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Pseudomonas aeruginosa Twitching Motility-Mediated Chemotaxis towards Phospholipids and Fatty Acids: Specificity and Metabolic Requirements▽‡

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***Pseudomonas aeruginosa* demonstrates type IV pilus-mediated directional twitching motility up a gradient of phosphatidylethanolamine (PE). Only one of four extracellular phospholipases C of *P. aeruginosa* (i.e., PlcB), while not required for twitching motility per se, is required for twitching-mediated migration up a gradient of PE or phosphatidylcholine. Whether other lipid metabolism genes are associated with this behavior was assessed by analysis of transcription during twitching up a PE gradient in comparison to transcription during twitching in the absence of any externally applied phospholipid. Data support the hypothesis that PE is further degraded and that the long-chain fatty acid (LCFA) moieties of PE are completely metabolized via β-oxidation and the glyoxylate shunt. It was discovered that *P. aeruginosa* exhibits twitching-mediated chemotaxis toward unsaturated LCFAs (e.g., oleic acid), but not saturated LCFAs (e.g., stearic acid) of corresponding lengths. Analysis of mutants that are deficient in glyoxylate shunt enzymes, specifically isocitrate lyase (Δ*aceA*) and malate synthase (Δ*aceB*), suggested that the complete metabolism of LCFAs through this pathway was required for the migration of *P. aeruginosa* up a gradient of PE or unsaturated LCFAs. At this point, our data suggested that this process should be classified as energy taxis. However, further evaluation of the ability of the Δ*aceA* and Δ*aceB* mutants to migrate up a gradient of PE or unsaturated LCFAs in the presence of an alternative energy source clearly indicated that metabolism of LCFAs for energy is not required for chemotaxis toward these compounds.**

Bacteria have evolved intricate systems that enable their movement in liquid environments as well as across solid surfaces. While flagella are usually associated with motility in fluids, gliding and twitching motility is most often associated with flagellum-independent mechanisms that enable some bacteria to virtually crawl across a solid surface (e.g., agar or tissues). For twitching motility, some bacteria utilize type IV pili (T4P), appendages that function as molecular grasping devices through their ability to extend, attach to a solid substrate, and then retract, effectively tugging the cell across a solid surface (9).

Even though flagella and T4P generate random movement of bacteria in their corresponding environments (i.e., liquid versus solid), each are required for preferential movement (i.e., chemotaxis) toward or away from specific signals, such as oxygen tension, pH, and specific chemicals (e.g., amino acids) that are either attractants or repellents. The molecular mechanisms involving flagellum-mediated motility and chemotaxis have been extensively characterized, but the relationship between twitching motility and chemotaxis has only recently been examined. T4P-dependent twitching motility has previously been associated with diverse biological processes in *Pseudomonas aeruginosa*, including nutrient acquisition, biofilm formation, and virulence (5, 10, 15, 16, 27), but the chemotactic signals involved in these phenotypes have not been identified. In fact, it was originally proposed that the prototypic gliding bacterium *Myxococcus xanthus* was unlikely to move by this mechanism through chemotaxis because it did not migrate toward an assortment of chemicals (e.g., amino acids and nucleotides) that were known attractants often used in flagellum-mediated chemotaxis experiments with other organisms (8). In spite of these observations early on, Kearns and Shimkets reported that *M. xanthus* is able to migrate up a phosphatidylethanolamine (PE) gradient by pilus-mediated twitching motion (11). This process is mediated through the FrzCD methyl-accepting chemotaxis protein, which aids adaptation, and a chemotaxis system called Dif, which is required for the production of cell appendages (fibrils) (13). Based on those observations, PE became the first chemoattractant identified for any gliding bacterium, and it was also the first lipid to be recognized as a chemoattractant for any prokaryote. In contrast, PE and derivatives of this lipid (i.e., dioleoyl-glycerol) have long been acknowledged to be chemoattractants for eukaryote organisms, including polymorphonuclear leukocytes and ants (17, 18).

Based on their original observations with *M. xanthus*, Kearns

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TABLE 1. Strain analysis of twitching and PE chemotaxis

Strain (PA no.) and/or annotation[a]	Source, reference, deleted amino acids,[b] and/or description	Twitching and/or chemotaxis[c]	
		General	PE directional
PAO1 wild type	3	P	P
PAO1 Δ*pilA* (PA4525)	Reuben Ramphal (2)	NM	NM
PAO1 Δ*plcB* (PA0026) extracellular zinc-dependent PLC	3	P	N
PAO1 Δ*plcA* (PA3464) extracellular zinc-dependent PLC	This study (aa 122 to 371)	P	P
PAO1 Δ*lipA* (PA2862) extracellular lipase	Karl-Erich Jaeger (25)	A	A
PAO1 Δ*lipC* (PA4813) extracellular lipase	Karl-Erich Jaeger (24)	A	P/A
PAO1 Δ*estA* (PA5112) extracellular esterase/lipase	Karl-Erich Jaeger (24)	NM	NM
PAO1 Δ*estA* pBBX *estA*$^+$	Karl-Erich Jaeger (24)	P	P
PAO1 Δ*fadL1* (PA1288) outer membrane LCFA transporter	This study (aa 14 to 414)[b]	P	P
PAO1 Δ*fadL2* (PA1764) outer membrane LCFA transporter	This study (aa 1 to 521)[b]	P	P
PA01 Δ*fadL3* (PA4589) outer membrane LCFA transporter	This study (aa 67 to 433)[b]	P	P
PAO1 Δ*fadL1* Δ*fadL2*	Same as PAO1 Δ*fadL1* and PAO1 Δ*fadL2* above	P	P
PAO1 Δ*fadL2* Δ*fadL3*	Same as PAO1 Δ*fadL2* and PAO1 Δ*fadL3* above	P	P
PAO1 Δ*fadL1* Δ*fadL3*	Same as PAO1 Δ*fadL1* and PAO1 Δ*fadL3* above	P	P
PAO1 Δ*fadL1* Δ*fadL2* Δ*fadL3*	Same as PAO1 Δ*fadL1*, PAO1 Δ*fadL2*, and PAO1 Δ*fadL3* above		
PAO1 Δ*aceA* (PA2634) isocitrate lyase, glyoxylate shunt	This study (aa 138 to 466)[b]	N/P**	N/P#
PAO1 Δ*aceA att*::*aceA*$^+$	This study (complementation of Δ*aceA* in single copy)	P	P
PAO1 Δ*aceB* (PA0482) malate synthase, glyoxylate shunt	This study (aa 83 to 442)[b]	N/P**	N/P#
PAO1 Δ*prpB* (PA0796) methyl isocitrate lyase	This study (aa 88 to 252)[b]	P	P

[a] The PA number corresponds to the *P. aeruginosa* genome annotation.
[b] Amino acids (aa) that were deleted in the mutant protein.
[c] P, positive (exhibits directional twitching toward PE, like PAO1 wild type [i.e., movement from the side proximal to the PE gradient was at least two times that observed on the distal side]); N, negative (strains like the Δ*plcB* mutant that still exhibit twitching but do not show directional twitching toward PE); A, irregular, weak twitching pattern (see Fig. 2); N/P**, does not show any general twitching on the side toward PE (see Fig. 3B) but does twitch on side toward LCFA (see Fig. 3D); N/P#, does not exhibit directional migration up a gradient of PE in plates without any nutrients in the assay plate (Fig. 3B and D) but does exhibit chemotaxis toward PE when 0.5% glucose is present throughout the basal media (Fig. 5); NM, nonmoving (those strains like the Δ*pilA* and Δ*estA* mutants that do not move at all from the site where they are placed on the top of the agar).

et al. (10) investigated whether other bacteria exhibited this behavior. They subsequently reported that the opportunistic bacterial pathogen *Pseudomonas aeruginosa* also shows directed movement (i.e., chemotaxis) up a PE gradient (10). Barker et al. extended these observations and reported that the directional twitching motility of *P. aeruginosa* toward PE, as well as phosphatidylcholine (PC), requires the expression of PlcB (3), which was the only one of the three extracellular phospholipases C (PLCs) known at that time to be produced by this organism that was required (20).

In the present study, we investigated the role of lipid metabolism, beyond that mediated by PlcB, in the twitching-mediated movement of *P. aeruginosa* up a gradient of phospholipids. Our data indicate that *P. aeruginosa* recognizes the long-chain fatty acid (LCFA) moiety of phospholipids (PE) as an attractant. More specifically, *P. aeruginosa* will exhibit twitching-mediated chemotaxis toward long-chain unsaturated fatty acids, but not long-chain saturated fatty acids of the same length (e.g., oleic versus stearic). We further show that the directed movement up an unsaturated LCFA gradient does not require that energy be generated from the metabolism of this compound (i.e., energy taxis). Rather, *P. aeruginosa* mutants that are unable to generate energy from unsaturated LCFAs via the glyoxylate shunt are still able to exhibit chemotaxis toward phospholipids, as well as LCFAs, if an unrelated energy source is provided (i.e., glucose). Thus, unsaturated LCFAs are not simply a target for energy taxis; rather they act as a classically defined chemoattractant. We also report that *P. aeruginosa* directionally twitches toward an acellular extract of pooled human bronchoalveolar lavage fluid (BALF) and that an assortment of independent isolates likewise exhibit chemotaxis toward PE.

MATERIALS AND METHODS

Bacterial strains and materials. The *P. aeruginosa* strains used in this study are listed in Table 1. Before inoculating the twitching motility assay plates, all strains were grown aerobically in brain heart infusion broth (Difco) with shaking at 37°C. When appropriate, medium was supplemented with the following antibiotics at the indicated concentrations: for *Escherichia coli*, ampicillin at 100 μg/ml and gentamicin at 15 μg/ml; and for *P. aeruginosa*, gentamicin at 75 μg/ml. The unlabeled phospholipids and LCFAs (C_{16} to C_{20}) used in these studies were purchased from Sigma or Avanti Polar Lipids. ^{14}C-labeled LCFAs (oleic and stearic) were purchased from Amersham Biosciences.

Twitching motility chemotaxis assays. Twitching motility chemotaxis assays were performed as previously described (3, 13). In brief, twitching assay plates containing buffered agar (10 mM Tris, pH 7.6; 8 mM $MgSO_4$; 1 mM $NaPO_4$, pH 7.6; and 1.5% agar) were allowed to dry overnight at 25°C before 4-μl spots of 10 mg/ml PE, 20 mg/ml LCFA, or 0.72 mg/ml BALF were placed on the agar

surface and incubated at 30°C for 24 h in order to establish the lipid gradient. The following day, wild-type or mutant strains were grown to an A_{590} of 1.2 and concentrated by centrifugation to 9×10^9 cells/ml in a morpholinepropanesulfonic acid (MOPS) buffer (10 mM MOPS, pH 7.6, and 8 mM $MgSO_4$). A total of 2.5 μl of this cell suspension was placed at an approximate distance of 5 mm from the center of the lipid drop and allowed to incubate at 37°C for 16 h before determining the twitching phenotype. When applicable, 0.5% glucose was supplemented into the buffered agar. If we provided glucose to *P. aeruginosa* without any phospholipid gradient, we observed an increased zone of nondirectional twitching, compared to when no carbon source was provided in the media. This observation is most likely due to the increased availability of an energy source (i.e., glucose) for the extension and retraction of T4P.

DNA microarray experiments. The DNA microarray experiments were performed as previously described (3, 14). However, in this study RNA was harvested from *P. aeruginosa* PAO1 growing on twitching motility agar plates, described above, either in the presence of PE or without PE. There were eight or nine individual twitching assays on each plate, and ~200 plates were used for each condition (i.e., PE versus no PE) in order to obtain enough RNA for microarray experiments. The plates without the PE gradient were supplemented with a small amount of glucose (0.5%) to mitigate growth phase differences between cells that had PE and those which had no available carbon source. Four separate experiments were performed with organisms that were incubated for 16 h at 37°C in the twitching assay (with or without PE), and one separate experiment was performed with cells that were incubated for 36 h in the twitching assay (with or without PE). After incubation, cells in the twitching assays were harvested with ~5 ml/plate of RNA*later* (Ambion), and the RNA was extracted using a Qiagen RNeasy mini kit. The mRNA was converted to cDNA as described in the Affymetrix GeneChip protocol. The cDNA was hybridized to the *P. aeruginosa* Affymetrix GeneChip according to the manufacturer's instruction manual. Analysis of hybridization data was performed with the GeneChip operating software v1.4.

Genetic construction of mutants. All mutants constructed during the course of this study were made using methods described previously (3, 6). All mutants, including the triple FadL mutant, have deletion mutations in the designated gene, and they do not contain antibiotic resistance cassettes in place of the deleted sequences. The ranges of the amino acids deleted in each protein are given in Table 1.

Isolation of pooled acellular human BALF. The BALF was obtained from healthy human subjects using a protocol (NJ166) approved by the institutional review board of National Jewish Medical and Research Center. The human subjects were sedated and subjected to fiber-optic bronchoscopy. Four 60-ml aliquots of sterile normal saline were instilled in the right middle lobe and sequentially aspirated back. The resulting BALF was centrifuged at 500 × *g* for 10 min to separate the cells from the acellular fluid. This acellular fluid was pooled from 80 human subjects. The University of Colorado—Denver institutional research review board for human research approved the transfer of the pooled BALF samples to M. L. Vasil (protocol 07-1195) at the University of Colorado—Denver. The phospholipid content of the material was originally 25 nmol/ml but was concentrated as follows: the final Ca^{2+} concentration was adjusted to 10 mM, and the acellular material was then centrifuged at 15,000 × *g* for 1 h. The pellet was then resuspended in chloroform to a final concentration of 0.72 mg/ml phospholipid. The free fatty acid concentration of this BALF is approximately 5% of its total phospholipid content (i.e., ~1.25 nmol/ml). This material was substituted for pure PE in twitching assays, where indicated.

RESULTS

Transcriptional analysis of *P. aeruginosa* during movement up a gradient of phospholipids. We had previously shown that a specific PLC (i.e., PlcB) is required for *P. aeruginosa* to exhibit twitching-mediated movement up a gradient of PE or PC (3). In order to determine if other lipid-modifying or -metabolizing enzymes might be involved in this process, we evaluated global transcription in *P. aeruginosa* PAO1 during twitching with and without phospholipids. Accordingly, we opted to compare the transcription of *P. aeruginosa* PAO1 genes in the presence and absence of PE, while it was exhibiting twitching motility on a solid surface (i.e., agar). In one case, *P. aeruginosa* was directionally twitching toward PE. In comparison, in the absence of PE, *P. aeruginosa* cells were twitching, but not in any preferential direction because no phospholipid gradient was provided. Due to the limited amount of intact mRNA that we could harvest from the twitching plates, it was necessary to isolate the RNA from cells relatively late in the process (i.e., 16 h), when there was a maximal number of bacteria in the population moving toward PE.

As presented in Table 2 and in Table S1 in the supplemental material, we identified several distinct classes of genes that showed increased expression in directionally twitching cells compared to cells that were twitching but not in any particular direction toward an attractant. By far the largest class of genes which showed increased expression while *P. aeruginosa* was moving up a gradient of PE were those associated with lipid metabolism (Table 2 and Table S1 in the supplemental material). Although this may not be particularly surprising, until these experiments were performed, it was entirely possible that metabolism of PE beyond the PlcB-generated diacylglycerol (DAG) would be unnecessary for *P. aeruginosa* to migrate up the PE gradient. It is also worthwhile to point out that the results obtained from the microarray experiments reported herein are very distinct from those recently reported, in which liquid cultures of planktonic cells were exposed to phospholipids (19). Most notably, in those studies neither the three genes encoding FadL nor any genes encoding FadD showed any increased expression. Moreover, there was no increase in expression of the genes encoding isocitrate lyase (*aceA*) or malate synthase (*aceB*).

We did find an assortment of genes that were repressed in PAO1 twitching toward PE, but we have not yet examined any of these further (i.e., analysis of mutants), as we have with the genes that were upregulated. Some of these genes were associated with glucose metabolism (e.g., PA2259 to PA2263, 2-ketogluconate utilization; PA2264 to PA2267, gluconate utilization) and interestingly a cluster of genes (PA1245 to PA1250) involved in alkaline protease production. Also, two clusters of genes involved in alginate biosynthesis (PA3540 to PA3551 and PA5483 to PA5484) were also repressed in PAO1 migrating toward PE. However, these observations will have to be further confirmed by independent means (e.g., mutagenesis or quantitative PCR). There were other repressed genes, but their significance with regard to this phenotype is not obvious at the present time.

Analysis of these microarray data revealed that, in addition to *plcB*, a notable assortment of genes encoding lipid-degrading enzymes, LCFA transport systems, and enzymes associated with β-oxidation, as well as glyoxylate shunt enzymes, showed significantly increased expression in cells moving up a gradient of PE (Table 2). These results essentially follow the diagram shown in Fig. 1, in which PlcB converts the phospholipids to DAG (3), and extracellular lipases (e.g., LipA, LipC, and EstA) cleave the DAG into LCFAs (e.g., oleic acid) and glycerol. Once the LCFAs are freed from DAG, multiple FadL transporters move these fatty acids through the outer membrane, ultimately leading to the transport and conversion of LCFAs to cytoplasmic acyl coenzyme A (Acyl-CoA) LCFAs, mediated by FadD (Fig. 1). Finally, *P. aeruginosa* converts these to long-chain acetyl-CoAs via β-oxidation to acetyl-CoA, which is then processed by isocitrate lyase and malate synthase through the glyoxylate shunt and ultimately through the tricar-

TABLE 2. Lipid/glycerol metabolism and regulatory genes showing increased expression during chemotaxis toward PE

PA no. or function	Gene[a]	Protein annotation	Mean fold increase[b]
Lipid/glycerol metabolism			
PA0026	*plcB*†	PlcB, extracellular PLC	2.6
PA0182		3-Oxoacyl (acyl carrier protein) reductase	2
PA0208		Acyl-CoA:acetate/3-ketoacid CoA transferase	12.5
PA0209		Triphosphoribosyl-dephospho-CoA synthetase	6.7
PA0211	*accD*	AccD, acetyl-CoA carboxylase beta subunit	6.7
PA0214	*fabD*	FabD, acyl-carrier-protein	5.7
PA0358		1-Acyl-*sn*-glycerol-3-phosphate acyltransferase	2.3
PA0482	*aceB*†	AceB, malate synthase, glyoxylate shunt	2.0
PA0506	*fadE**	FadE, acyl-CoA dehydrogenase, β-oxidation	2
PA0507	*fadE**	FadE, acyl-CoA dehydrogenases, β-oxidation	2.7
PA0508	*fadE**	FadE, acyl-CoA dehydrogenases, β-oxidation	2.6
PA0743	*mmsB*	MmsB, 3-hydroxyisobutyrate dehydrogenase	2.0
PA0744	*fadN**	FadN, enoyl-CoA hydratase, β-oxidation	2
PA0745	*fadN**	FadN, enoyl-CoA hydratase, β-oxidation	2
PA0746	*fadE**	FadE, acyl-CoA dehydrogenases, β-oxidation	2
PA0792	*prpD*	PrpD methylcitrate dehydratase, propionate catabolism	2
PA0795	*prpC* (*gltA*)	PrpC (GltA), methylcitrate synthase	2.8
PA0796	*prpB*†	PrpB, methylisocitrate lyase	2.7
PA0797	*fadR* (*gntR*)	FadR-like regulator, GntR class of transcriptional regulators	2.4
PA0887	*acs*	Acs, acyl-coenzyme A synthetases/AMP-fatty acid ligase	3.9
PA1137		Crotonyl-CoA reductase	4.6
PA1288	*fadL*†	FadL, LCFA transport protein	2
PA1736		Acetyl-CoA acyltransferase	2.8
PA1737	*fadB**	FadB 3-hydroxyacyl-CoA dehydrogenase, β-oxidation	2.6
PA2011	*liuE*	LiuE, 3-hydroxy-3-methylglutaryl-CoA lyase	4.2
PA2012	*liuD*	LiuD, alpha subunit of geranoyl-CoA carboxylase	7.7
PA2013	*fadN**	FadN, enoyl-CoA hydratase, β-oxidation	3.9
PA2014	*liuB*	LiuB, methylmalonyl-CoA decarboxylase alpha subunit	4.6
PA2015	*liuA*	LiuA, isovaleryl-CoA dehydrogenase	4.1
PA2142	*fabG*	FabG, 3-oxoacyl-(acyl-carrier-protein) reductase	2
PA2552	*fadE**	FadE, acyl-CoA dehydrogenases, β-oxidation	4.7
PA2553	*paaJ*	PaaJ, acetyl-CoA acetyltransferase	7.0
PA2554	*fabG*	FabG, 3-oxoacyl-(acyl-carrier-protein) reductase	5.9
PA2555	*acs*	Acs, acyl-coenzyme A synthetases/AMP-fatty acid ligases	6.0
PA2557	*fadD* (*caiC*)	FadD (CaiC), acyl-CoA synthetases	5.7
PA2634	*aceA*†	AceA, isocitrate lyase, glyoxylate shunt	5.0
PA2764	*mhpC*	MhpC, acyl transferase	2.5
PA2862	*lipA*†	LipA, lipases, serine active site	8.9
PA2863	*lipH*	LipH, lipase chaperone for LipA and LipC	4.9
PA2887		3-Oxoacyl-(acyl-carrier-protein) reductase	2.1
PA2888		Acetyl-CoA carboxylase	2.0
PA2889	*caiA**	CaiA, acyl-CoA dehydrogenases, β-oxidation	2.0
PA3013	*fadA**	FadA, 3-ketoacyl-CoA thiolase, β-oxidation	2.0
PA3014	*fadB**	FadB, 3-hydroxyacyl-CoA dehydrogenase, β-oxidation	2.0
PA3267		1-Acyl-*sn*-glycerol-3-phosphate acyltransferase	2.0
PA3277	*fabG*	FabG, 3-oxoacyl-(acyl-carrier-protein) reductase	2.2
PA3300	*fadD2*	FadD2, LCFA CoA ligase	2.0
PA3415		Probable dihydrolipoamide acetyltransferase	2.0
PA3427	*fabG*	FabG, 3-oxoacyl-(acyl-carrier-protein) reductase, putative	2.9
PA3429	*mhpC*	MhpC, predicted hydrolases or acyltransferases	2.5
PA3567		Crotonyl-CoA reductase	4.6
PA3568		Acyl-coenzyme A synthetases/AMP-(fatty) acid ligases	4.8
PA3569	*mmsB*	MmsB, 3-hydroxyisobutyrate dehydrogenase	2.4
PA3581	*glpF*	GlpF, glycerol uptake facilitator	4.4
PA3582	*glpK*	GlpK, glycerol kinase	2.5
PA3994	*mhpC*	MhpC, predicted acyltransferases	3.4
PA4148	*fabG*	FabG, dehydrogenases with different specificities	3.9
PA4152	*mhpC*	MhpC, predicted hydrolase or acyltransferases	3.7
PA4198	*caiC*	CaiC, acyl-CoA synthetases	2.3
PA4338		Predicted acyltransferases	2.0
PA4589	*fadL*†	FadL, LCFA transport protein	2.8
PA4733	*acsA*	AcsA, acetate CoA ligase	3.1
PA4813	*lipC*†	LipC, lipase	9.6
PA5112	*estA*†	EstA, lipase, G-D-S-L family, serine active site	2.6

Continued on following page

TABLE 2—*Continued*

PA no. or function	Gene[a]	Protein annotation	Mean fold increase[b]
Regulatory genes			
PA0176	*tar*	Tar, methyl-accepting chemotaxis protein	2.1
PA1285	*marR*	MarR, transcriptional regulators	2.0
PA1300	*rpoE*	RpoE, sigma 24 homolog	2.0
PA1354		Transcription factor, NusG proteins	2
PA1363	*rpoE*	RpoE, sigma 24 homolog	2
PA1437	*ompR*	OmpR, response regulators, CheY-like receiver domain and winged-helix DNA-binding domain	2.4
PA1438	*baeS*	BaeS, signal transduction histidine kinase	2
PA1760	*gerE*	GerE, bacterial regulatory proteins, LuxR family	2.7
PA1912	*rpoE*	RpoE, sigma 24 homolog	2.4
PA1976	*baeS*	BaeS, signal transduction histidine kinase	8.2
PA1978	*citB*	CitB, response regulator containing a CheY-like receiver domain and an HTH DNA-binding domain	6.3
PA1979		Signal transduction histidine kinase; part of operon with PA1980	6.3
PA1989	*citB*	CitB, response regulator containing a CheY-like receiver domain and HTH DNA-binding domain, PhoB-like	6.8
PA1992	*baeS*	BaeS, signal transduction histidine kinase	5.3
PA2016	*liuR*	LiuR, transcriptional regulator of *liuABCDE* genes, SoxR-like	3.8
PA2072		Diguanylate cyclase GGDEF domain and EAL domain	2
PA2137	*atoC*	AtoC, response regulator containing CheY-like receiver	2.3
PA2556	*araC*	AraC type regulator	2.4
PA3133	*tetR*	Bacterial regulatory proteins, TetR family	2.2
PA3233		Predicted signal transduction; cAMP-binding and CBS domains	4.5
PA4094	*araC*	Transcriptional regulator AraC type	3.7
PA4203	*lysR*	LysR type, transcriptional regulator	2.5
PA4147	*acoR*	AcoR, transcriptional activator of acetoin/glycerol metabolism	5.1
PA4290	*tar*	Tar, methyl-accepting chemotaxis protein	3.5
PA4436	*araC*	Transcriptional regulator, AraC type	2.1
PA4787	*araC*	Transcriptional regulator, AraC type	2
PA5324	*araC*	Transcriptional regulator, AraC type	2.9
PA5550	*glpR*	GlpR, transcriptional regulators of sugar metabolism	3.6

[a] †, gene was mutated in this study to determine whether it is required for *P. aeruginosa* to preferentially move up a PE or LCFA gradient. *, gene is associated with β-oxidation.

[b] All genes listed showed an average of a twofold or greater increase ($P \leq 0.004$) in expression in *P. aeruginosa* twitching toward PE, compared to *P. aeruginosa* twitching in the absence of PE. Four independent comparisons with each condition were run on separate days. Cells were harvested for RNA at 16 h after inoculation to the twitching plates.

boxylic acid cycle to generate ATP (Fig. 1). At first glance, this pattern of gene expression suggests that significant degradation of PE is associated with movement up a PE gradient and that energy produced from the metabolism of LCFAs may also be necessary. This process would then be tentatively called "energy taxis" but will be further examined below.

The second class of genes that showed increased expression were regulatory genes (Table 2). There were genes encoding several RpoE sigma factor homologs (PA1300, PA1363, and PA1912), AraC regulators, and two LysR regulators that showed increased expression. There was only one TetR gene that showed increased expression during twitching-mediated migration up a gradient of phospholipids. FadR regulators belong to the more general TetR class of prokaryotic regulators, but it is not clear at this time whether this particular *tetR* gene (PA3133) encodes a FadR-type regulator (Fig. 1). Finally, there was an assortment of chemotaxis-related genes showing increased expression, including receptors (Tar) and Che genes. However, there is no clear pattern associated with these results.

There is also an increased expression, during phospholipid chemotaxis, of an assortment of iron starvation-responsive genes, including several that are involved in the biosynthesis of pyoverdine and its uptake (e.g., *pvdA*, *fpvA*, and *tonB*) (14) (Table S1 in the supplemental material). It is worthwhile to note in this context that the expression of *plcB* is also iron starvation-dependent (i.e., there is a >10-fold increase in expression under iron-deficient conditions versus iron-replete conditions) (A. P. Barker and M. L. Vasil, unpublished observations).

Analysis of selected mutants for chemotaxis toward PE. While analysis of our microarray data suggests that *P. aeruginosa* might actually be completely degrading and utilizing the entire PE molecule during its migration up the gradient of this phospholipid, these data obviously cannot reveal whether any of the genes that showed increased transcription in this situation are actually required for this complex behavior. Consequently, extant mutants or newly constructed mutants with deletions in genes whose products might be required for the directional twitching toward PE were examined. There are basically three different outcomes for the chemotaxis assay used in this study and described in Table 1. First, the strains that behave like the wild type and migrate toward the lipid at least two times further than toward the opposite side, where no lipid is placed, are considered positive. Second, the strains that behave like the Δ*plcB* mutant in the presence of a PE gradient, where the radii of the twitching zones are equal on all sides, are considered negative, and third, those that do not move at all,

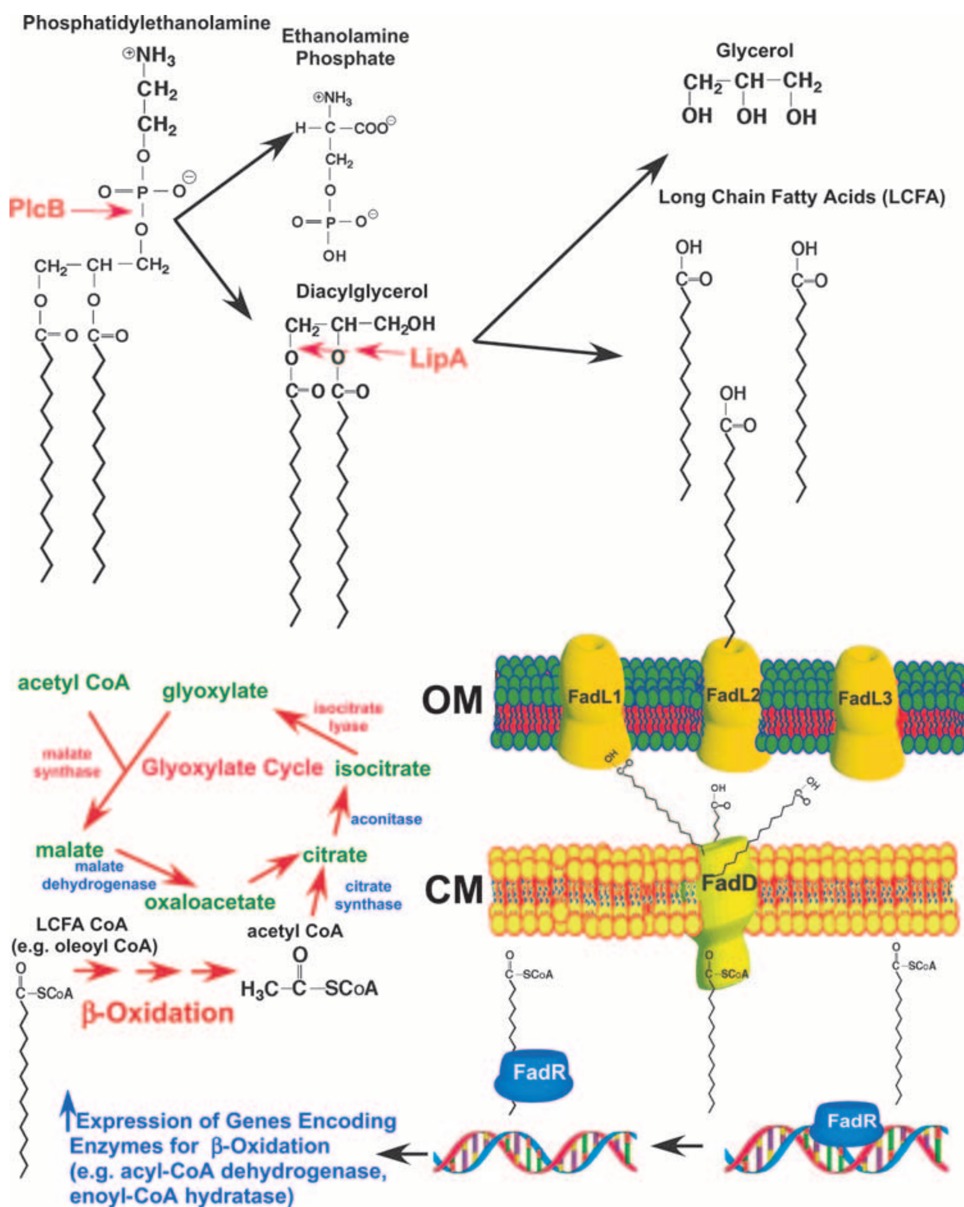

FIG. 1. This diagram provides a representation of the pathways relevant to this study and discussed in the text by which (i) PE is degraded by PlcB and lipases to generate LCFAs, (ii) LCFAs are transported through the inner and outer membrane of *P. aeruginosa*, (iii) LCFAs are metabolized via β-oxidation to acetyl-CoA, and (iv) acetyl-CoA is processed by the glyoxylate shunt to gene rate energy.

like the Δ*pilA* mutant, are designated nonmoving. However, as noted in Table 1, there are some variances of these phenotypes. In the context of this study, we were particularly interested in mutants that are still able to exhibit twitching motility but unable to preferentially move up the PE gradient.

Recently we discovered the existence of a gene encoding a fourth extracellular PLC, PlcA, of *P. aeruginosa* that can hydrolyze PC and PE (M. J. Stonehouse and M. L. Vasil, unpublished observations). While the gene encoding PlcA (PA3464) was not upregulated in the cells twitching toward the PE gradient, it was still possible that it might contribute, along with PlcB, to *P. aeruginosa*'s ability to migrate up a gradient of these phospholipids. Nonetheless, the Δ*plcA* mutant is still able to demonstrate chemotaxis toward PE, thereby supporting the hypothesis that PlcB is the only one of the now four extracellular PLCs of *P. aeruginosa* that is required for this phenotype (Table 1).

As represented in Fig. 1, it seems likely that extracellular lipases (e.g., LipA, LipC, and EstA) of *P. aeruginosa* would contribute to its ability to migrate up a gradient of PE. Consequently, several lipase mutants were examined for their ability to exhibit this behavior. The Δ*lipA* mutant shows an irregular, weak twitching pattern (Fig. 2A; Table 1), and it does not seem to preferentially move toward either PE or unsaturated LCFAs (see below). On the other hand, while the Δ*lipC* mutant also has an irregular twitching pattern, it consistently forms very large bleb-like growths only on the side toward the PE gradient (Fig. 2B and Table 1). This highly reproducible

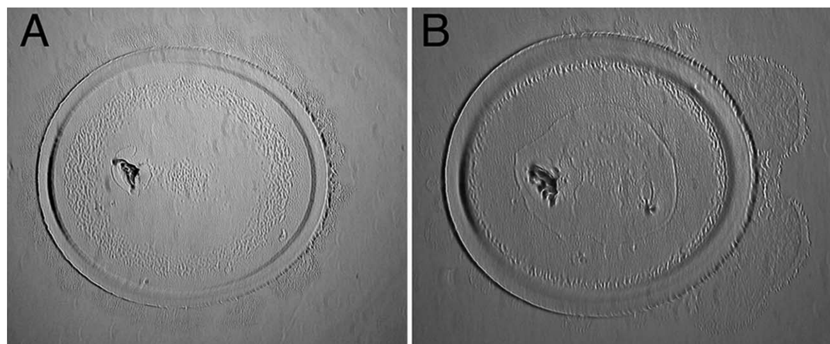

FIG. 2. T4P-mediated twitching assay for migration of the *P. aeruginosa* Δ*lipA* mutant (A) and Δ*lipC* mutant (B) up a gradient of PE. The PE gradient was first established to the right of the round/oval ring marking where the mutant cells were placed on the top of the agar 24 h later (see Materials and Methods).

observation supports the impression that this mutant is still able to preferentially move up a PE gradient despite its unusual twitching pattern. Another gene encoding an additional lipase, EstA, also showed significantly increased expression in the microarray experiments. However, a Δ*estA* mutant did not exhibit any twitching motility in this assay (Table 1). This phenotype is consistent with the observations of others (24) working with the more widely used subsurface agar twitching assay. Taken together, analyses of lipase mutants demonstrate unusual defects in twitching and in their ability to migrate up a gradient of PE.

As represented in the diagram shown in Fig. 1, once the LCFAs are released from DAG, they would be available for uptake via the outer membrane LCFA transport system. *P. aeruginosa* has three genes annotated as encoding proteins homologous to the *E. coli* FadL transporter. Notably, the expression of two of these three annotated genes showed upregulation in the microarray experiments (Table 2). Single, double, and triple mutations (Δ*fadL1*, Δ*fadL2*, and Δ*fadL3*) were constructed in all of the genes encoding FadL homologs in PAO1 (PA1288, PA1764, and PA4589) and tested in the PE chemotaxis assay. Surprisingly, all FadL mutants, including the triple mutant, showed the same ability to migrate up the PE gradient as the wild-type parent (Table 1). These data suggest that an alternative mechanism is responsible for the movement of LCFAs through the inner and outer membranes and into the cytoplasm of *P. aeruginosa*.

Once LCFAs are transported through the outer and inner membranes and converted to the long-chain acyl-CoA forms, they could then be metabolized via the β-oxidation pathway to acetyl-CoA (Fig. 1). Based on the microarray data, there was a notable increase in the expression of a significant number of genes encoding enzymes associated with β-oxidation (Table 2) during chemotaxis up the PE gradient. However, β-oxidation pathway mutants were not examined in the twitching assay due to the relatively large number of genes annotated as participating in β-oxidation, including acyl-CoA dehydrogenases (PA0506, PA0507, PA0508, and PA2889) and enoyl-CoA hydratases (e.g., PA0744, PA0745, and PA2013) in the PAO1 genome.

Once LCFAs are converted to acetyl-CoA via β-oxidation, they would then be metabolized through the glyoxylate shunt. The two key enzymes of this pathway, which only exists in prokaryotes and plants, are isocitrate lyase (PA2634, *aceA*) and malate synthase (PA0482, *aceB*). In the microarray experiments, the expression levels of the genes encoding both enzymes were significantly increased during migration up the PE gradient (Table 2). Moreover, both the Δ*aceA* and Δ*aceB* mutants exhibited identical, but unusual, phenotypes in the chemotaxis assay with PE. On the side of the inoculum toward the PE gradient, the Δ*aceA* and Δ*aceB* mutants were completely unable to exhibit twitching motility and therefore appear to behave as a Δ*pilA* mutant, but only on the edge facing PE (Fig. 3B). By contrast, on the side of the inoculum away from the PE gradient, both mutants exhibited normal twitching motility, as seen with the corresponding side of the wild-type parent (Table 1; Fig. 3A and 3B). Complementation of the Δ*aceA* mutant with a single copy of the wild-type *aceA* gene at the *att* site restores the ability of this mutant to migrate up the PE gradient, as seen with the wild-type parent (Table 1; Fig. 3C). We also examined the phenotype of these mutants with regard to their ability to utilize acetate. In *E. coli*, the Δ*aceA* and Δ*aceB* mutants are unable to utilize acetate as a sole carbon source. They also cannot utilize LCFAs for energy because LCFAs must be degraded to this two-carbon compound via β-oxidation before it can be metabolized through the glyoxylate shunt and TCA pathways to generate ATP. As shown in Fig. S1 in the supplemental material, both the Δ*aceA* and the Δ*aceB* mutants are unable to use acetate as a sole source of carbon, thereby confirming that the Δ*aceA* and Δ*aceB* mutants of *P. aeruginosa* are unable to use LCFAs to generate energy via the glyoxylate shunt.

We also examined whether a mutant deficient in one of the enzymes associated with the methylisocitrate pathway is able to exhibit preferential migration up a PE or LCFA gradient. The enzymes of this pathway (e.g., methylisocitrate lyase) metabolize propionyl-CoA generated from the β-oxidation of odd numbered LCFAs. Although we always used even-chain LCFAs and PE, which contains only even-chain LCFAs (i.e., dioleyl-PE), the expression of methylisocitrate lyase in our microarray experiments showed a significant increase in *P. aeruginosa* moving up a gradient of dioleyl-PE (Table 2). However, as shown in Table 1, a methyl isocitrate lyase mutant (PAO1 Δ *prpB*) is still able to twitch toward PE.

Analysis of twitching-mediated chemotaxis toward LCFAs. Taken together, our data suggest that *P. aeruginosa* is actually

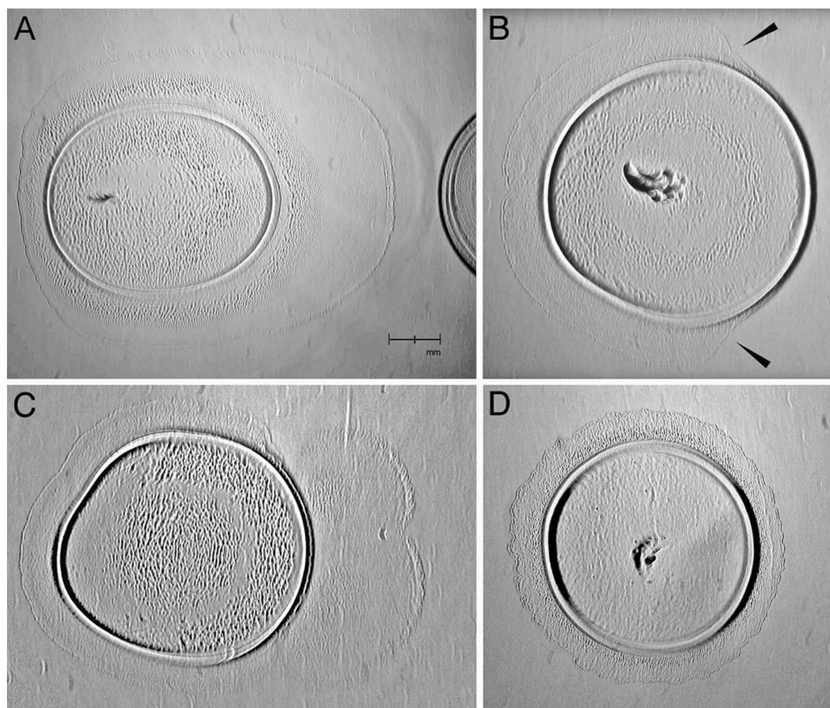

FIG. 3. T4P-mediated twitching assay for migration up a gradient of PE and oleic acid. A PE (A, B, and C) or oleic acid (D) gradient was first established to the right of the round/oval ring marking where the *P. aeruginosa* strains were placed on the top of the agar 24 h after the placement of the lipid (see Materials and Methods). The PAO1 wild-type strain (A) and PAO1 strains (Δ*aceA* [B and D] and Δ*aceA att*::*aceA*$^+$ [C]) are depicted. The arrows in panel B point to the transitional region between the area where there is a complete inhibition of twitching motility (toward the PE gradient) and the area where general twitching is occurring in the absence of PE. Note that the area of complete inhibition of twitching motility is not seen when the chemoattractant is an LCFA (i.e., oleic acid) as shown in panel D. The bar in panel A represents 2 mm and provides a gauge of distance between the phospholipid shown to the right and the bacteria on the left.

attracted to the LCFA moiety of PE during twitching up a gradient of PE. We therefore examined whether an assortment of saturated and unsaturated LCFAs of corresponding chain lengths (C_{16} to C_{20}) could also mediate directional twitching like PE. Interestingly, *P. aeruginosa* is able to exhibit this twitching-mediated chemotaxis only toward the unsaturated versions (e.g., oleic, palmitoleic, and arachidonic acids), not the saturated versions (stearic, palmitic, and arachidic acids) of C_{16}, C_{18}, or C_{20} LCFAs (Fig. 4). It should be pointed out that the PE we used throughout this study contained two unsaturated LCFA oleic acid moieties (i.e., dioleyl-PE). *P. aeruginosa* also exhibits chemotaxis toward linoleic acid ($C_{18:3}$), another unsaturated LCFA (data not shown).

One possible factor that could skew the interpretation of these data is that *P. aeruginosa* could not come in contact with saturated LCFAs because they do not form a gradient in the agar. In order to mitigate this concern, LCFA gradients of ^{14}C-radiolabeled oleic or stearic acids were established on agar plates and the levels of cell-associated radioactivity were compared after cells had the opportunity to move up the gradient. The entire cell population from a single assay was harvested by a sterile cotton swab at 16 h and measured for ^{14}C counts of radioactivity. Significant ^{14}C counts above the background (subtracted from the following values) were detected in the bacteria exposed to both the radiolabeled oleic acid and stearic acid (counts from *P. aeruginosa* cells exposed to [^{14}C]oleic acid were 1,126 cpm and counts from *P. aeruginosa* cells exposed to [^{14}C]steric acid were 364 cpm), thereby demonstrating that *P. aeruginosa* was indeed exposed to both kinds of LCFAs in the twitching assay, yet it was able to migrate up the gradient of only unsaturated LCFAs. When *P. aeruginosa* PAO1 was incubated for as long as 72 h in the twitching assay, it never demonstrated directional twitching toward stearic acid. Also, when we have inoculated *P. aeruginosa* ~ 2.5 mm away from the saturated LCFA spot, no directional migration toward the saturated LCFA occurs.

Another issue that could have an impact on the differences between saturated and unsaturated fatty acids noted above would be if *P. aeruginosa* cannot utilize saturated fatty acids as an energy source via the glyoxylate shunt. However, it has previously been demonstrated that PAO1 is able to grow on fully saturated LCFAs, such as stearate or palmitate (26), even though it does not exhibit twitching-mediated chemotaxis toward these saturated LCFAs.

Since it is now clear that *P. aeruginosa* twitches toward the unsaturated LCFA moiety of PE, we examined selected mutants in twitching assays using an unsaturated LCFA. As predicted from the diagram shown in Fig. 1, the Δ*plcB* mutant exhibited wild-type preferential migration up a gradient of unsaturated LCFA (data not shown). These results support the hypothesis that the unsaturated LCFA or a modified unsaturated LCFA (see below) moiety of PE is the attractant perceived by *P. aeruginosa* during its twitching-mediated migration up a gradient of this phospholipid.

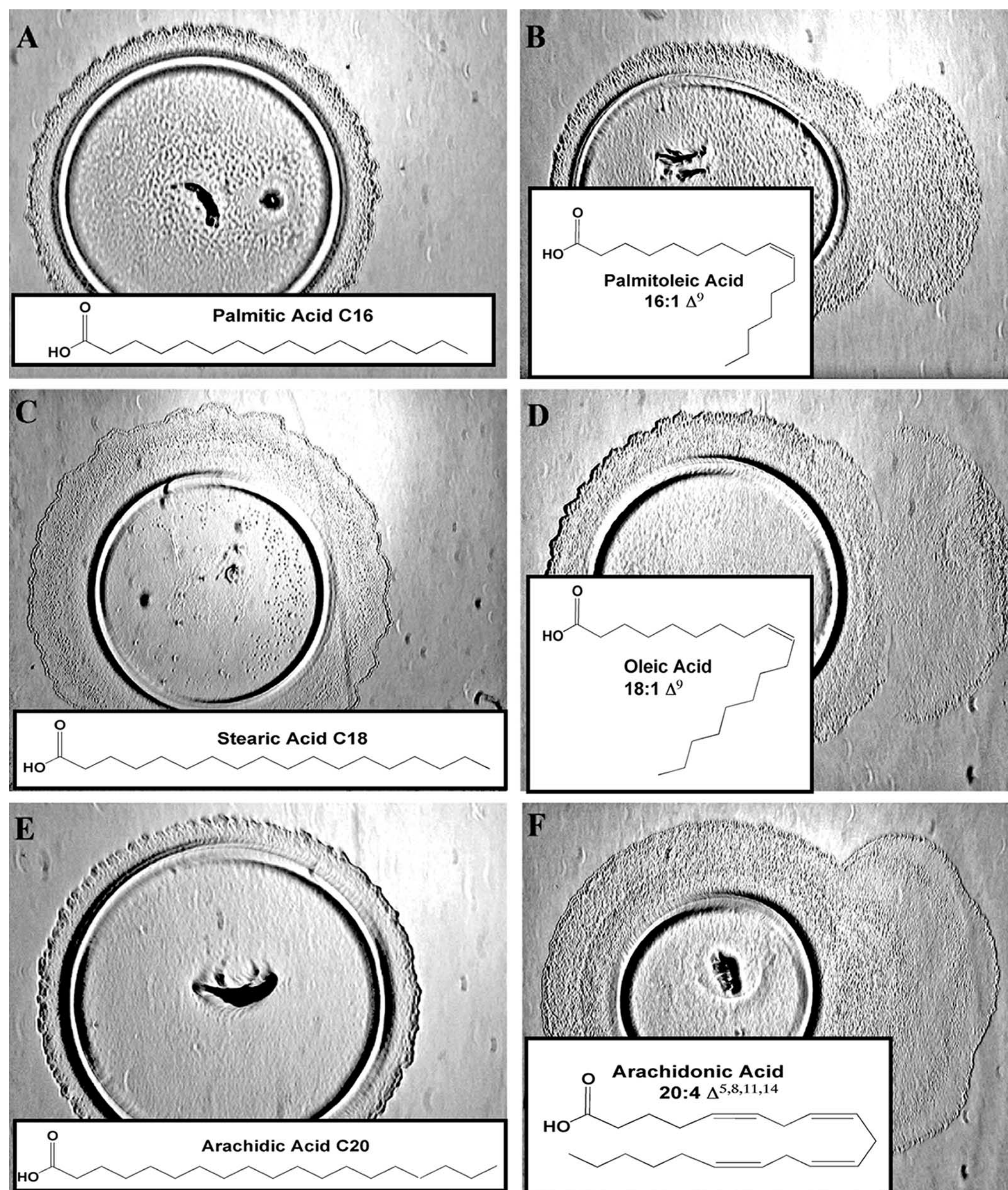

FIG. 4. Lipid chemotaxis assay toward saturated (palmitic, stearic, and arachidic acids [left panels]) and unsaturated (palmitoleic, oleic, and arachidonic acids [right panels]) LCFAs of the same corresponding lengths. The assay is performed exactly as it is for PE except that saturated and unsaturated LCFAs were used to generate the lipid gradients instead of PE. In all cases, the LCFA gradients were first established to the right of the round/oval rings marking where the culture of *P. aeruginosa* was placed on the top of the agars 24 h after placement of the LCFAs (see Materials and Methods).

We examined the behavior of the Δ*aceA* and Δ*aceB* mutants in our assay using unsaturated LCFA as the chemoattractant. Although the Δ*aceA* or Δ*aceB* mutants were still able to demonstrate twitching motility in this assay, they were not able to move up the gradient of the unsaturated LCFA (Fig. 3D). However, the phenotype of these mutants with respect to unsaturated LCFAs showed an interesting variation in comparison to their behavior when PE was used as the attractant. While these mutants are unable to migrate up a gradient of unsaturated LCFA, they do not show a failure to twitch on the side toward the unsaturated LCFA gradient, like they do when PE is the chemoattractant (Fig. 3B). The significance of these different phenotypes when PE, rather than unsaturated LCFA, is used is not clear at the present time.

The migration up a gradient of PE or unsaturated LCFA is not due solely to energy taxis. The observation that neither the Δ*aceA* nor the Δ*aceB* mutant can exhibit directional twitching toward unsaturated LCFAs in our assay suggests that these compounds must be metabolized for directional twitching to occur. Thus, for the time being, this process was best classified as energy taxis. Whether this hypothesis was entirely true was further examined by providing the glyoxylate shunt mutants (i.e., Δ*aceA* and Δ*aceB*) with an alternative energy source so that they would not need to derive their energy from unsatur-

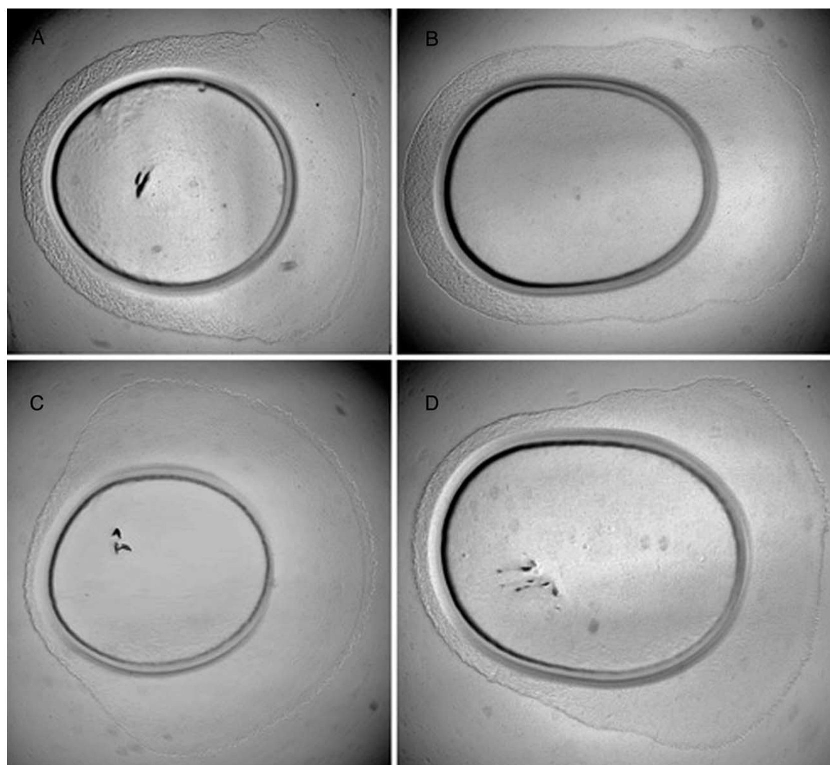

FIG. 5. T4P-mediated chemotaxis of *P. aeruginosa* strains up a gradient of either PE or LCFA (palmitoleic acid, C_{16}) in the presence of 0.5% glucose that was incorporated throughout the agar. In all cases, the PE or palmitoleic acid gradient was first established to the right of the round/oval ring marking where the culture of *P. aeruginosa* was placed on the top of the agar 24 h later (see Materials and Methods). (A) PAO1 Δ*aceA* mutant with palmitoleic acid as the chemoattractant; (B) PAO1 wild type with palmitoleic acid as the chemoattractant; (C) PAO1 Δ*aceA* mutant with PE as the chemoattractant; (D) PAO1 wild type with PE as the chemoattractant.

ated LCFAs in this assay in order to migrate up a gradient of PE or LCFA. Glucose was therefore incorporated throughout the assay plates in addition to establishing the localized PE or the unsaturated LCFA gradient. Remarkably, as shown in Fig. 5, the Δ*aceA* mutant then demonstrated a phenotype in this chemotaxis assay identical to that of the wild-type parent, despite the fact that the Δ*aceA* mutant cannot utilize LCFAs for energy (Fig. S1 in the supplemental material). This is also true for the Δ*aceB* mutant (data not shown). These results provide a compelling argument that the migration of *P. aeruginosa* up a gradient of PE or unsaturated LCFA is not merely due to energy taxis, but represents the classically defined chemotaxis in which the target attractant does not need to be metabolized.

Analysis of clinical strains and BALF: possible clinical relevance. We considered whether this ability to exhibit chemotaxis toward PE would also be observed in other *P. aeruginosa* strains, including clinical isolates from cystic fibrosis patients and the frequently used research strains PAK, PA14, and PA103. In all cases, these strains, like PAO1, exhibited movement up a gradient of PE (M. L. Vasil and A. P. Barker, unpublished observations) on a solid surface. While pure PE, PC, or LCFAs have exclusively been used as attractants in the twitching assays used in this study, we felt that it might be worthwhile to examine whether *P. aeruginosa* would exhibit preferential migration up a gradient of a more biologically relevant mix of lipids. A cell extract of pooled human BALF (see Materials and Methods) was substituted for pure PE or PC in the typical twitching assay (3, 12). When human BALF was used as an attractant in our twitching-mediated assay, *P. aeruginosa* clearly exhibited preferential migration up the gradient of this more clinically relevant material (see Fig. S2 in the supplemental material), as it does with pure PE, PC, or LCFAs.

DISCUSSION

We previously demonstrated that PlcB is required for twitching-mediated migration up a gradient of either PE or PC (3). However, those data do not address questions about whether further degradation of these phospholipids is associated with this phenotype. We began to examine these issues by comparing the global transcription in *P. aeruginosa* during twitching in the presence or absence of PE. Notably, the largest class of genes that showed increased expression in the presence of PE were those associated with the complete metabolism of a phospholipid (i.e., PE) through the glyoxylate shunt. Thus, the outcome of our microarray experiments provided a pattern consistent with our initial hypothesis that complete degradation of PE occurs during twitching-mediated migration up a phospholipid gradient (e.g., PE). This hypothesis was further tested through an analysis of selected mutants.

The substantial increase in the expression of the three known extracellular lipases of *P. aeruginosa* during twitching-mediated migration up a gradient of PE suggested that one or

more of these lipases might be required for the generation of LCFAs from the DAG that was released by the action of PlcB on PE (Fig. 1). However, an examination of the lipase mutants revealed that the deletion of these genes imparts an unusual phenotype with regard to their basic twitching response. The Δ*estA* mutant, as previously observed in an alternative twitching assay, is completely defective in its ability to move by a twitching-mediated mechanism and has a Δ*pilA*-like phenotype (24). On the other hand, the Δ*lipA* mutant shows an irregular twitching pattern (Fig. 2A). That is, it does not move up the PE gradient nor is it able to twitch up a gradient of unsaturated LCFAs (unpublished data). By contrast, it appears that despite a similar irregular twitching pattern, the Δ*lipC* mutant is still able to preferentially move toward the PE gradient. It is entirely possible that these results are somehow related to previous observations of EstA lipase, which is involved in rhamnolipid production (24). Rhamnolipids have also been shown to play a role in twitching motility. Perhaps the other extracellular lipases (i.e., LipA and LipC) are likewise associated with rhamnolipid biosynthesis or modification. In any case, these and other data support a role for extracellular lipases in the ability of PAO1 to exhibit twitching-mediated migration up a gradient of PE.

The next stage that would be required for the metabolism of LCFAs generated by lipases would be their movement across the outer membrane by an LCFA transporter. In *E. coli*, this is accomplished by a transporter designated FadL. In *P. aeruginosa*, there are three genes annotated as encoding a homolog of FadL. Surprisingly, the deletion of all three FadL genes failed to abolish twitching-mediated migration up a gradient of PE or unsaturated LCFAs. These somewhat unanticipated results, and others discussed below, suggest that there must be an alternative mechanism by which these attractants (i.e., unsaturated LCFAs) are able to traverse the outer membrane. As noted above, we demonstrated that unsaturated, but not saturated, LCFAs function as attractants in our assay. However, we previously reported that *P. aeruginosa* migrates up a gradient of dilauryl-PE, a phospholipid with two saturated fatty acids (C_{12}, lauric acid). While it would seem that this outcome is contradictory to our hypothesis that *P. aeruginosa* cannot twitch toward saturated fatty acids, there are two facts indicating that directional twitching toward this particular phospholipid does not run counter to our conclusions. That is, there is clearly a difference between migrating toward dilauryl-PE (i.e., PE with lauric acid) and PE with any of the saturated fatty acids (e.g., palmitic acid or stearic acid) used in this study. First, migration up a gradient of dilauryl-PE is not dependent on PlcB, thereby indicating that it was not necessary to break down dilauryl-PE for it to serve as an attractant. Moreover, lauric acid (C_{12}) is not an LCFA; rather, it is a medium-chain-length fatty acid. Only fatty acids with an acyl chain length of >14 C (i.e., long chain) normally support growth. In *E. coli*, mutations in the regulatory gene *fadR* allow for growth on both medium-chain (C_8 to C_{12}) and long-chain (>14 C) fatty acids. This is due to the fact that only LCFAs inhibit FadR binding and prevent the repression of genes encoding proteins required for growth on fatty acids (4).

With regard to chemotaxis toward the unsaturated LCFAs used in this study, it is worthwhile to note that *P. aeruginosa* expresses an extracellular double-bond fatty acid-modifying enzyme called a lipoxygenase (PA1169, LoxA) that has been shown to be active on arachidonic, oleic, and linoleic acids (22, 23). This bacterial enzyme has been shown to synthesize the same derivative from arachidonic acid as the human enzyme (i.e., 15-hydroxyeicosatetraenoic acid [15-HETE]). Perhaps such a modification of any of the unsaturated LCFAs used in this study might enable them to be transported by a currently unidentified FadL-independent mechanism. Such modified unsaturated LCFAs could also act as powerful signaling factors or as chemoattractants, like oxylipins of fungi or eicosanoids in mammals (21). Any one of the vast number of arachidonic acid derivatives generated by host cells, including prostaglandins, thromboxanes, leukotrienes, hydroxy and epoxy fatty acids, lipoxins, and isoprostanes, could actually be a chemoattractant for *P. aeruginosa* (7). Moreover, lipoxygenases can generate proinflammatory as well as anti-inflammatory derivatives, some of which are potent neutrophil and macrophage chemoattractants (7). It is not difficult to imagine that twitching-mediated chemotaxis of *P. aeruginosa* toward an anti-inflammatory lipoxygenase derivative could provide a selective advantage to this opportunist during an infection.

Finally, it has been observed by others that in *P. aeruginosa*, in contrast to *E. coli*, energy taxis can dominate the classical metabolism-independent chemotactic responses that occur at the same time (1). Our transcriptional and mutational data initially suggested that movement up a gradient of PE or unsaturated LCFAs would best be classified as energy taxis. This conjecture was further supported by the observations that the Δ*aceA* and Δ*aceB* glyoxylate shunt mutants were unable to directionally move toward unsaturated LCFAs because they were unable to use the PE moieties as a source of energy (Fig. S1 in the supplemental material). However, we ultimately showed that the Δ*aceA* and Δ*aceB* mutants manifested a robust wild-type directional movement up a gradient of PE or unsaturated LCFA when they were provided with glucose as an alternative energy source (Fig. 5). These observations provide compelling evidence not only that the process described herein is "energy taxis," but that it can also be classified as metabolically independent chemotaxis toward unsaturated LCFAs or possibly a modified unsaturated LCFA.

The mechanisms associated with this T4P-mediated chemotaxis-like process in *P. aeruginosa* will be further scrutinized. While some molecular and biochemical aspects of this complex behavior could turn out to be similar to the classical Che-mediated mechanisms that govern flagellum-mediated chemotaxis, it seems just as likely that there will be fascinating new processes revealed about how T4P mediate chemotaxis on a solid surface.

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REFERENCES

1. **Alvarez-Ortega, C., and C. S. Harwood.** 2007. Identification of a malate chemoreceptor in *Pseudomonas aeruginosa* by screening for chemotaxis de-

fects in an energy taxis-deficient mutant. Appl. Environ. Microbiol. **73:**7793–7795.
2. **Arora, S. K., B. W. Ritchings, E. C. Almira, S. Lory, and R. Ramphal.** 1998. The *Pseudomonas aeruginosa* flagellar cap protein, FliD, is responsible for mucin adhesion. J. Bacteriol. **66:**1000–1007.
3. **Barker, A. P., A. I. Vasil, A. Filloux, G. Ball, P. J. Wilderman, and M. L. Vasil.** 2004. A novel extracellular phospholipase C of *Pseudomonas aeruginosa* is required for phospholipid chemotaxis. Mol. Microbiol. **53:**1089–1098.
4. **Black, P. N., N. J. Faergeman, and C. C. DiRusso.** 2000. Long-chain acyl-CoA-dependent regulation of gene expression in bacteria, yeast and mammals. J. Nutr. **130:**305S–309S.
5. **Chiang, P., and L. L. Burrows.** 2003. Biofilm formation by hyperpiliated mutants of *Pseudomonas aeruginosa*. J. Bacteriol. **185:**2374–2378.
6. **Choi, K. H., and H. P. Schweizer.** 2005. An improved method for rapid generation of unmarked *Pseudomonas aeruginosa* deletion mutants. BMC Microbiol. **5:**30.
7. **De Caterina, R., and A. Zampolli.** 2004. From asthma to atherosclerosis—5-lipoxygenase, leukotrienes, and inflammation. N. Engl. J. Med. **350:**4–7.
8. **Dworkin, M., and D. Eide.** 1983. *Myxococcus xanthus* does not respond chemotactically to moderate concentration gradients. J. Bacteriol. **154:**437–442.
9. **Eisenbach, M., and J. W. Lengeler.** 2004. Chemotaxis. Imperial College Press, London, United Kingdom.
10. **Kearns, D. B., J. Robinson, and L. J. Shimkets.** 2001. *Pseudomonas aeruginosa* exhibits directed twitching motility up phosphatidylethanolamine gradients. J. Bacteriol. **183:**763–767.
11. **Kearns, D. B., and L. J. Shimkets.** 1998. Chemotaxis in a gliding bacterium. Proc. Natl. Acad. Sci. USA **95:**11957–11962.
12. **Kearns, D. B., and L. J. Shimkets.** 2001. Directed movement and surface-borne motility of Myxococcus and Pseudomonas. Methods Enzymol. **336:**94–102.
13. **Kearns, D. B., and L. J. Shimkets.** 2001. Lipid chemotaxis and signal transduction in *Myxococcus xanthus*. Trends Microbiol. **9:**126–129.
14. **Ochsner, U. A., P. J. Wilderman, A. I. Vasil, and M. L. Vasil.** 2002. GeneChip expression analysis of the iron starvation response in *Pseudomonas aeruginosa*: identification of novel pyoverdine biosynthesis genes. Mol. Microbiol. **45:**1277–1287.
15. **Ramsey, M. M., and M. Whiteley.** 2004. *Pseudomonas aeruginosa* attachment and biofilm development in dynamic environments. Mol. Microbiol. **53:**1075–1087.
16. **Rashid, M. H., and A. Kornberg.** 2000. Inorganic polyphosphate is needed for swimming, swarming, and twitching motilities of *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. USA **97:**4885–4890.
17. **Sahu, S., and W. S. Lynn.** 1977. Lipid chemotaxins isolated from culture filtrates of *Escherichia coli* and from oxidized lipids. Inflammation **2:**47–54.
18. **Skidmore, B. A., and E. R. Heithaus.** 1988. Lipid cues of seed-carrying by ants in *Hepatica americana*. J. Chem. Ecol. **14:**2185–2196.
19. **Son, M. S., W. J. Matthews, Jr., Y. Kang, D. T. Nguyen, and T. T. Hoang.** 2007. In vivo evidence of *Pseudomonas aeruginosa* nutrient acquisition and pathogenesis in the lungs of cystic fibrosis patients. Infect. Immun. **75:**5313–5324.
20. **Stonehouse, M. J., A. Cota-Gomez, S. K. Parker, W. E. Martin, J. A. Hankin, R. C. Murphy, W. Chen, K. B. Lim, M. Hackett, A. I. Vasil, and M. L. Vasil.** 2002. A novel class of microbial phosphocholine-specific phospholipases C. Mol. Microbiol. **46:**661–676.
21. **Tsitsigiannis, D. I., and N. P. Keller.** 2007. Oxylipins as developmental and host-fungal communication signals. Trends Microbiol. **15:**93–142.
22. **Vance, R. E., S. Hong, K. Gronert, C. N. Serhan, and J. J. Mekalanos.** 2004. The opportunistic pathogen *Pseudomonas aeruginosa* carries a secretable arachidonate 15-lipoxygenase. Proc. Natl. Acad. Sci. USA **101:**2135–2139.
23. **Vidal-Mas, J., M. Busquets, and A. Manresa.** 2005. Cloning and expression of a lipoxygenase from *Pseudomonas aeruginosa* 42A2. Antonie Leeuwenhoek **87:**245–251.
24. **Wilhelm, S., A. Gdynia, P. Tielen, F. Rosenau, and K.-E. Jaeger.** 2007. The autotransporter esterase EstA of *Pseudomonas aeruginosa* is required for rhamnolipid production, cell motility, and biofilm formation. J. Bacteriol. **189:**6695–6703.
25. **Windgassen, M., A. Urban, and K. E. Jaeger.** 2000. Rapid gene inactivation in *Pseudomonas aeruginosa*. FEMS Microbiol. Lett. **193:**201–205.
26. **Zhu, K., K. H. Choi, H. P. Schweizer, C. O. Rock, and Y. M. Zhang.** 2006. Two aerobic pathways for the formation of unsaturated fatty acids in *Pseudomonas aeruginosa*. Mol. Microbiol. **60:**260–273.
27. **Zolfaghar, I., D. J. Evans, and S. M. Fleiszig.** 2003. Twitching motility contributes to the role of pili in corneal infection caused by *Pseudomonas aeruginosa*. Infect. Immun. **71:**5389–5393.