Mutation of *rpiA* in *Enterobacter cloacae* Decreases Seed and Root Colonization and Biocontrol of Damping-Off Caused by *Pythium ultimum* on Cucumber

Scott M. Lohrke,¹ Pierre D. Dery,¹ Wei Li,² Ralph Reedy,³ Donald Y. Kobayashi³, and Daniel P. Roberts¹

¹ Sustainable Agricultural Systems Laboratory, USDA-ARS, Building 001, Room 140, 10300 Baltimore Avenue, Beltsville, MD 20705, U.S.A.; ² Department of Plant Science, Hubei University, Wuhan, People's Republic of China; ³ Department of Plant Pathology, Rutgers University, New Brunswick, NJ 08903, U.S.A.

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Strains of *Enterobacter cloacae* show promise as biocontrol agents for *Pythium ultimum*-induced damping-off on cucumber and other crops. *E. cloacae* A145 is a mini-Tn5 Km transposon mutant of strain 501R3 that was significantly reduced in suppression of damping-off on cucumber caused by *P. ultimum*. Strain A145 was deficient in colonization of cucumber, sunflower, and wheat seeds and significantly reduced in colonization of corn and cowpea seeds relative to strain 501R3. Populations of strain A145 were also significantly lower than those of strain 501R3 at all sampling times in cucumber, wheat, and sunflower rhizosphere. Populations of strain A145 were not detectable in any rhizosphere after 42 days, while populations of strain 501R3 remained at substantial levels throughout all experiments. Molecular characterization of strain A145 indicated mini-Tn5 Km was inserted in a region of the *E. cloacae* genome with a high degree of DNA and amino acid sequence similarity to *rpiA*, which encodes ribose-5-phosphate isomerase. In *Escherichia coli*, RpiA catalyzes the interconversion of ribose-5-phosphate and ribulose-5-phosphate and is a key enzyme in the pentose phosphate pathway. Ribose-5-phosphate isomerase activity in cell lysates from strain A145 was approximately 3.5% of that from strain 501R3. In addition, strain A145 was a ribose auxotroph, as expected for an *rpiA* mutant. Introduction of a 1.0-kb DNA fragment containing only the *rpiA* homologue into strain A145 restored ribose phosphate isomerase activity, prototrophy, seedling colonization, and disease suppression to levels similar to those associated with strain 501R3. Experiments reported here indicate a key role for *rpiA* and possibly the pentose phosphate pathway in suppression of damping-off and colonization of subterranean portions of plants by *E. cloacae*.

Our knowledge of the behavior of microbial inoculants in the spermosphere and rhizosphere needs to be expanded for

effective application of biocontrol agents of plant fungal diseases. A number of studies indicate that colonization of subterranean plant parts is a limiting step in certain biocontrol interactions (Bull et al. 1991; Chin-A-Woeng et al. 2000; Weller 1988). A number of diverse traits have been correlated with colonization of plant surfaces, including motility, chemotaxis, salt tolerance, root attachment, putrescine uptake, O-antigen side chain of lipopolysaccharide (LPS), and a site specific recombinase (Anderson et al. 1988; Dekkers et al. 1998a, b; de Weger et al. 1987, 1989; Gamliel et al. 1992; Heinrich and Hess 1985; Kuiper et al. 2001; Polonenko et al. 1981). It has also been demonstrated that growth of the microbial inoculant is indispensable for efficient colonization to occur (O'Sullivan et al. 1992; Roberts et al. 1996b, 1996c, 2000; Simons et al. 1996, 1997). Seed and root exudates are a complex and rich source of carbohydrates, amino acids, organic acids, and other nutrients that are thought to promote the metabolic activity and growth of beneficial bacteria in the spermosphere and rhizosphere (Curl and Truelove 1986). However, there is a significant gap in our knowledge of the role played by the metabolic (catabolic and anabolic) pathways of plant growth-promoting rhizobacteria and the nutrients supplied by host plants in growth, seed colonization, and root colonization.

Strains of *Enterobacter cloacae* have been shown to protect cucumber and other plants from damping-off caused by *Pythium ultimum* (Hadar et al. 1983; Howell et al. 1988; Nelson 1988; Nelson et al. 1986). The traits required for suppression of *P. ultimum* damping-off by *E. cloacae* are not completely understood. There is some evidence suggesting that the production of ammonia by *E. cloacae* is involved in disease suppression (Howell et al. 1988). However, the most compelling evidence comes from the work of Nelson and associates who showed that *P. ultimum* germinates rapidly in response to fatty acids released by germinating seeds (Nelson 1987, 1990; Rutledge et al. 1997) and that inactivation of these fatty acids by *E. cloacae* is involved in suppression of *P. ultimum* seed rot (Van Dijk and Nelson 2000). It appears that root colonization is not required for suppression of *P. ultimum* damping-off of cucumber. A root-colonization deficient strain of *Escherichia coli* provided disease suppression that was similar to a root-colonization proficient strain of *E. cloacae* (Roberts et al. 1997).

E. cloacae is also an efficient colonizer of the spermosphere and rhizosphere of a wide range of plant species (Hadar et al. 1983; Kleeberger et al. 1983; Ladha et al. 1983; Nelson 1988; Rennie et al. 1982; Roberts et al. 1996c, 1997, 1999). Our ap-

Corresponding author: Daniel P. Roberts; Sustainable Agricultural Systems Laboratory, USDA-ARS; E-mail: robertsd@ba.ars.usda.gov; Telephone: +301-504-5680.

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proach to identify bacterial genes and metabolic pathways important for both growth and colonization on plant parts has been to construct metabolic mutants of *E. cloacae* strain 501R3 and screen for mutants affected in seedling colonization. We previously reported the isolation of *E. cloacae* strain A-11, which contained a transposon insertion in *pfkA*, which encodes phosphofructokinase. Strain A-11 was reduced in colonization on cucumber and radish seeds, but colonized pea, sunflower, and sweet corn at wild-type levels (Roberts et al. 1992, 1999). A number of other catabolic and anabolic mutants of strain 501R3 have been identified, which are reduced in colonization of one or two seed types (Roberts et al. 1996b, 1996c; D. P. Roberts, W. Li, S. M. Lohrke, and P. D. Dery, *unpublished data*). To date, none of these mutants have been shown to be altered in biocontrol of damping-off on cucumber (D. P. Roberts, W. Li, S. M. Lohrke, and P. D. Dery, *unpublished data*).

E. cloacae strain A145 was selected for further study after the preliminary screening of a library of mini-Tn5 Km mutants of *E. cloacae* strain 501R3 (Roberts et al. 1996c). In initial screens, *E. cloacae* A145 was drastically reduced in colonization of cucumber seedlings relative to the parental strain 501R3 (data not shown). Southern hybridization analysis demonstrated the presence of a single mini-Tn5 Km insertion in the chromosome of strain A145 (data not shown). A cosmid library of A145 DNA was constructed, and cosmid pECL830, containing the mini-Tn5 Km insertion, was isolated. Subsequent subcloning from pECL830 yielded plasmids pP6 and pT145, each of which contains transposon mini-Tn5 Km (Fig. 1, Table 1). Sequencing outward from mini-Tn5 Km in pT145, using primers specific for the ends of mini-Tn5 Km, indicated that this transposon was inserted in a region of the strain A145 genome with a high degree of sequence similarity to *rpiA*, which encodes ribose-5-phosphate isomerase (Hove-Jensen and Maigaard 1993). The complete nucleotide sequence of *rpiA* was obtained by sequencing the *E. cloacae* inserts in pT145, pGK3.5, and pGK1.7. Plasmids pGK3.5 and pGK1.7 were also derived by subcloning from pP6 (Fig. 1). Analysis of the combined sequences from pT145, pGK3.5, and pGK1.7 revealed the presence of an open reading frame of 657 nucleotides.

sufficient to encode a polypeptide of 219 amino acids (data not shown). A consensus -10 promoter sequence (TATAAT) extending from residues -41 to -36 relative to the predicted ATG translation initiation site was identified. A consensus -35 promoter sequence was not observed at the correct spacing to the -10 sequence, which is consistent with the structure of the *Escherichia coli rpiA* gene. A proposed Shine-Dalgarno sequence (AGG) was identified 11 to 13 nucleotides upstream of the predicted start codon. An inverted repeat, representing a potential transcriptional termination site, was identified immediately downstream of the translational stop codon. The inverted repeat was located five bases from the stop codon and consisted of the 7-bp repeat CGGGGGG-N₆-CCCCCG, followed by six A residues. Comparison of this sequence with the *rpiA* sequence from *Escherichia coli* indicated 98.1% DNA sequence similarity and 96.8% amino acid sequence identity (data not shown). Analysis of the complete DNA sequence from pT145, pGK3.5, and pGK1.7 also indicated the presence of regions upstream and downstream of *rpiA* with a high degree of DNA sequence similarity to *iciA* and *serA* (Fig. 1). These genes encode an inhibitor of chromosome initiation (Thöny et al. 1991) and 3-phosphoglycerate dehydrogenase (Tobey and Grant 1986), respectively. The *rpiA*, *serA*, and *iciA* genes have identical organization and direction of transcription in *E. cloacae* and *Escherichia coli* (Hove-Jensen and Maigaard 1993).

To confirm that the transposon insertion affected RpiA activity, an enzymatic assay was carried out. Ribose-5-phosphate isomerase activity in cell lysates from *E. cloacae* A145 was approximately 3.5% of the ribose-5-phosphate isomerase activity in cell lysates from the parental strain, *E. cloacae* 501R3 (Table 2). Based on sequence data obtained from pT145, pGK3.5, and pGK1.7, the wild-type *rpiA* homologue from *E. cloacae* 501R3 was polymerase chain reaction (PCR) amplified using *Pfu* polymerase and primers rpiAUP and rpiADOWN (Fig. 1), and the resulting 1.3-kb blunt-end fragment was subcloned into the *Sma*I site of pGEM-7Zf(+). Both strands of the 1.3-kb PCR product were sequenced to confirm that the PCR product contained no mutations (data not shown). *rpiA*, containing all putative upstream promoter sequences, was subsequently subcloned as a 1.0-kb *Hind*III-*Xba*I fragment into pRK415 to generate pKR1.0 (Fig. 1, Table 1) and was introduced into strains A145 and 501R3 by electroporation. The direction of transcription of *rpiA* in pKR1.0 is opposite to that of the *lac* promoter of pRK415 (Fig. 1), preventing expression of *rpiA* from the strong inducible *lac* promoter. Introduction of the *rpiA* homologue on pKR1.0 into strains A145 and 501R3 resulted in a dramatic increase in ribose-5-phosphate isomerase

activities for both strains. Enzyme activity increased approximately 4.1-fold for 501R3(pKR1.0) and 107-fold for A145(pKR1.0) compared with strains 501R3 and A145, respectively (Table 2). Specific ribose-5-phosphate isomerase activity in A145(pKR1.0) was significantly greater than that in 501R3 and similar to that in 501R3(pKR1.0) (Table 2).

Strains A145 and A145(pRK415) grew in M56 minimal salts broth plus 0.2% glucose or xylose only in the presence of ribose (ribose was added at 0.0025%). This growth profile is expected for ribose auxotrophy resulting from a mutation in *rpiA* (Hove-Jensen and Maigaard 1993; Skinner and Cooper 1971; 1974). *E. cloacae* strain A145(pKR1.0) containing the wild-type *rpiA* homologue grew on M56 minimal salts broth plus either 0.2% glucose, arabinose, or xylose in the absence of ribose (data not shown). In addition, *E. cloacae* A145 did not grow on M56 minimal salts broth amended with 0.5% glycerol and 26 D,L- amino acids, 21 growth factors, or 6 nucleotide precursors added individually or in combinations (data not shown). *E. cloacae* 501R3 was able to grow under all nutritional conditions examined.

Seed and root colonization.

E. cloacae A145 was substantially reduced in short term colonization ability of corn (*Zea mays* cv. Stowells Evergreen), cowpea (*Vigna sinensis* cv. Pinkeye Purple Hull), cucumber (*Cucumis sativum* cv. Marketmore 76), sunflower (*Helianthus giganteus*), and wheat (*Triticum aestivum*) seeds in natural soil compared with strain 501R3 (Table 3). Populations of *E. cloacae* 501R3, the parental strain, increased substantially ($P \leq 0.001$), on all seed types over the initial 45 h after application. In contrast, populations of *E. cloacae* A145 increased ($P \leq 0.05$) on only corn and cowpea seeds. Populations of strain A145 were similar at 0 and 45 h after application on all other seed types.

E. cloacae A145 was also significantly impaired in colonization of cucumber, sunflower, and wheat rhizosphere relative to strain 501R3 (Fig. 2). Populations of strain A145 were significantly lower ($P \leq 0.05$) than those of 501R3 at all time points after being added to natural soil as a seed treatment. Populations of strain A145 were not detectable in the rhizosphere of any crop tested after 42 days. In contrast, populations of strain 501R3 remained at substantial levels during the entire experiment (Fig. 2).

Plant disease suppression.

E. cloacae A145 was significantly reduced in suppression of damping-off of cucumber caused by *P. ultimum* relative to *E. cloacae* 501R3 (Table 4). Mean plant stand per pot was significantly lower ($P \leq 0.05$) when seeds were treated with strain

Table 1. Bacterial strains and plasmids

Strain or Plasmid	Relevant Characteristics ^a	Reference or source
Strains		
<i>E. cloacae</i> 501R3	Spontaneous Rif ^r mutant of <i>E. cloacae</i> EcCT501	Roberts et al. 1992
<i>E. cloacae</i> A145	Mini-Tn5 Km mutant of 501R3; Rif ^r , Kan ^r	This study
<i>Escherichia coli</i> DH5 α	(ϕ 80dlacZ Δ M15) Δ (<i>lacZYA-argF</i>) U169 <i>glnV44 deoR gyrA96 recA1 relA91 endA1 thi-1 hsdR17</i>	Sambrook et al. 1989
Plasmids		
pRK415	Tc ^r ; cloning vector; IncP	Keen et al. 1988
pGEM-7Zf(+)	Ap ^r ; cloning vector; ColE1	Promega Corp.
pLAFR3	Tc ^r ; cosmid cloning vector; IncP	Staskawicz et al. 1986
pECL830	Tc ^r ; cosmid clone containing <i>rpiA</i> ::mini-Tn5 Km	This study
pP6	Tc ^r ; contains 9-kb <i>Bgl</i> III fragment from pECL830 containing <i>rpiA</i> ::mini-Tn5 Km in pRK415	This study
pGK3.5	Ap ^r ; contains 3.5-kb <i>Kpn</i> I fragment from pP6 in pGEM-7Zf(+). Contains <i>rpiA</i> ::mini-Tn5 Km	This study
pGK1.7	Ap ^r ; contains 1.7-kb <i>Kpn</i> I fragment from pP6 in pGEM-7Zf(+)	This study
pGR1.3	Ap ^r ; contains WT 1.3-kb <i>rpiA</i> PCR product from <i>E. cloacae</i> 501R3 in pGEM-7Zf(+)	This study
pT145	Tc ^r , Km ^r ; contains 3.5-kb <i>Clal</i> fragment from <i>E. cloacae</i> A145 containing <i>rpiA</i> ::mini-Tn5 Km in pGEM-7Zf(+)	This study
pKR1.0	Tc ^r ; contains 1.0-kb <i>Bam</i> HI- <i>Xba</i> I fragment from pGR1.3 in pRK415. Contains WT <i>rpiA</i>	This study

^a Ap^r, ampicillin resistant; Kan^r, kanamycin resistant; Rif^r, rifampicin resistant; Tc^r, tetracycline resistant; WT, wild type.

A145 than with the healthy check or when strain 501R3 was applied, with all levels of *P. ultimum* tested (Table 4). This was the case when similar levels of strains A145 and 501R3 were applied in seed treatments and when approximately threefold higher populations of strain A145 were applied. Mean plant stand per pot was similar to the pathogen check when cucumber seeds were treated with A145 and were sown in potting mix containing the higher levels of *P. ultimum* inoculum. There was no evidence of phytotoxicity with any seed treatment. Similar results were obtained from repeated experiments.

Genetic restoration of seedling colonization and disease suppression.

Introduction of the wild-type *rpiA* homologue into *E. cloacae* A145 on plasmid pKR1.0 resulted in a significant increase in colonization of cucumber seedlings when compared with both strains A145 and A145(pRK415) in the presence of *P. ultimum* (Table 5). By day 14, log₁₀ CFU per seedling was essentially identical for both 501R3 and A145(pKR1.0) (7.11 vs. 7.08 log₁₀ CFU per seedling, respectively), whereas populations of A145 and A145(pRK415) were both substantially and significantly lower (5.71 and 4.21 log₁₀ CFU per seedling, respectively). The presence of control plasmid pRK415 appeared to have a detrimental effect on the colonization of both A145 and 501R3. Interestingly, the presence of extra copies of *rpiA* in the wild-type strain 501R3 did not increase colonization, suggesting that increasing the copy number of *rpiA* does not improve colonization of the wild-type strain. Similar results were obtained with repeated experiments in the presence of and the absence of *P. ultimum* (data not shown).

Introduction of the wild-type *rpiA* homologue into *E. cloacae* A145 also resulted in a significant increase in disease

suppression (Table 4). Mean plant stand per pot with cucumber seeds treated with strain A145(pKR1.0) was similar to the healthy check and to treatments where strain 501R3 was applied. In addition, mean plant stand per pot was significantly greater ($P \leq 0.05$) in treatments containing A145(pKR1.0) than with treatments where A145 or A145(pRK415) were applied at similar or threefold greater populations.

DISCUSSION

E. cloacae A145, a transposon mutant derivative of *E. cloacae* 501R3, was severely impaired in seed (Table 3) and root (Fig. 2) colonization of several diverse plant species and was reduced in biocontrol of *P. ultimum*-induced damping-off on cucumber (Table 4). Genetic and biochemical data presented here demonstrate that the defect in colonization and biocontrol of A145 is the result of inactivation of *rpiA*, the gene encoding

Table 2. Ribose phosphate isomerase activity in *Enterobacter cloacae* strains 501R3 and A145 containing various plasmids

Strain	Functional <i>rpiA</i> ^a		Specific activity ($\mu\text{mol/min/mg protein}$) ^b
	Chromosomal	Plasmid	
501R3	+	–	1.14 $\pm$ 0.02
501R3 (pRK415)	+	–	0.82 $\pm$ 0.07
501R3 (pKR1.0)	+	+	4.71 $\pm$ 0.22
A145	–	–	0.04 $\pm$ 0.04
A145 (pRK415)	–	–	0.16 $\pm$ 0.01
A145 (pKR1.0)	–	+	4.25 $\pm$ 0.45

^a Indicates the presence (+) or absence (–) of a wild-type *rpiA* gene on the chromosome or a plasmid.

^b Values are the means of four replicate cultures with standard deviations included and measure the conversion of ribose-5-phosphate to ribulose-5-phosphate.

Table 3. Growth of *Enterobacter cloacae* 501R3 and A145 on various seeds in natural soil^a

Seed	Time (h)	Log ₁₀ CFU/seedling		
		501R3	A145	Pr > F ^b
Corn	0	4.26	4.81	0.09
	45	8.05	5.77	0.0002*
Wheat	0	4.29	4.71	0.24
	45	7.07	4.62	0.0002*
Cucumber	0	4.83	4.95	0.51
	45	5.97	5.04	0.0001*
Cowpea	0	4.44	4.66	0.57
	45	7.40	5.93	0.008*
Sunflower	0	4.19	4.78	0.21
	45	6.84	5.13	0.006*

^a Values are the means of three replicate experiments carried out in a natural Galestown gravelly loamy sand soil.

^b Pr > F log₁₀ CFU/seed values in this row. * Indicates a statistically significant ($P \leq 0.05$) difference in population between strains.

Fig. 2. Root colonization by *Enterobacter cloacae* strains 501R3 and A145 in natural soil. Values are the mean of three replicate experiments carried out in natural soil and expressed as mean log₁₀ CFU per g of fresh weight root. Error bars represent one standard deviation from the mean. Limit of detection is log₁₀ 2.0 and is indicated by a horizontal dashed line. Populations of A145 are significantly lower ($P \leq 0.05$) than 501R3 at all time points after addition to soil.

ribose-5-phosphate isomerase. *E. cloacae* A145 contains a single mini-Tn5 Km insertion that is inserted in a region of the *E. cloacae* genome with very high DNA and amino acid sequence similarity to *rpiA* from *Escherichia coli* (data not shown). *E. cloacae* A145 is significantly reduced in RpiA activity relative to strain 501R3 and is a ribose auxotroph, as expected for an *rpiA* mutant (Hove-Jensen and Maigaard 1993). A 1.0-kb DNA fragment containing only *rpiA* restored prototrophy, RpiA activity, seedling colonization, and disease suppression, indicating that the *E. cloacae* A145 phenotype is due solely to a mutation in *rpiA* and not to other physically linked genes (Tables 2, 4, and 5). In addition, the presence of an inverted repeat indicative of a transcriptional termination signal downstream of *rpiA* suggests this gene is expressed as a monocistronic mRNA.

Strain A145 is unique among all the *E. cloacae* metabolic mutants we have identified to date because it is the only mutant that exhibited impaired colonization on all plant seeds tested and also was reduced for biocontrol of damping-off on cucumber (Roberts et al. 1996c; 1999; D. P. Roberts, W. Li, S. M. Lohrke, and P. D. Dery, unpublished data). In order to explain the defects in colonization and biocontrol of strain A145, it is necessary to examine the role of *rpiA* in bacterial metabolism (Fig. 3). RpiA plays a key role in the pentose phosphate pathway. One function of the pentose phosphate pathway is to allow for growth on pentoses as a sole carbon source (Fraenkel et al. 1996; Lin 1996). It is unlikely that the loss of catabolic capabilities due to the mutation in *rpiA* results in the strain A145 phenotype. Ribose is a minor constituent in exudates of seeds we have tested (Roberts et al. 1999). In addition, strain A-11, another mutant derived from strain 501R3, lost the ability to catabolize a much broader range of exudate than strain A145, and strain A-11 was affected in colonization of a more narrow range of seed types compared to A145 and was not affected in disease suppression (Roberts et al. 1999; D. P. Roberts, W. Li, S. M. Lohrke, and P. D. Dery, unpublished data).

Another, and probably more relevant, function of *rpiA* to colonization and to disease suppression is to provide intermediates for several essential anabolic pathways (Fig. 3). One of these intermediates is 5-phosphoribosyl 1-pyrophosphate (PRPP), which is derived directly from ribose-5-phosphate and is required for the biosynthesis of purine and pyrimidine nucleotides, histidine, tryptophan, and NAD. Ribose-5-phosphate in combination with xylulose-5-phosphate is required for the synthesis of the core polysaccharides of LPS and for aromatic amino acids and certain vitamins. In *Escherichia coli*, another gene that encodes ribose-5-phosphate isomerase activity (*rpiB*) has been identified, but the majority of the isomerase activity is provided by *rpiA* (Sørensen et al. 1996). The enzyme assay data from Table 2 suggests that, if *E. cloacae* contains an *rpiB* homologue, the activity is very low. Examination of the pen-

tose phosphate pathway outlined in Fig. 3 suggests that in an *rpiA* mutant, very little ribose-5-phosphate should be formed from ribulose-5-phosphate in the absence of exogenous ribose, resulting in insufficient PRPP, sedoheptulose-7-phosphate and erythrose-4-phosphate for essential anabolic pathways. The low levels of ribose in the seed exudates are irrelevant to strain 501R3 which possesses ribose phosphate isomerase activity, and is able to generate sufficient ribose-5-phosphate for growth from other, more prevalent, compounds present in exudate. However, the role of the anabolic pathways branching off from the pentose phosphate pathway in colonization and disease suppression is still unclear. There has been one report documenting the importance of LPS to root colonization (Dekkers et al. 1998b).

In order to develop microbial inoculants that are effective biocontrol agents, it is imperative that inconsistencies in the performance of inoculant strains be resolved (Cook and Baker 1983; Weller 1988). There is a consensus that these inconsistencies may be due, at least in part, to an inability of the introduced strain to compete with indigenous microorganisms for nutrients and other resources, resulting in a failure to effectively colonize the rhizosphere (Lugtenberg and Dekkers 1999; O'Sullivan et al. 1992; Roberts et al. 1996a; 1996c; 1999). One approach to improving colonization is to increase expression of genes involved in the colonization process. The concept of metabolic engineering of microorganisms to increase either the productivity or quality, or both, of microbial fermentations has attracted increased attention from industry (Bailey 1991; Brown 1997; Emmerling et al. 1999). Within any given pathway, the metabolic flow is determined to a large extent by the activities of one or more key enzymatic steps. It is argued that by increased expression of these key enzymes, metabolic flow through the pathway can be increased, which may result in increased production of one or more desired metabolites. Given the central role of the pentose phosphate pathway in the synthesis of many key metabolites, metabolic engineering of this pathway may allow for improved colonization capabilities. However, overexpression of *rpiA* in *E. cloacae* did not significantly improve colonization or biocontrol, suggesting that while *rpiA* is essential for these processes, it does not appear to be a rate-limiting step. It is possible that overexpression of other genes in the pentose phosphate pathway, either alone or in combination with *rpiA*, may allow for increased metabolic flow, resulting in increased anabolic activity that may improve colonization or biocontrol, or both.

MATERIALS AND METHODS

Bacterial strains and culture conditions.

All strains and plasmids used in this study are listed in Table 1. *E. cloacae* 501R3 is a prototrophic, spontaneous, rifampicin-

Table 4. Biocontrol of damping-off on cucumber by *Enterobacter cloacae* strains

Treatment	Log ₁₀ CFU/seed	Mean plant stand per pot ^a			
		0 ^b	25 ^b	40 ^b	60 ^b
501R3	8.52 ± 0.10	5.0 A	4.4 AB	4.1 AB	3.9 B
A145 (normal) ^c	8.68 ± 0.20	4.9 AB	2.4 CD	0.6 FG	0.3 G
A145 (high) ^c	9.15 ± 0.15	5.0 A	2.6 C	1.1 EFG	1.4 DEF
A145 (pRK415) (normal) ^c	8.59 ± 0.10	5.0 A	2.5 C	1.0 EFG	1.0 EFG
A145 (pRK415) (high) ^c	9.07 ± 0.19	4.9 AB	1.8 CDE	0.3 G	0.6 FG
A145 (pKR1.0)	8.34 ± 0.34	4.9 AB	4.5 AB	4.0 AB	4.1 AB
No bacteria	ND ^d	5.0 A	0.8 EFG	0.1 G	0.5 FG

^a Values are the means of eight replicates, each containing five seeds carried out in soilless mix and expressed as plant stand per pot. Numbers followed by the same letter are not statistically different ($P \leq 0.05$). Least significant difference ($P \leq 0.05$) for this experiment was 1.0.

^b Quantity of *Pythium ultimum* sporangia per cm³ of soilless mix inoculum.

^c Relative levels of inoculum/seed.

^d ND indicates no Rif^r bacteria detected.

resistant mutant of *E. cloacae* strain EcCT501 (Nelson 1988; Roberts et al. 1992). *E. cloacae* A145 is a mini-Tn5 Km mutant of 501R3. *E. cloacae* strains used in this report were grown in either Luria-Bertani (LB) broth or agar (Miller 1972) or M56 basal salts broth or agar (Nguyen et al. 1983). Unless indicated otherwise, strains were grown to stationary phase at 250 rpm and 37°C. When appropriate, the medium was supplemented with kanamycin (50 µg/ml), rifampicin (100 µg/ml) or tetracycline (12.5 µg/ml).

Molecular techniques.

DNA isolations, transformations, electroporation, restriction enzyme digests, electrophoresis, ligations, and hybridizations were performed as previously described (Sambrook et al. 1989). A cosmid clone library of strain A145 was con-

structed in pLAFR3 using standard procedures (Sambrook et al. 1989). Cosmid clone pECL830 was identified by selection on LB media supplemented with tetracycline and kanamycin. The 9-kb *Bgl*II fragment containing mini-Tn5 Km was subcloned from pECL830 into pRK415, generating subclone pP6 (Fig. 1). Plasmid pP6 was digested with *Cla*I, and a 3.5-kb fragment containing mini-Tn5 Km plus flanking *E. cloacae* DNA was cloned into pGEM-7Zf(+), generating pT145. Primers designed to the right (MT5R2096: 5'-GGG CCT TGA TGT TAC CGA GAG C-3') and the left (MT5L294: 5'-TAA GCG TGC ATA ATA AGC CCT ACA-3') ends of mini-Tn5 Km were used to sequence outwards from mini-Tn5 Km in pT145. Plasmid pP6 was digested with *Kpn*I and the resulting *Kpn*I fragments subcloned into pGEM-7Zf(+), generating pGK3.5 and pGK1.7 (Fig. 1), which were subsequently sequenced. Wild-type *rpiA*, plus all transcriptional control elements, was PCR amplified from strain 501R3 using primers *rpiAUP*: 5'-GCT TAT TCT GCA GTA TGA CAA A and *rpiADOWN*: 5'-CAT CCA TGC CCC CGA ACT-3' and *Pfu* polymerase. Primer *rpiAUP* is located at residues -610 to -586 relative to the translation start site and primer *rpiADOWN* is located 80 to 103 residues downstream of the translation termination site. The resulting 1.3-kb PCR product containing *rpiA* was cloned into the *Sma*I site of pGEM-7Zf(+), generating pGR1.3. The complete nucleotide sequence of pGR1.3 was determined on both strands. DNA sequencing was carried out by PCR-mediated Taq DyeDeoxy terminator cycle sequencing. The LASERGENE (DNASTAR, Inc., Madison, WI, U.S.A.) sequence analysis software package and the BLAST software package (Altschul et al. 1997) were used for all DNA and protein sequence analysis. For

Table 5. Colonization of cucumber seedlings by *Enterobacter cloacae* A145 strains^a

Treatment	Day 0	Day 4	Day 14
	log ₁₀ CFU	log ₁₀ CFU	log ₁₀ CFU
501R3	8.71 A	8.40 BCD	7.11 F
501R3 (pRK415)	8.76 A	8.23 D	6.41 H
501R3 (pKR1.0)	8.58 AB	7.81 E	6.76 G
A145	8.47 BC	7.67 E	5.71 I
A145 (pRK415)	8.25 CD	7.16 F	4.21 J
A145 (pKR1.0)	8.62 AB	8.25 CD	7.08 F

^a Values are the means of six replicates carried out in a soilless mix containing 60 *Pythium ultimum* sporangia per cm³ and expressed as log₁₀ CFU/seed or seedling. Means with the same letter are not significantly different ($P \leq 0.05$). Least significant difference ($P \leq 0.05$) = 0.24.

Fig. 3. Pentose phosphate metabolism in *Escherichia coli*. Metabolism of pentose phosphates in *Escherichia coli*, with emphasis placed on the anabolic portion of the pathway. PRPP = 5-phosphoribosyl 1-pyrophosphate; EMP = Embden-Meyerhoff Pathway; LPS = lipopolysaccharide; NAD = nicotinamide adenine dinucleotide; *deoB* = phosphodeoxyribomutase; *deoD* = purine nucleoside phosphorylase; *gnd* = 6-phosphogluconate dehydrogenase; *prs* = phosphoribosyl diphosphate synthetase; *rbsK* = ribokinase; *rpe* = ribose phosphate epimerase; *rpiA* = ribose phosphate isomerase A; *rpiB* = ribose phosphate isomerase B; *tal* = transaldolase; *tkt* = transketolase; and *zwf* = glucose-6-phosphate dehydrogenase.

complementation studies, insert DNA from pGR1.3 containing *rpiA* was subcloned into pRK415 as a 1.0-kb *HindIII*-*XbaI* fragment to generate pKR1.0. The location of the *HindIII* site is 210 residues upstream of the translation start site, and the *XbaI* site is located downstream of the *rpiA* gene in pGEM7Zf(+).

RpiA enzyme assays.

Ribose-5-phosphate isomerase activity was determined by the method of MacElroy and Middaugh (1982). *E. cloacae* strains were grown overnight at 37°C with shaking in 5 ml of LB broth containing appropriate antibiotics. Overnight culture (1 ml) was removed, centrifuged, and resuspended in 1 ml of assay buffer (0.1 M Tris-HCl pH 7.4, 1 mM EDTA, 1 mM mercaptoethanol). This suspension was diluted 1:3 in assay buffer and the cells disrupted by sonication on ice (Vibra Cell Sonicator with a micro tip at a setting of 3.0 for 3 × 15 s; Sonics and Materials, Danbury, CT, U.S.A.). Cell debris was pelleted by centrifugation in a microcentrifuge for 2 min at 4°C, the supernatant transferred to a clean tube and then incubated on ice. Crude lysate (0.1 ml) or assay buffer (as control) were added to 0.3 ml of assay buffer and 0.1 ml of 30 mM ribose-5-phosphate (in assay buffer) and then incubated at 37°C for 15 min. Reactions were stopped by the sequential addition of 0.5 ml 30 mM cysteine, 5 ml 70% H₂SO₄ and 0.2 ml 0.1% carbazole and then incubated at 37°C for 30 min for color development. The optical density at 540 nm (OD₅₄₀) of the samples was determined. An OD₅₄₀ of 0.14 corresponds to the formation of 0.1 μmol of ribulose-5-phosphate (MacElroy 1982). The protein content in cell lysates was determined using the Bio-Rad Protein Assay kit (Bio-Rad, Hercules, CA, U.S.A.) and was used to determine specific activity (μmoles ribulose-5-phosphate/min/mg protein).

In vitro growth assays.

Strains 501R3, A145, A145(pRK415), and A145(pKR1.0) were grown overnight at 37°C with shaking in 100 ml of M56 minimal salts medium containing 0.2% glucose and 0.0025% ribose. Overnight cultures were centrifuged, washed once with sterile M56 minimal salts containing no supplements, and resuspended to an OD₅₄₀ of 0.8 in M56 minimal salts containing no supplements. The washed cell suspension (400 μl) was used to inoculate in triplicate 20 ml of M56 minimal salts medium in 250 ml flasks amended with either 0.2% ribose, 0.2% xylose, 0.2% glucose, 0.2% ribose plus 0.2% xylose, 0.2% ribose plus 0.2% glucose or 0.2% glucose plus 0.5% yeast extract. The initial OD₅₄₀ of the cultures was determined, and the flasks were incubated at 37°C with constant shaking at 250 rpm. Optical densities of the cultures were taken at 2 h postinoculation and at subsequent 1 h intervals for a total of 7 h. For biochemical complementation of auxotrophy, ribose was added to M56 basal salts medium at 0.0025%.

Disease suppression assays.

E. cloacae strains were grown overnight in LB broth containing the appropriate antibiotics (Table 1), were washed in sterile distilled water (SDW), and were incorporated into treatments of cucumber seeds with pelgel (LiphaTech Inc., Milwaukee, WI, U.S.A.) and peat as described (Roberts et al. 1997). Seeds coated with pelgel, peat, and SDW, but no bacteria were used as controls. *P. ultimum* was grown on corn meal agar at 25°C for 3 days, were flooded with soil extract (Ayers and Lumsden 1975), and were incorporated into nonsterile soilless mix (Redi-earth peat-lite mix, Scott's Horticultural Products, Marysville, OH, U.S.A.). Soilless mix, soilless mix amended with sporangia of *P. ultimum* or with SDW, treated cucumber seeds, and soilless mix amended with sporangia of *P. ultimum*

or SDW, were added as sequential layers to 120 cm³ cups as described (Roberts et al. 1997). Eight replicate cups were sown with five seeds each and were incubated in a growth chamber at 22°C for 14 days with a 12-h photoperiod. Plant stand per cup was determined, analysis of variance (ANOVA) carried out, and differences among means estimated using a protected least significant difference (LSD) test (SAS, Cary, NC, U.S.A.). The experiment was performed three times. Repeated experiments were not combined prior to analysis.

Populations of strains were monitored during the course of the biocontrol assay by sowing seeds treated for the biocontrol assay in 4 cm³ nonsterile soilless mix (prepared for the biocontrol assay) in 14 ml sterile snap cap tubes and then incubating the tubes in the growth chamber along with the biocontrol assay. CFU were determined at various times by dilution plating the entire contents of the tubes onto LB agar containing the appropriate antibiotics. The mean log₁₀ CFU per seedling for each strain was determined, ANOVA was carried out, and differences among means were estimated using LSD (SAS). Treatments were replicated six times, and the experiment was performed three times. Repeated experiments were not combined prior to analysis.

Seed colonization experiments in natural soil.

E. cloacae strains were grown, washed, resuspended in SDW, and applied to single cucumber, corn, cowpea, sunflower, or wheat seeds as aqueous suspensions as previously described (Roberts et al. 1992). Seeds were buried in a natural Galestown gravelly loamy sand soil (77.8% sand, 12.6% silt, 9.6% clay, 0.6% organic matter, pH 5.8) that had been previously equilibrated to -75 kPa and were incubated at 22°C. Seeds were not surface sterilized. CFU were determined at 0 and 45 h by dilution plating onto LB agar containing the appropriate antibiotics as described above. Experiments were performed three times with two replicates per treatment. The mean log₁₀ CFU per seedling for each seed type for each strain was determined and compared using the Student's *t* test (SAS). Repeated experiments were combined prior to statistical analysis.

Root colonization experiments in natural soil.

Cucumber, sunflower, and wheat seeds treated with approximately 10⁸ CFU per seed of *E. cloacae* strains in a pelgel/peat formulation as described above were sown in the natural Galestown gravelly loamy sand soil in 6.5 cm diameter × 25 cm deep pots and were incubated in a growth chamber at 22°C with a 12-h photoperiod. Plants were removed at sampling time and the entire root system sampled by cutting the root at the soil line. The entire root system and adhering soil were placed in SDW, sonicated, and CFU per root system determined as described (Roberts et al. 1997). Root system fresh weight was determined for each sample. Log₁₀ CFU per gram of fresh weight root tissue was determined for each treatment and compared using the Student's *t* test (SAS). The experiment was performed three times with six replicates per treatment for each crop species. Repeated experiments were combined prior to analysis.

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LITERATURE CITED

- Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* 25:3389-3402.

- Anderson, A. J., Habibzadegah-Tari, P., and Tepper, C. S. 1988. Molecular studies on the role of root surface agglutinin in adherence and colonization by *Pseudomonas putida*. Appl. Environ. Microbiol. 54:375-380.
- Ayers, W. A., and Lumsden, R. D. 1975. Factors affecting production and germination of oospores of three *Pythium* species. Phytopathology 65:1094-1100.
- Bailey, J. E. 1991. Toward a science of metabolic engineering. Science 252:1668-1675.
- Brown, A. J. P. 1997. Control of metabolic flux in yeasts and fungi. Trends Biotechnol. 15:445-447.
- Bull, C. T., Weller, D. M., and Thomashow, L. S. 1991. Relationship between root colonization and suppression of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* strain 2-79. Phytopathology 81:954-959.
- Chin-A-Woeng, T. F. C., Bloemberg, G. V., Mulders, I. H. M., Dekkers, L. C., and Lugtenberg, B. J. J. 2000. Root colonization by phenazine-1-carboxamide-producing bacterium *Pseudomonas chlororaphis* PCL1391 is essential for biocontrol of tomato foot and root rot. Mol. Plant-Microbe Interact. 13:1340-1345.
- Cook, R. J. and Baker, K. F. 1983. The Nature and Practice of Biological Control of Plant Pathogens. American Phytopathological Society, St. Paul, MN, U.S.A.
- Curl, E. A., and Truelove, B. 1986. The Rhizosphere. Springer-Verlag, New York.
- Dekkers, L. C., Phoelich, C. C., van der Fits, L., and Lugtenberg, B. J. J. 1998a. A site-specific recombinase is required for competitive root colonization by *Pseudomonas fluorescens* WCS365. Proc. Natl. Acad. Sci. U.S.A. 95:7051-7056.
- Dekkers, L. C., van der Bij, A. J., Mulders, I. H. M., Phoelich, C. C., Wentwoord, R. A. R., Glandorf, D. C. M., Wijffelman, C. A., and Lugtenberg, B. J. J. 1998b. Role of the O-antigen of lipopolysaccharide, and possible roles of growth rate and of NADH:ubiquinone oxidoreductase (*nuo*) in competitive tomato root-tip colonization by *Pseudomonas fluorescens* WCS365. Mol. Plant-Microbe Interact. 11:763-771.
- de Weger, L. A., van der Vlugt, C. I. M., Wijffges, A. H. M., Bakker, P. A. H. M., Schippers, B., and Lugtenberg, B. J. J. 1987. Flagella of a plant growth-stimulating *Pseudomonas fluorescens* are required for colonization of potato roots. J. Bacteriol. 169:2769-2773.
- de Weger, L. A., Bakker, P. A. H. M., Schippers, B., van Loosdrecht, M. C. M., and Lugtenberg, B. J. J. 1989. *Pseudomonas* spp. with mutational changes in the O-antigenic side chain of their lipopolysaccharide and affected in their ability to colonize potato roots. NATO Adv. Study Inst. Ser. H. 36:197-202.
- Emmerling, M., Bailey, J. E., and Sauer, U. 1999. Glucose catabolism of *Escherichia coli* strains with increased activity and altered regulation of key glycolytic enzymes. Metabolic Eng. 1:117-127.
- Fraenkel, D. G. 1996. Glycolysis. Pages 189-198 in: *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology, Vol. 2. F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger, eds., American Society for Microbiology, Washington, D.C.
- Gamliel, A., and Katan, J. 1992. Chemotaxis of fluorescent pseudomonads towards seed exudates and germinating seeds in solarized soil. Phytopathology 82:328-332.
- Hadar, Y., Harman, G. E., Taylor, A. G., and Norton, J. M. 1983. Effects of pregermination of pea and cucumber seeds and of seed treatment with *Enterobacter cloacae* on rots caused by *Pythium* spp. Phytopathology 73:1322-1325.
- Heinrich, D., and Hess, D. 1985. Chemotactic attraction of *Azospirillum lipoferum* by wheat roots and characterization of some attractants. Can. J. Microbiol. 31:27-31.
- Howell, C. R., Beir, R. C., and Stipanovic, R. D. 1988. Production of ammonia by *Enterobacter cloacae* and its possible role in the biological control of *Pythium* preemergence damping off by the bacterium. Phytopathology 78:1075-1078.
- Hove-Jensen, B., and Maigaard, M. 1993. *Escherichia coli* *rpiA* encoding ribose phosphate isomerase A. J. Bacteriol. 175:5628-5635.
- Keen, N. T., Tamaki, S., Kobayashi, D., and Trollinger, D. 1988. Improved broad-host-range plasmids for DNA cloning in gram-negative bacteria. Gene 70:191-197.
- Kleeberger, A., Castorph, H., and Klingmüller, W. 1983. The rhizosphere microflora of wheat and barley with special reference to gram-negative bacteria. Arch. Microbiol. 136:306-311.
- Kuiper, I., Bloemberg, G. V., Noreen, S., Thomas-Oates, J. E., and Lugtenberg, B. J. J. 2001. Increased uptake of putrescine in the rhizosphere inhibits competitive root colonization by *Pseudomonas fluorescens* strain WCS365. Mol. Plant-Microbe Interact. 14:1096-1104.
- Ladha, J. K., Barraquio, L., and Watanabe, I. 1983. Isolation and identification of nitrogen-fixing *Enterobacter cloacae* and *Klebsiella planticola* associated with rice plants. Can. J. Microbiol. 29:1301-1308.
- Lin, E. C. C. 1996. Dissimilatory pathways for sugars, polyols, and carbohydrates. Pages 307-342 in: *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology, Vol. 2. F. C. Neidhardt, R. Curtiss, III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger, eds. American Society for Microbiology, Washington, D.C.
- Lugtenberg, B. J. J., and Dekkers, L. C. 1999. What makes *Pseudomonas* bacteria rhizosphere competent? Environ. Microbiol. 1:9-13.
- MacElroy, R. D., and Middaugh, C. R. 1982. Bacterial ribosephosphate isomerase. Meth. Enzymol. 89:571-579.
- Miller, J. H. 1972. Experiments in Molecular Genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, U.S.A.
- Nelson, E. B. 1988. Biological control of *Pythium* seed rot and preemergence damping-off of cotton with *Enterobacter cloacae* and *Erwinia herbicola* applied as seed treatments. Plant Dis. 72:140-142.
- Nelson, E. B. 1987. Rapid germination of sporangia of *Pythium* species in response to volatiles from germinating seeds. Phytopathology 77:1108-1122.
- Nelson, E. B. 1990. Exudate molecules initiating fungal responses to seeds and roots. Plant Dis. 72:140-142.
- Nelson, E. B., Chao, W.-L., Norton, J. M., Nash, G. T., and Harman, G. E. 1986. Attachment of *Enterobacter cloacae* to hyphae of *Pythium ultimum*: Possible role in the biological control of *Pythium* preemergence damping-off. Phytopathology 76:327-335.
- Nguyen, N. D., Gottfert, M., Singh, M., and Klingmüller, W. 1983. Nif hybrids of *Enterobacter cloacae*: Selection for *nif* gene integration with *nif* plasmids containing the Mu transposon. Mol. Gen. Genet. 192:439-443.
- O'Sullivan, D. J., and O'Gara, F. 1992. Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root pathogens. Microbiol. Rev. 56:662-676.
- Polonenko, D. R., Dumbroff, E. B., and Mayfield, C. I. 1981. Microbial responses to salt-induced osmotic stress. II. Population changes in the rhizoplane and rhizospheres. Plant Soil 63:415-426.
- Rennie, J. R., de Freitas, J. R., Ruschel, A. P., and Vose, P. B. 1982. Isolation and identification of N₂-fixing bacteria associated with sugar cane (*Saccharum* sp.). Can. J. Microbiol. 28:462-467.
- Roberts, D. P., Sheets, C. J., and Hartung, J. S. 1992. Evidence for proliferation of *Enterobacter cloacae* on carbohydrates in cucumber and pea spermospheres. Can. J. Microbiol. 38:1128-1134.
- Roberts, D. P., Dery, P. D., and Hartung, J. S. 1996a. Peptide utilization and colonization of corn, radish and wheat spermospheres by *Enterobacter cloacae*. Soil Biol. Biochem. 28:1109-1111.
- Roberts, D. P., Marty, A. M., Dery, P. D., and Hartung, J. S. 1996b. Isolation and modulation of growth of a colonization-impaired strain of *Enterobacter cloacae* in cucumber spermosphere. Can. J. Microbiol. 42:196-201.
- Roberts, D. P., Marty, A. M., Dery, P. D., Yucel, I., and Hartung, J. S. 1996c. Amino acids as reduced carbon sources for *Enterobacter cloacae* during colonization of the spermospheres of crop plants. Soil. Biol. Biochem. 28:1015-1020.
- Roberts, D. P., Dery, P. D., Hebbbar, P. K., Mao, W., and Lumsden, R. D. 1997. Biological control of damping-off of cucumber caused by *Pythium ultimum* with a root-colonization-deficient strain of *Escherichia coli*. J. Phytopathol. 145:383-388.
- Roberts, D. P., Dery, P. D., Yucel, I., Buyer, J., Holtman, M. A., and Kobayashi, D. Y. 1999. Role of *pfkA* and general carbohydrate catabolism in seed colonization by *Enterobacter cloacae*. Appl. Environ. Microbiol. 65:2513-2519.
- Roberts, D. P., Dery, P. D., Yucel, I., and Buyer, J. S. 2000. Importance of *pfkA* for rapid growth of *Enterobacter cloacae* during colonization of crop seeds. Appl. Environ. Microbiol. 66:87-91.
- Ruttledge, T. R., and Nelson, E. B. 1997. Extracted fatty acids from *Gossypium hirsutum* stimulatory to the seed-rotting fungus, *Pythium ultimum*. Phytochemistry 46:77-82.
- Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Press, Cold Spring Harbor, NY, U.S.A.
- Simons, M., Permentier, M. P., de Weger, L. A., Wijffelman, C. A., and Lugtenberg, B. J. J. 1997. Amino acid synthesis is necessary for tomato root colonization by *Pseudomonas fluorescens* strain WCS365. Mol. Plant-Microbe Interact. 10:102-106.
- Simons, M., van Der Bij, A. J., Brand, I., de Weger, L. A., Wijffelman, C. A., and Lugtenberg, B. J. J. 1996. Gnotobiotic system for studying rhizosphere colonization by plant growth-promoting *Pseudomonas* bacteria. Mol. Plant-Microbe Interact. 9:600-607.

- Skinner, A. J., and Cooper, R. A. 1971. The regulation of ribose-5-phosphate isomerization in *Escherichia coli* K-12. FEBS (Fed. Eur. Biochem. Soc.) Lett. 12:293-296.
- Skinner, A. J., and Cooper, R. A. 1974. Genetic studies on ribose-5-phosphate isomerase mutants of *Escherichia coli* K-12. J. Bacteriol. 118:1183-1185.
- Sørensen, K. I., and Hove-Jensen, B. 1996. Ribose catabolism of *Escherichia coli*: Characterization of the *rpiB* gene encoding ribose phosphate isomerase B and of the *rpiR* gene, which is involved in regulation of *rpiB* expression. J. Bacteriol. 178:1003-1011.
- Sprenger, G. E. 1995. Genetics of pentose-phosphate pathway enzyme of *Escherichia coli* K-12. Arch. Microbiol. 164:324-330.
- Staskawicz, B., Dahlbeck, D., Keen, N., and Napoli, C. 1986. Molecular characterization of cloned avirulence genes from race 0 and race 1 of *Pseudomonas syringae* pv. Glycinea. J. Bacteriol. 169:5789-5794.
- Thöny, B., Huang, D. S., Fradkin, L., and Kornberg, A. 1991. *iciA*, an *Escherichia coli* gene encoding a specific inhibitor of chromosomal initiation of replication *in vitro*. Proc. Natl. Acad. Sci. U.S.A. 88:4066-4070.
- Tobey, K. L., and Grant, G. A. 1986. The nucleotide sequence of the *serA* gene of *Escherichia coli* and the amino acid sequence of the encoded protein, D-3-phosphoglycerate dehydrogenase. J. Biol. Chem. 261:12179-12183.
- Van Dijk, K., and Nelson, E. B. 1998. Inactivation of seed exudate stimulants of *Pythium ultimum* sporangium germination by biocontrol strains of *Enterobacter cloacae* and other seed-associated bacteria. Soil Biol. Biochem. 30:183-192.
- Weller, D. M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annu. Rev. Phytopath. 26:379-407.