1 was reduced in the presence of the protonophore CCCP when compared with the untreated one (Fig. 5). Phase-contrast microscopic observation further confirmed that CCCP-treated biofilms contained fewer cells in small clusters that sparsely adhered to the PVC surface as in the cases of Δ*fliC* and Δ*motA* mutants (data not shown).

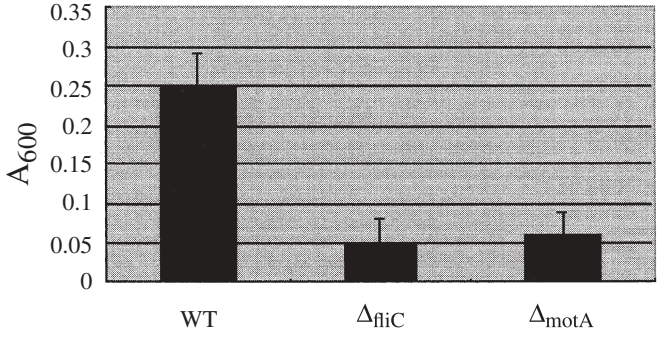

Fig. 3. Quantification of bacteria in biofilms formed by WT, and Δ*fliC* and Δ*motA* mutants. The biofilms were stained with crystal violet solubilized in 95% ethanol. The optical density of the resultant solution was measured at 600nm

Discussion

Of the many factors that influence biofilm formation, the nutritional status of the environment is considered an important factor (Dewanti and Wong 1995; Hood and Zottola 1997). For example, *Salmonella* spp. produce better biofilms in nutrient-limited medium, while *Listeria monocytogenes* produces better biofilms in nutrient-rich medium (Hood and Zottola 1997). We observed that YP medium containing salts of minimal medium supported biofilm formation to a larger extent, whereas YP and LB medium only slightly supported formation. This result suggests that biofilm formation by *E. carotovora* subsp. *carotovora* EC1 seems to be stimulated by unidentified components in the minimal medium in the presence of peptone or tryptone and yeast extract.

Motility has been suggested to be involved in biofilm formation in several cases (O'Toole and Kolter 1998a,b; Choy et al. 2004). In the case of *P. fluorescens*, the flagellum itself was reported to play a direct role in adhesion (Lawrence et al. 1987). Here, we observed that not only the aflagellate mutant (Δ*fliC*) but also the paralyzed mutant (Δ*motA*) were equally reduced in their ability to form a biofilm on the surface of PVC. These results suggest that functional flagella rather than nonfunctional flagella are involved in biofilm formation. Furthermore, after bacterial growth in the presence of the protonophore CCCP, biofilm formation was greatly reduced. Thus, motility itself seems to be involved in biofilm formation. In *E. coli*, motility was reported to be involved in attachment to the surface and in movement on the surface (Pratt and Kolter 1998). However, in the case of *P. aeruginosa*, flagella were reported to be uninvolved in the attachment to the abiotic surface (Klausen et al. 2003). Considering the equal inefficiency of both the Δ*fliC* and Δ*motA* mutants to form a biofilm, we suggest that flagella-mediated motility plays an important role in biofilm formation of *E. carotovora* subsp. *carotovora* EC1.

The biofilm forming ability of bacteria may promote host colonization as shown for several animal and plant pathogenic bacteria (Hoyle et al. 1992; Marques et al. 2002; Walker et al. 2004). However, the ability of biofilm formation did not influence virulence of *Staphylococcus aureus* (Kristian et al. 2004). We reported that Δ*fliC* and Δ*motA* mutants of *E. carotovora* subsp. *carotovora* EC1 had

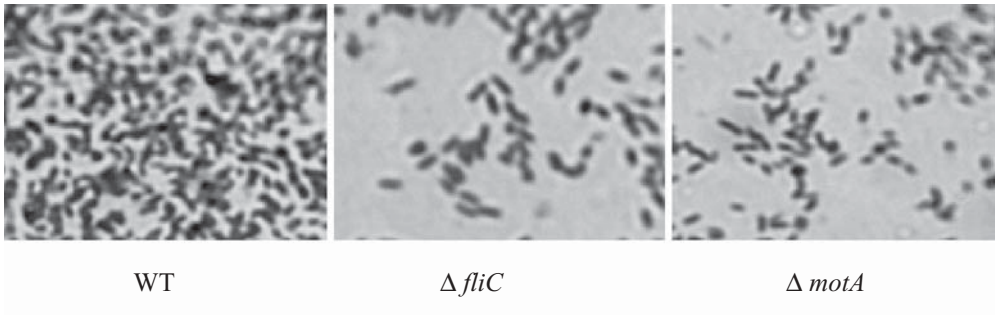

Fig. 4. Direct visualization of biofilm formation on the surface of the wells of microtiter plates at the air–liquid interface by phase-contrast microscopy under 100× oil immersion objective lens after 12h of incubation in the biofilm-inducing medium

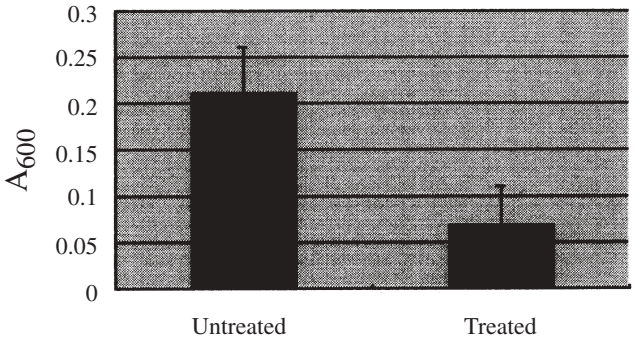

Fig. 5. Biofilm formation by *Erwinia carotovora* subsp. *carotovora* EC1 after treatment with carbonyl cyanide *m*-chlorophenylhydrazone (CCCP). The bacteria were cultured and resuspended in biofilm-inducing medium with or without 10 μM protonophore CCCP, and biofilm formation was assayed 12 h after addition into wells of microtiter plates as described in Materials and methods

reduced pathogenicity due to their lack of motility (Hossain et al. 2005). In this article, we demonstrated that both mutants were also reduced in their ability to form biofilms when compared with the wild type. Furthermore, in the presence of the protonophore CCCP, the level of biofilm formation by the wild-type strain on the PVC surface was drastically reduced. Thus, we must consider the new possibility that flagellar motility may be indirectly involved in pathogenicity by contributing to the formation of the biofilm, a pathogenicity factor.

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References

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (1987) Current protocols in molecular biology. Wiley, New York

Barras F, Gijsegem FV, Chatterjee AK (1994) Extracellular enzymes and pathogenesis of soft-rot *Erwinia.* Annu Rev Phytopathol 32:201–234

Boyd A, Chakrabarty AM (1995) *Pseudomonas aeruginosa* biofilms: role of the alginate exopolysaccharide. J Ind Microbiol 15:162–168

Choy WK, Zhou L, Syn CK, Zhang LH, Swarup S (2004) MorA defines a new class of regulators affecting flagellar development and biofilm formation in diverse Pseudomonas species. J Bacteriol 186:7221–7228

Condemine G, Castillo A, Passeri F, Enard C (1999) The PecT repressor coregulates synthesis of exopolysaccharides and virulence factors in *Erwinia chrysanthemi*. Mol Plant Microbe Interact 12:45–52

Costerton JW (1995) Overview of microbial biofilms. J Ind Microbiol 15:137–140

Costerton JW, Cheng K-J, Geesey GG, Ladd TI, Nickel JC, Dasgupta M, Marrie TJ (1987) Bacterial biofilms in nature and disease. Annu Rev Microbiol 41:435–464

Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM (1995) Microbial biofilms. Annu Rev Microbiol 49:711–745

Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: a common cause of persisent infections. Science 284:1318–1322

Danese PN, Pratt LA, Dove SL, Kolter R (2000) The outer membrane protein, antigen 43, mediates cell-to-cell interactions within *Escherichia coli* biofilms. Mol Microbiol 37:424–432

Davies DG, Geesey GG (1995) Regulation of the alginate biosynthesis gene *algC* in *Pseudomonas aeruginosa* during biofilm development in continous culture. Appl Environ Microbiol 61:860–867

Davies DG, Chakrabarty AM, Geesey GG (1993) Exopolysaccharide production in biofilms: substratum activation of alginate gene expression by *Pseudomonas aeruginosa*. Appl Environ Microbiol 59:1181–1186

Dewanti R, Wong AC (1995) Influence of culture conditions on biofilm formations by *Escherichia coli* 0157:H7. Int J Food Microbiol 26:147–164

Figurski DH, Helinski DR (1979) Replication of an origin containing derivative of plasmid RK2 dependent on a plasmid function provided in trans. Proc Natl Acad Sci USA 76:1648–1652

Geesey GG, Richardson WT, Yeomans HG, Irvin RT, Costerton JW (1977) Microscopic examination of natural sessile bacterial populations from an alpine stream. Can J Microbiol 23:1733–1736

Herroro M, de Lorenzo V, Timmis KN (1990) Transposon vectors containing non-antibiotic resistance selection markers for cloning and stable chromosomal insertion of foreign genes in gram-negative bacteria. J Bacteriol 172:6557–6567

Hood SK, Zottola EA (1997) Adherence to stainless steel by food borne microorganisms during growth in model food systems. Int J Food Microbiol 37:145–153

Hossain MM, Shibata S, Aizawa SI, Tsuyumu S (2005) Motility is an important determinant for pathogenesis of *Erwinia carotovora* subsp. *carotovora*. Physiol Mol Plant Pathol 66:134–143

Hoyle BD, Costerton JW (1991) Bacterial resistance to antibiotics: the role of biofilms. Prog Drug Res 37:91–105

Hoyle BD, Alcantara J, Costerton JW (1992) *Pseudomonas aeruginosa* biofilm as a diffusion barrier to piperacillin. Antimicrob Agents Chemother 36:2054–2056

Ichimiya T, Yamasaki T, Nasu M (1994) In vitro effects of antimicrobial agents on *Pseudomonas aeruginosa* biofilm formation. J Antimicrob Chemother 34:331–341

Kaniga K, Delor I, Cornelis GR (1991) A wide-host range suicide vector for improving reverse genetics in Gram-negative bacteria: inactivation of the *blaA* gene of *Yersinia enterocolitica*. Gene 109:137–141

Kharazmi A (1991) Mechanisms involved in the evasion of host-defence by *Pseudomonas aeruginosa*. Immunol Lett 30:201–205

Klausen M, Heydorn A, Ragas P, Lambertsen L, Aaes-Jorgensen A, Molin S, Tolker-Nielsen T (2003) Biofilm formation by *Pseudomonas aeruginosa* wild type, flagella and type IV pili mutants. Mol Microbiol 48:1511–1524

Kristian SA, Golda T, Ferracin F, Cramton SE, Neumeister B, Peschel A, Gotz F, Landmann R (2004) The ability of biofilm formation does not influence virulence of *Staphylococcus aureus* and host response in a mouse tissue cage infection model. Microb Pathogenesis 36:237–245

Lawrence JR, Delaquis PJ, Korber DR, Caldwell DE (1987) Behavior of *Psedomonas fluorescens* within the hydrodynamic boundary layers of surface microenvironments. Microb Ecol 14:1–14

Leid JG, Shirtliff ME, Costerton JW, Stoodley AP (2002) Human leukocytes adhere to, penetrate, and respond to *Staphylococcus aureus* biofilms. Infect Immun 70:6339–6345

Marques LLR, Ceri H, Manfio GP, Reid DM, Olson ME (2002) Characterization of biofilm formation by *Xylella fastidiosa* in vitro. Plant Dis 86:633–638

Mulholland V, Hinton JCD, Sidebotham J, Toth IK, Hyman LJ, Perombelon MCM, Reeves PJ, Salmond GPC (1993) A pleiotropic reduced virulence (Rvi^-) mutant of *Erwinia carotovora* subspecies *atroseptica* is defective in flagella assembly proteins that are conserved in plant and animal bacterial pathogens. Mol Microbiol 9:343–356

O'Toole GA, Kolter R (1998a) Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. Mol Microbiol 28:449–461

O'Toole GA, Kolter R (1998b) Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. Mol Microbiol 30:295–304

O'Toole GA, Gibbs KA, Hager PW, Phibbs PV Jr, Kolter R (2000) The global carbon metabolism regulator Crc is a component of a signal transduction pathway required for biofilm development by *Pseudomonas aeruginosa*. J Bacteriol 182:425–431

Perombelon MCM, Kelman A (1980) Ecology of the soft-rot erwinias. Annu Rev Phytopathol 18:361–387

Pratt LA, Kolter R (1998) Genetic analysis of *Escherichia coli* biofilm formation: roles of flagella, motility, chemotaxis and type I pili. Mol Microbiol 30:285–293

Sambrook J, Fristsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Schoonejans E, Expert D, Toussaint A (1987) Characterization and virulence properties of *Erwinia chrysanthemi* lipopolysaccharide-defective, phiEC2-resistant mutants. J Bacteriol 169:4011–4017

Tsuyumu S (1977) Inducer of pectic acid lyase in *Erwinia carotovora*. Nature 269:237–238

Verhoef J (1997) Host defense against infection. In: Crossley KB, Archer GL (eds) The staphylococci in human disease. Churchill Livingstone, NY, pp 213–232

Walker TS, Bais HP, Deziel E, Schweizer HP, Rahme LG, Fall R, Vivanco JM (2004) *Pseudomonas aeruginosa*-plant root interactions. Pathogenicity, biofilm formations, and root exudation. Plant Physiol 134:320–331

Watnick PI, Fullner KJ, Kolter R (1999) A role for the mannose-sensitive hemagglutinin in biofilm formation by *Vibrio cholerae* E1Tor. J Bacteriol 181:3606–3609

Yildiz FH, Schoolnik GK (1999) *Vibrio cholerae* 01 E1 Tor: identification of a gene cluster required for the rugose colony type, exopolysaccharide production, chlorine resistance, and biofilm formation. Proc Natl Acad Sci USA 96:4028–4033

Zottola EA, Sasahara KC (1994) Microbial biofilms in the food processing industry – should they be a concern? Int J Food Microbiol 23:125–148