

# PAS domain containing chemoreceptor couples dynamic changes in metabolism with chemotaxis

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**Chemoreceptors provide sensory specificity and sensitivity that enable motile bacteria to seek optimal positions for growth and metabolism in gradients of various physicochemical cues. Despite the abundance of chemoreceptors, little is known regarding the sensory specificity and the exact contribution of individual chemoreceptors to the lifestyle of bacteria. *Azospirillum brasilense* are motile bacteria that can fix atmospheric nitrogen under microaerophilic conditions. Here, we characterized a chemoreceptor in this organism, named AerC, which functions as a redox sensor that enables the cells to seek microaerophilic conditions that support optimum nitrogen fixation. AerC is a representative of a widespread class of soluble chemoreceptors that monitor changes in the redox status of the electron transport system via the FAD cofactor associated with its PAS domains. In *A. brasilense*, AerC clusters at the cell poles. Its cellular localization and contribution to the behavioral response correlate with its expression pattern and with changes in the overall cellular FAD content under nitrogen-fixing conditions. AerC-mediated energy taxis in *A. brasilense* prevails under conditions of nitrogen fixation, illustrating a strategy by which cells optimize chemosensing to signaling cues that directly affect current metabolic activities and thus revealing a mechanism by which chemotaxis is coordinated with dynamic changes in cell physiology.**

FAD | nitrogen fixation | signal transduction

**M**otile bacteria detect changes in environmental conditions and respond by navigating toward niches where conditions are optimal for growth. This widespread behavior is collectively known as chemotaxis (1). Various physicochemical cues are detected by dedicated chemoreceptors (methyl-accepted chemotaxis proteins or MCPs) that relay information to the flagellar motors via a signal transduction cascade (2). The model organism for chemotaxis, *Escherichia coli*, possesses five transmembrane chemoreceptors (Tar, Tsr, Tap, Trg, and Aer) and the sensory specificities for each of these have been determined (2). Most bacterial species have a greater number of chemoreceptors than *E. coli* (3); however, their sensory specificities and contribution to the lifestyle of bacteria are virtually unknown.

*Azospirillum brasilense* are motile bacteria that can fix atmospheric nitrogen under microaerophilic conditions. These bacteria respond tactically to various chemoeffectors that affect their metabolism and the resulting changes in energy levels function as signals (4). In oxygen gradients, *A. brasilense* cells quickly navigate to a specific zone where oxygen concentration is low enough (3–5  $\mu$ M) to support their microaerobic lifestyle and to be compatible with nitrogen fixation (5). Chemotactic responses to changes in energy metabolism have been identified in several bacterial species and are collectively referred to as “energy taxis” (6). In *E. coli*, two of the five chemoreceptors have been implicated in mediating energy taxis: Aer, which senses the redox status via an FAD cofactor (7, 8) and Tsr, which likely monitors the proton motive force (8, 9). In *A. brasilense*, a transmembrane chemoreceptor Tlp1 modulates many energy taxis responses (10). The sensory domain of Tlp1 is located in the periplasm and

lacks a recognizable motif for redox or energy sensing, and thus the energy-related parameter sensed by Tlp1 is unknown (10).

Here, we describe a chemoreceptor, named AerC (transducer for aerotaxis and related responses, cytoplasmic), which functions as an energy taxis transducer in *A. brasilense*. Strikingly, the cellular localization of AerC and its contribution to behavior correlate with metabolic changes under nitrogen-fixing conditions suggesting a mechanism by which chemotaxis is coordinated with dynamic changes in cell physiology. Given the widespread distribution of AerC-like chemoreceptors revealed by comparative genomics, these findings are directly relevant to many other bacterial species.

## Results

**Identification of AerC, a Cytoplasmic Chemoreceptor with Elevated Expression Under Nitrogen-Fixing Conditions.** The available genome sequence of *A. brasilense* (<http://genome.ornl.gov/microbial/abra/19sep08/>) revealed 48 chemoreceptor genes. Five chemoreceptors were predicted to contain PAS domains that often serve as redox and oxygen sensors (11), including one encoded by a gene located between two *nif* operons, which was named *aerC* (Fig. 1). Although the chemoreceptor-encoding gene in this genomic region was identified previously (12), its function has not been elucidated. AerC consists of a C-terminal MCP signaling domain (3) and two N-terminal PAS domains, but lacks transmembrane regions (Fig. 1A). We compared the cellular levels of the AerC protein in the presence of ammonium and under nitrogen-fixing conditions. A polyclonal antibody targeted against the N-terminal PAS domain of the *E. coli* Aer (Aer<sub>2–166</sub>) protein cross-reacted with a protein of about 40 kDa (the predicted molecular weight of AerC) in the wild type, but not in a  $\Delta$ *aerC*::Kan mutant strain (AB301), suggesting that it specifically recognizes AerC (Fig. 1B). Using this antibody, we found that AerC is present under both growth conditions but it is upregulated under conditions of nitrogen fixation (Fig. 1B). AerC was only found in the soluble fraction, an observation consistent with its predicted topology. A C-terminal translational fusion of the yellow fluorescent protein (Yfp) with AerC, expressed under the control of its native promoter from a broad host range low-copy plasmid, was used to determine the subcellular localization of this chemoreceptor (Fig. 2). When expressed in the  $\Delta$ *aerC*::Kan mutant background (strain AB301), the plasmid could restore chemotaxis and aerotaxis to wild-type levels (Fig. S1). In plasmid-containing wild-type and AB301 cells grown in the presence

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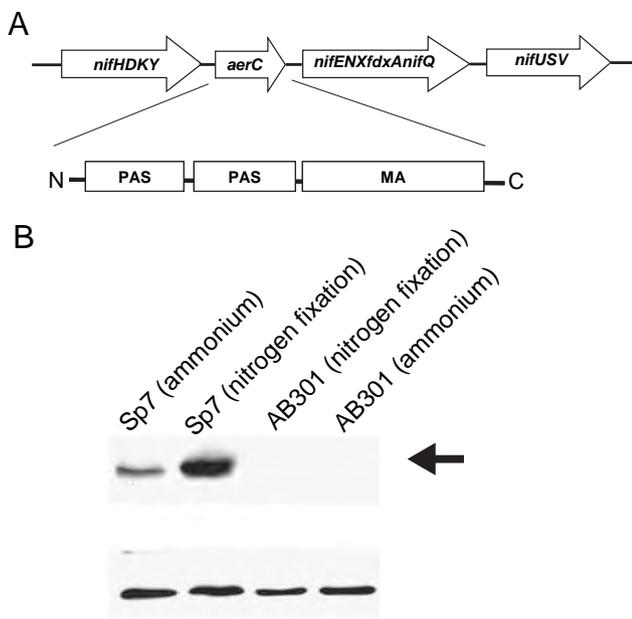
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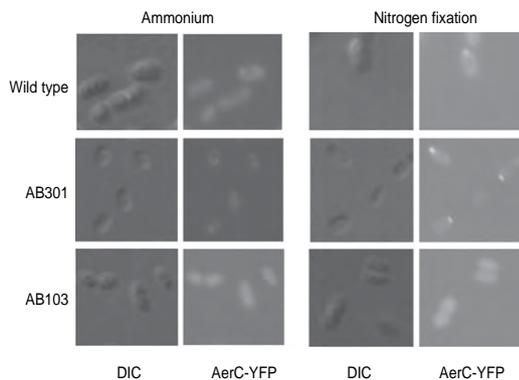
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**Fig. 1.** AerC is a chemoreceptor homolog found in the *nifHfix* gene region of the genome of *A. brasilense*. (A) Organization of the genomic region around *aerC* (Upper) and protein domains found in AerC (Lower). The arrows indicate the direction of transcription. (B) Expression of AerC in *A. brasilense* strain Sp7 (wild type) and its  $\Delta$ *aerC* mutant derivative (strain AB301) grown in presence of ammonium and under nitrogen-fixation conditions. An equivalent amount of protein extracted from whole cells was analyzed. AerC expression was detected by using an affinity-purified *E. coli* anti Aer<sub>2-166</sub> antibody that cross-reacts with a protein of about 40 kDa in Sp7 but not AB301 (indicated by an arrow on the Right). The unidentified low molecular weight cross-reacting band present in all lanes was used as an internal control. The experiment was repeated three times and representative results are shown.

of ammonium, fluorescence was seen within the cell cytoplasm and often in dim polar foci. Under conditions of nitrogen fixation, however, bright fluorescent foci were detected at either one or both cell poles in more than 90% of the cells examined (Fig. 2). No localization of the AerC–Yfp fusion in polar foci was detected when AerC–Yfp was expressed in strain AB103, which is deleted for the Che1 chemotaxis pathway (13) (Fig. 2). Bright



**Fig. 2.** AerC–Yfp localization in *A. brasilense*. The wild-type and mutant derivative strains were grown in presence of ammonium (Left) and under nitrogen-fixation conditions (Right). The AB301 strain is a  $\Delta$ *aerC*::Kan derivative of Sp7 and the AB103 strain is a  $\Delta$ *cheOp1*::Cm derivative of Sp7 (13) (Table S2). In each panel, DIC images are shown on the Left and fluorescent images on the Right. Representative images are shown.

fluorescence was observed throughout the cytoplasm indicating that the AerC–Yfp fusion was expressed. The expression levels of AerC–Yfp were similar in the wild-type and AB301 strains, but lower in the AB103 strain (Fig. S2), suggesting that AerC–Yfp is less stably expressed and/or at reduced levels in this strain.

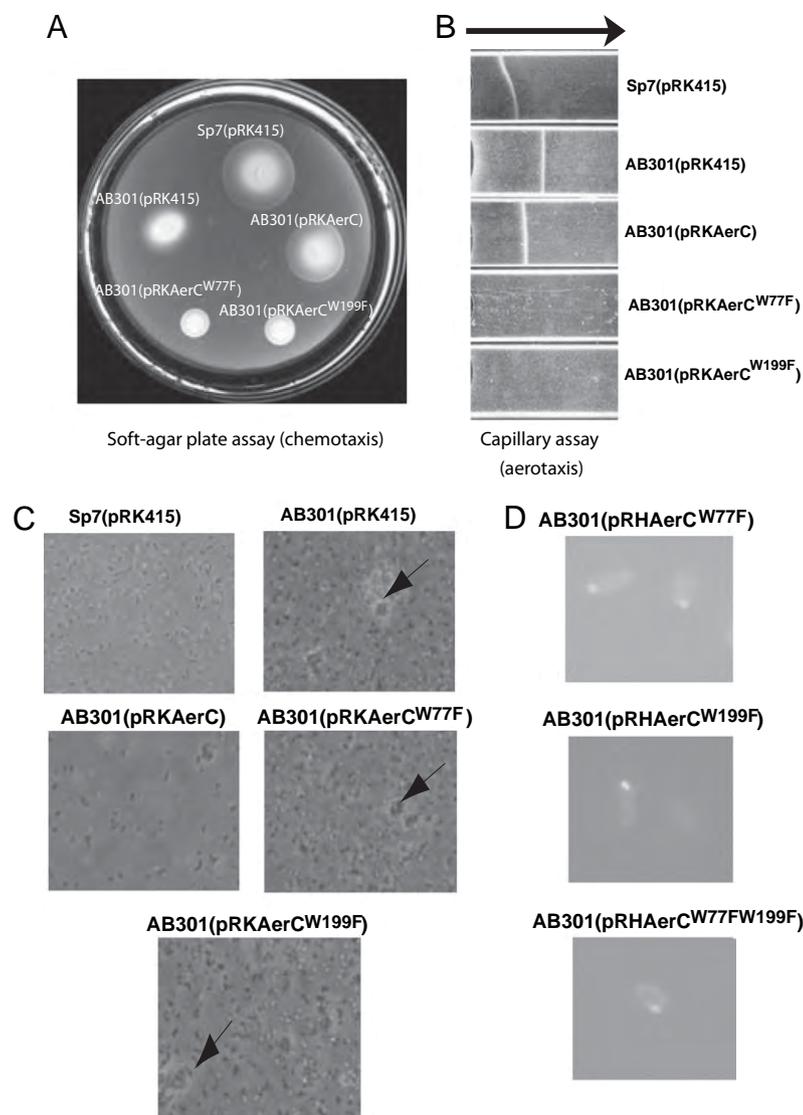
**AerC Is a FAD-Binding Chemoreceptor.** In contrast to the best studied PAS-domain containing chemoreceptor, Aer of *E. coli*, and similar chemoreceptors characterized in other bacteria (14, 15), AerC is a soluble protein. The PAS domain of Aer contains FAD (16, 17); however, PAS domains are ubiquitous and contain various other cofactors (11). To determine which cofactor is associated with the PAS domains of AerC, we compared PAS domains from bacterial chemoreceptors identified in public databases. A total of 1,056 chemoreceptor sequences containing 1,649 PAS domains have been analyzed with respect to their domain architecture and PAS domain conservation (Fig. 3; Figs. S3 and S4). Three groups of PAS domains were identified: (i) a conserved class I exemplified by the PAS domain of the *E. coli* Aer chemoreceptor; (ii) a conserved class II exemplified by both PAS domains of the *A. brasilense* AerC chemoreceptor, and (iii) a diverse group of apparently unrelated PAS domains. All MCPs containing class I PAS domains were predicted to be membrane bound, whereas >98% of MCPs containing class II PAS domains were predicted to be cytoplasmic (Fig. S3).

The pattern of sequence conservation indicates that class I and II PAS domains are closely related in structure and function (Fig. S4). To predict the cofactor bound to PAS domains in AerC, we analyzed the multiple sequence alignment of the PAS domain for the presence of conserved residues that are involved in cofactor binding in PAS domains containing known cofactors: FAD (18), FMN (19), heme (20), hydroxycinnamic acid (21), and citrate (22). Strikingly, only one such residue, a tryptophan corresponding to Trp87 of the FAD-containing PAS domain in the NifL protein of *Azotobacter vinelandii* (18), was conserved in both class I and class II PAS domains (Fig. 3). In NifL, Trp87 participates in multiple interactions with FAD, specifically with its adenine moiety and the ribityl chain (18). We conclude that this residue is the only irreplaceable position specifically involved in FAD binding and its presence in both PAS domains of AerC (Trp77 in PAS1 and Trp199 in PAS2) strongly suggests that they both contain FAD. This prediction was verified experimentally as shown below.

The PAS domain of *E. coli* Aer contains a noncovalently bound FAD that does not copurify well with the native protein, and an indirect assay was developed to confirm the association of FAD with the PAS domain (7, 16, 17, 23). Overexpression of a functional Aer protein (but not nonfunctional Aer alleles) from a plasmid is associated with an increase in the FAD content of cell membranes (7, 23). Overexpressing AerC in wild-type *A. brasilense* cells causes a significant increase in the FAD content of cells, which was not observed in wild-type cells carrying an empty vector (Table S1). Overexpressing AerC alleles that carried either W77F or W199F substitutions yielded a modest increase in the FAD content, whereas no significant increase was detected in cells expressing an AerC<sup>W77FW199F</sup> allele (Table S1). The cellular levels of the expressed mutant AerC proteins were comparable to the wild type and similar between all strains (Fig. S5). These results support the computational prediction that conserved tryptophan residues in the PAS1 and PAS2 domains of AerC bind FAD.

**AerC Is an Energy Taxis Transducer.** We compared the motile behavior of strain AB301 with that of the wild-type strain in a set of behavioral assays performed in the presence of ammonium and under nitrogen-fixing conditions (Fig. S6). Strain AB301 was significantly impaired in chemotaxis to organic acids, glycerol, and certain sugars under all growth conditions (Fig. 4A and Fig. S6A). Interestingly, the  $\Delta$ *aerC* mutant strain was null for chemotaxis to succinate when grown with combined nitrogen, but





**Fig. 4.** Role of tryptophan residues W77 and W199 of the PAS1 and PAS2 domains of AerC in chemoreceptor function. (A) Chemotaxis in the soft agar plate assay of the wild-type *A. brasilense*, the AB301 strain carrying an empty pRK415 vector (controls), or complemented with wild-type AerC and AerC alleles expressed from their own promoter on pRK415, after 24 h incubation at 28 °C. The soft agar plates contained malate as the carbon and energy source and ammonium chloride as the source of combined nitrogen. Longer incubation times did not change the results. (B) Aerotaxis in the capillary assay. An equivalent number of cells were inoculated in each capillary tube and the photograph was taken after a 5-min incubation period. The arrow indicates the direction of the air gradient from the air-liquid interface. (C) Effect of AerC on the propensity of cells to aggregate by cell-to-cell interaction and to form clumps. Pictures were taken directly from actively growing cultures in MMAB with 10 mM malate. Magnification,  $\times 400$ . Representative pictures within a field of view are shown. The arrows point to clumps. (D) Subcellular localization of AerC<sup>W77F</sup>-Yfp, AerC<sup>W199F</sup>-Yfp, and AerC<sup>W77FW199F</sup>-Yfp in the AB301 strain under nitrogen-fixation conditions by fluorescence.

## Discussion

The ability of motile cells to locate preferred niches for energy generation is essential for their survival. Given the ecological advantage provided by energy taxis (24), energy taxis transducers are expected to be widespread in bacteria; however, only a few

have been identified thus far (7, 8, 10, 14, 15). Here we characterize an energy taxis chemoreceptor, AerC, in the nitrogen-fixing bacterium, *A. brasilense*. We show that AerC has elevated expression and localizes to bright polar foci at the cell poles under nitrogen-fixing conditions, but not in the presence of combined nitrogen.

**Table 1. Role of AerC in aerotaxis and redox taxis in *A. brasilense***

Growth conditions/strains	Response time in seconds to adaptation* in a temporal assay for aerotaxis, $\pm$ SD		Threshold concentration for a repellent response in a miniplug assay, $\mu$ M
	-Air <sup>†</sup>	+Air	
Ammonium			
Sp7	62.4 $\pm$ 5.1	58.1 $\pm$ 1.1	50
AB301	76.7 $\pm$ 7.2	62.4 $\pm$ 2.0	50
Nitrogen fixation			
Sp7	71.6 $\pm$ 1.7	62.0 $\pm$ 2.3	50
AB301	45.3 $\pm$ 3.3	37.2 $\pm$ 2.1	100

\*The response time of free-swimming cells until they return to a prestimulus swimming pattern was measured. The removal of air triggers a repellent response (increase in the reversal frequency of swimming cells) and the addition of air triggers an attractant response (decrease in the reversal frequency) under these conditions.

<sup>†</sup>The “-” and “+” signs refer to the removal (repellent) or addition (attractant) of air, respectively, in the temporal assay.



was cloned into the pFUS1 vector (33) to generate a transcriptional fusion with the promoterless *gusA*, yielding *PaerC-gusA* (pFUSPaerC; Table S2). The *nifH* promoter activity was assessed using a *PnifH-gusA* fusion from the pFAJ318 vector (Table S2). The  $\beta$ -glucuronidase activity was determined according to Reeve et al. (33).

**Behavioral Assays.** Semisoft plates, capillary, miniplug, and temporal assays were performed essentially as previously described (4).

**Computational Analysis.** Database searches and protein sequence analyses were carried out using standard bioinformatics techniques (see *SI Materials and Methods* for details).

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