Elastin degradation product isodesmosine is a chemoattractant for *Pseudomonas aeruginosa*

Inmaculada Sampedro,¹ Junichi Kato² and Jane E. Hill¹

¹Thayer School of Engineering, Dartmouth College, 14 Engineering Drive, Hanover, NH 03755, USA
²Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, Higashi-Hiroshima, Hiroshima 739-8530, Japan

Previous studies have demonstrated that *Pseudomonas aeruginosa* PAO1 is chemotactic towards proteinogenic amino acids, however, the chemotaxis response of this strain towards non-proteinogenic amino acids and the specific chemoreceptors involved in this response are essentially unknown. In this study, we analysed the chemotactic response of PAO1 towards two degradation products of elastin, the lysine-rich, non-proteinogenic amino acids, desmosine and isodesmosine. We observed that isodesmosine, a potential biomarker for different diseases, served as a chemoattractant for PAO1. A screen of 251 methyl-accepting chemotaxis proteins mutants of PAO1 identified PctA as the chemoreceptor for isodesmosine. We also showed that the positive chemotactic response to isodesmosine is potentially common by demonstrating chemotraction in 12 of 15 diverse (in terms of source of isolation) clinical isolates, suggesting that the chemotactic response to this non-proteinogenic amino acid might be a conserved feature of acute infection isolates and thus could influence the colonization of potential infection sites.

INTRODUCTION

*Pseudomonas aeruginosais*, an opportunistic human pathogen that can cause acute and chronic infections, e.g. skin (Andonova & Urumova, 2013), lung (Munder et al., 2011), eye (Zegans et al., 2012), cystic fibrosis airway (Bethesda, 2011), chronic obstructive pulmonary disease airway (Murphy et al., 2008) and atherogenesis (Turkay et al., 2004). *P. aeruginosa* can sense chemical gradients using a chemosensory pathway linked to flagella or pili (Sampedro et al., 2014). *P. aeruginosa* PAO1 has 26 putative methyl-accepting chemotaxis proteins (MCPs). Thirteen of its 26 MCP-like proteins have been functionally characterized. Ten MCPs have been shown to mediate a flagella-based positive chemotaxis response to malate (PA2652), oxygen (Aer and Aer-2), ethylene (TlpQ), chloroethylenes (McpA), amino acids and gamma aminobutyrate (GABA) (PctABC) and inorganic phosphate (CtpH and CtpL) (Alvarez-Ortega & Harwood, 2007; Hong et al., 2004; Kim et al., 2006, 2007; Rico-Jiménez et al., 2013; Shitashiro et al., 2005; Taguchi et al., 1997; Wu et al., 2000). The MCPs responsible for detecting the 20 natural L-amino acids in PAO1 are the paralogous PctA, PctB and PctC receptors (Kuroda et al., 1995; Taguchi et al., 1997). PctA recognizes and mediates the response to most of the natural L-amino acids, while PctB is primarily involved in mediating taxis to glutamine and PctC to histidine and proline. Only one natural amino acid has been investigated as a chemotactic stimulant for *P. aeruginosa*, the human neurotransmitter GABA (Rico-Jiménez et al., 2013). GABA was shown to generate a positive chemotactic response in the concentration range 0.01–1 mM GABA, similar to that of the 20 natural amino acids investigated previously.

Elastin is an insoluble extracellular matrix protein that provides elasticity to tissues and organs (Debelle & Tamburro, 1999; Mithieux & Weiss, 2005; Rosenbloom et al., 1993). This stretchy, flexible protein is enriched with glycine, valine, alanine and proline and cross-linked by lysine residues. Elastin does not undergo significant turnover in healthy tissues (Urbán & Boyd, 2000), however, tissue damage caused by physical injury, infection or an underlying chronic pathology results in the degradation of elastin. Elastinolytic enzymes may be produced by the host [e.g. in response to inflammation the neutrophil elastase, a serine protease, degrades lung connective tissue (Bruce et al., 1985; Kuraki et al., 2002; Stockley, 2002; Wright et al., 2002)] or from an external source, such as snake venom (Komori et al., 2011) or bacterial infection [e.g. *P. aeruginosa* (Amitani et al., 1991; Azghani et al., 2014; Yoo et al., 2014)] or pulmonary tuberculosis (Greenlee et al., 2004).

Abbreviations: GABA, gamma aminobutyrate; MCP, methyl-accepting chemotaxis protein

Two supplementary tables are available with the online Supplementary Material.

Thayer School of Engineering, Dartmouth College, 14 Engineering Drive, Hanover, NH 03755, USA

Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, Higashi-Hiroshima, Hiroshima 739-8530, Japan

Jane E. Hill

Jane.E.Hill@dartmouth.edu

Thayer School of Engineering, Dartmouth College, 14 Engineering Drive, Hanover, NH 03755, USA

Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, Higashi-Hiroshima, Hiroshima 739-8530, Japan

Jane E. Hill

Jane.E.Hill@dartmouth.edu
<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant characteristics</th>
<th>Source of reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strain</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. aeruginosa</em> PAO1</td>
<td>Prototroph, FP&quot; (sex factor minus)</td>
<td>Holloway <em>et al.</em> (1979)</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> PAO1 ΔpctA</td>
<td>PAO1 derivative,Cb&quot;</td>
<td>This study</td>
</tr>
<tr>
<td>pMAI18-1(pctA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCTA1</td>
<td>PAO1 derivative, pctA:Cbr&quot;</td>
<td>Kuroda <em>et al.</em> (1995)</td>
</tr>
<tr>
<td>PCTB1</td>
<td>PAO1 derivative, pctB:Cbr&quot;</td>
<td>Taguchi <em>et al.</em> (1997)</td>
</tr>
<tr>
<td>PCTC1</td>
<td>PAO1 derivative, pctC:Cbr&quot;</td>
<td>Taguchi <em>et al.</em> (1997)</td>
</tr>
<tr>
<td>tlpA</td>
<td>PAO1 derivative, tlpA:Cbr&quot;</td>
<td>PA1646*</td>
</tr>
<tr>
<td>tlpB</td>
<td>PAO1 derivative, tlpB:Cbr&quot;</td>
<td>PA1608*</td>
</tr>
<tr>
<td>tlpE</td>
<td>PAO1 derivative, tlpE:Cbr&quot;</td>
<td>PA1251*</td>
</tr>
<tr>
<td>tlpH</td>
<td>PAO1 derivative, tlpH:Cbr&quot;</td>
<td>PA5072*</td>
</tr>
<tr>
<td>tlpI</td>
<td>PAO1 derivative, tlpI:Cbr&quot;</td>
<td>PA4915*</td>
</tr>
<tr>
<td>tlpJ</td>
<td>PAO1 derivative, tlpJ:Cbr&quot;</td>
<td>PA4633*</td>
</tr>
<tr>
<td>tlpK</td>
<td>PAO1 derivative, tlpK:Cbr&quot;</td>
<td>PA4520*</td>
</tr>
<tr>
<td>tlpL</td>
<td>PAO1 derivative, tlpL:Cbr&quot;</td>
<td>PA4290*</td>
</tr>
<tr>
<td>wspA (tlpM)</td>
<td>PAO1 derivative, tlpM:Cbr&quot;</td>
<td>PA3708*</td>
</tr>
<tr>
<td>tlpN</td>
<td>PAO1 derivative, tlpN:Cbr&quot;</td>
<td>PA2920*</td>
</tr>
<tr>
<td>tlpO</td>
<td>PAO1 derivative, tlpO:Cbr&quot;</td>
<td>PA2867*</td>
</tr>
<tr>
<td>tlpP</td>
<td>PAO1 derivative, tlpP:Cbr&quot;</td>
<td>PA2788*</td>
</tr>
<tr>
<td>tlpQ</td>
<td>PAO1 derivative, tlpQ:Cbr&quot;</td>
<td>Kim <em>et al.</em> (2007)</td>
</tr>
<tr>
<td>mcps (tlpR)</td>
<td>PAO1 derivative, tlpR:Cbr&quot;</td>
<td>Alvarez-Ortega &amp; Harwood (2007)</td>
</tr>
<tr>
<td>tlpS</td>
<td>PAO1 derivative, tlpS:Cbr&quot;</td>
<td>PA2573*</td>
</tr>
<tr>
<td>tlpT</td>
<td>PAO1 derivative, tlpT:Cbr&quot;</td>
<td>PA1930*</td>
</tr>
<tr>
<td>ctpP</td>
<td>PAO1 derivative, ctpP:Cbr&quot;</td>
<td>Kim <em>et al.</em> (2006)</td>
</tr>
<tr>
<td>ctpL</td>
<td>PAO1 derivative, ctpL:Cbr&quot;</td>
<td>Wu <em>et al.</em> (2000)</td>
</tr>
<tr>
<td>ctpH</td>
<td>PAO1 derivative, ctpH:Cbr&quot;</td>
<td>Wu <em>et al.</em> (2000)</td>
</tr>
<tr>
<td>bdlA</td>
<td>PAO1 derivative, bdlA:Cbr&quot;</td>
<td>This study</td>
</tr>
<tr>
<td>per (tlpG)</td>
<td>PAO1 derivative, tlpG:Cbr&quot;</td>
<td>Hong <em>et al.</em> (2004)</td>
</tr>
<tr>
<td>aor2 (tlpG)</td>
<td>PAO1 derivative, tlpG:Cbr&quot;</td>
<td>Hong <em>et al.</em> (2004)</td>
</tr>
<tr>
<td><strong>P. aeruginosa</strong> acute infections strains</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. aeruginosa</em> PA14</td>
<td>Wild-type</td>
<td>Rahme <em>et al.</em> (1995)</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> clinical isolate</td>
<td>Ear isolate</td>
<td>PA3†</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> clinical isolate</td>
<td>Trachea isolate</td>
<td>PA6†</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> clinical isolate</td>
<td>Peritoneal fluid isolate</td>
<td>PA8†</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> clinical isolate</td>
<td>Urine isolate</td>
<td>PA10†</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> clinical isolate</td>
<td>Abdominal fluid isolate</td>
<td>PA36†</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> clinical isolate</td>
<td>Foot isolate</td>
<td>PA2†</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> clinical isolate</td>
<td>Toe isolate</td>
<td>PA1†</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> clinical isolate</td>
<td>Sputum isolate</td>
<td>PA38†</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> clinical isolate</td>
<td>Tracheal aspiration isolate</td>
<td>PA42†</td>
</tr>
<tr>
<td>PA715</td>
<td>Ocular isolate</td>
<td>Zegans <em>et al.</em> (2012)</td>
</tr>
<tr>
<td>PA738</td>
<td>Ocular isolate</td>
<td>Zegans <em>et al.</em> (2012)</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> clinical isolate</td>
<td>PA2192</td>
<td>PA2192‡</td>
</tr>
<tr>
<td>PA 6077</td>
<td>Ocular isolate</td>
<td>Wolfgang <em>et al.</em> (2003)</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> clinical isolate</td>
<td>Ocular isolate</td>
<td>PA4699/11§</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pUCP18</td>
<td>Broad-host-range cloning vector; Cbr&quot;</td>
<td>Schweizer (1991)</td>
</tr>
<tr>
<td>pMAI18-1</td>
<td>pUCP18 with a 2.1 kb PCR fragment containing pctA:Cbr&quot;</td>
<td>Shitashiro <em>et al.</em> (2005)</td>
</tr>
</tbody>
</table>

*Km", kanamycin; Cbr", carbenicillin.

*Culture collections obtained from Dr Kato (Hiroshima University, Japan).
†Culture collections obtained from Dr Schwartzman (Geisel School of Medicine, Dartmouth).
‡Culture collections obtained from Dr O’Toole (Geisel School of Medicine, Dartmouth).
§Culture collections obtained from Dr Zegans (Geisel School of Medicine, Dartmouth).

http://mic.sgmjournals.org
et al., 2007)]. In addition to releasing larger pieces of the polymer, the degradation of elastin generates two non-natural amino acids, desmosine and isodesmosine (Afedhal et al., 1997; Albarbarawi et al., 2013; Boutin et al., 2009; Kaga et al., 2003; Laguna et al., 2009, 2012; Lamerz et al., 2013; Ma et al., 2003, 2007, 2011, 2013; Miliotis et al., 2013; Ongay et al., 2014; Osakabe et al., 1999). Desmosine and isodesmosine are 1,2,3,5- and 1,3,4,5-tetrasubstituted pyridinium amino acids, respectively that serve as the cross-linking molecules that bind the polymeric chains in elastin into a three-dimensional network (Partridge et al., 1963; Thomas et al., 1963). Desmosine and isodesmosine can be measured in clinical samples, such as urine, sputum and plasma (Ferrari et al., 2012; Ma et al., 2011, 2013) during pathogenesis. Both amino acids are potential biomarkers for the diagnosis of chronic obstructive pulmonary disease (Huang et al., 2012; Ongay et al., 2014; Usuki et al., 2014) and inflammatory arterial diseases, including atherosclerosis and abdominal aortic aneurysm (Umeda et al., 2011). Sputum desmosine may serve as a marker of structural lung damage occurring during exacerbations of lung disease in cystic fibrosis (Laguna et al., 2009). We hypothesize that lysine-rich desmosine and isodesmosine, expected at sites of injury, serve as chemoattractants to the opportunistic pathogen P. aeruginosa, potentially setting off a vicious cycle of elastase degradation and bacterial recruitment. The objective of this paper was to initiate an investigation of this hypothesis by assessing the chemoresponse of P. aeruginosa to desmosine and isodesmosine.

Fig. 1. Concentration response curves for chemotaxis to isodesmosine (IS), desmosine (D) and lysine (Lys) by P. aeruginosa PAO1. The average background accumulation in capillaries containing CB only is indicated by the broken line. Results are averages of at least 12 capillaries from five independent experiments; error bars indicate SEM.

METHODS

Bacterial strains and growth conditions. The strains and plasmids used in this study are shown in Table 1. P. aeruginosa PAO1 and the 25 mcp single mutants of P. aeruginosa PAO1 were kindly provided by Dr Junichi Kato (Hiroshima University, Japan). The clinical strains of P. aeruginosa from acute infections were kindly provided by Dr Joseph D. Schwartzman and Dr Michael Zegans (Geisel School of Medicine, Dartmouth) and P. aeruginosa PA14 was provided courtesy of Dr Fred Ausubel (Massachusetts General Hospital). For chemotaxis assays, strains were grown overnight at 37 °C in minimal salts medium (Stanier et al., 1966) supplemented with 27.5 mM glucose and 0.5 % (w/v) Casamino acids. The strain P. aeruginosa PAO1 ΔpctA pMA18-1 (pctA) was grown in the presence of 50 µg carbenicillin ml⁻¹.

DNA manipulation and electroporation. Standard methods were used for the manipulation of plasmid DNA (Ausubel et al., 1993). To create P. aeruginosa PAO1 ΔpctA pMA18-1 (pctA), P. aeruginosa PAO1 ΔpctA was transformed by electroporation with the vector pMA18-1 (carrying pctA) (Shitashiro et al., 2005) provided by Dr Kato.

HPLC analyses. To confirm the purity of isodesmosine and desmosine, LC/MS/MS analyses were performed without chemical derivatization on a Waters Quattro micro mass spectrometer coupled to Shimadzu HPLC pumps as described by Gu et al. (2012).

Chemotaxis assays. The chemoattractants used in these experiments were isodesmosine and desmosine, obtained from MP Biomedicals (purity ≥ 99 and ≥99.5 %, respectively), and L-lysine, L-leucine, succinic acid and Casamino acids were obtained from Amresco at the highest purity commercially available. The chemoattractants were prepared in chemotaxis buffer (CB; 50 mM sodium phosphate buffer (pH 7.0), 10 µM disodium EDTA and 0.05 % (w/v) glycerol).

Bacterial cells were harvested in mid-exponential phase (OD₆₆₀ 0.3–0.4) by centrifugation at 4600 r.p.m. for 5 min and washed once with CB. Cells were resuspended to an OD₆₆₀ of 0.1. Quantitative capillary assays were carried out as described previously (Adler, 1973). Briefly, the capillary tubes were 1 µl disposable micropipettes with one end sealed via flame and filled with the chemotactrant resuspended in CB. After incubation in the pool of cells for 30 min at room temperature, the capillary was removed, the exterior rinsed with sterile water and the contents of the capillaries were transferred to tubes of CB via centrifugation (8000 r.p.m.). Dilution in CB and then plating allowed for determination of c.f.u. per capillary tube. In all experiments, negative controls (CB) and positive controls (0.2 %, w/v, Casamino acid) were used. The amino acids desmosine, isodesmosine and lysine were tested at 0.01, 0.1, 1, 5 and 10 mM. Data are represented as the mean ± SEM of at least five independent experiments with three technical replicates each.

The qualitative capillary assay was carried out as previously described by Parales et al. (2000). Washed cells were suspended in CB to OD₆₆₀ 0.1, then placed in a chamber formed by a coverslip and a glass U-tube. Microcapillaries (1 µl) were filled with 10 µM of each chemoattractant in a gel of 2 % low-melting-temperature agarose (Nusieve GTG; Lonza) in CB and inserted into the pool of bacterial cells. In all experiments, negative controls (CB) and positive controls (0.2 %, w/v, Casamino acids and 10 mM succinate) were included. The response was visualized at 0 and 5 min on an Olympus IX73 inverted microscope with an Olympus TH4-100 halogen illuminator and photographed using an Olympus DP73 CCD camera with Olympus cellSens standard version 1.8 software. The dark-field illumination was generated using a Ph2 ring in the long-working distance condenser NA 0.55 with a UPlanFLN 4X NA. 0.13 objective. Images were processed (contrast and brightness) as well as centered using Adobe Photoshop Lightroom.

Downloaded from www.microbiologyresearch.org by Dr. Joseph D. Schwartzman and Dr. Michael Zegans (Geisel School of Medicine, Dartmouth) and P. aeruginosa PA14 was provided courtesy of Dr Fred Ausubel (Massachusetts General Hospital). For chemotaxis assays, strains were grown overnight at 37 °C in minimal salts medium (Stanier et al., 1966) supplemented with 27.5 mM glucose and 0.5 % (w/v) Casamino acids. The strain P. aeruginosa PAO1 ΔpctA pMA18-1 (pctA) was grown in the presence of 50 µg carbenicillin ml⁻¹.

DNA manipulation and electroporation. Standard methods were used for the manipulation of plasmid DNA (Ausubel et al., 1993). To create P. aeruginosa PAO1 ΔpctA pMA18-1 (pctA), P. aeruginosa PAO1 ΔpctA was transformed by electroporation with the vector pMA18-1 (carrying pctA) (Shitashiro et al., 2005) provided by Dr Kato.

HPLC analyses. To confirm the purity of isodesmosine and desmosine, LC/MS/MS analyses were performed without chemical derivatization on a Waters Quattro micro mass spectrometer coupled to Shimadzu HPLC pumps as described by Gu et al. (2012).

Chemotaxis assays. The chemoattractants used in these experiments were isodesmosine and desmosine, obtained from MP Biomedicals (purity ≥ 99 and ≥99.5 %, respectively), and L-lysine, L-leucine, succinic acid and Casamino acids were obtained from Amresco at the highest purity commercially available. The chemoattractants were prepared in chemotaxis buffer (CB; 50 mM sodium phosphate buffer (pH 7.0), 10 µM disodium EDTA and 0.05 % (w/v) glycerol).

Bacterial cells were harvested in mid-exponential phase (OD₆₆₀ 0.3–0.4) by centrifugation at 4600 r.p.m. for 5 min and washed once with CB. Cells were resuspended to an OD₆₆₀ of 0.1. Quantitative capillary assays were carried out as described previously (Adler, 1973). Briefly, the capillary tubes were 1 µl disposable micropipettes with one end sealed via flame and filled with the chemotactrant resuspended in CB. After incubation in the pool of cells for 30 min at room temperature, the capillary was removed, the exterior rinsed with sterile water and the contents of the capillaries were transferred to tubes of CB via centrifugation (8000 r.p.m.). Dilution in CB and then plating allowed for determination of c.f.u. per capillary tube. In all experiments, negative controls (CB) and positive controls (0.2 %, w/v, Casamino acid) were used. The amino acids desmosine, isodesmosine and lysine were tested at 0.01, 0.1, 1, 5 and 10 mM. Data are represented as the mean ± SEM of at least five independent experiments with three technical replicates each.

The qualitative capillary assay was carried out as previously described by Parales et al. (2000). Washed cells were suspended in CB to OD₆₆₀ 0.1, then placed in a chamber formed by a coverslip and a glass U-tube. Microcapillaries (1 µl) were filled with 10 µM of each chemoattractant in a gel of 2 % low-melting-temperature agarose (Nusieve GTG; Lonza) in CB and inserted into the pool of bacterial cells. In all experiments, negative controls (CB) and positive controls (0.2 %, w/v, Casamino acids and 10 mM succinate) were included. The response was visualized at 0 and 5 min on an Olympus IX73 inverted microscope with an Olympus TH4-100 halogen illuminator and photographed using an Olympus DP73 CCD camera with Olympus cellSens standard version 1.8 software. The dark-field illumination was generated using a Ph2 ring in the long-working distance condenser NA 0.55 with a UPlanFLN 4X NA. 0.13 objective. Images were processed (contrast and brightness) as well as centered using Adobe Photoshop Lightroom.
Isodesmosine is a chemoattractant for PAO1 and utilizes pctA as a receptor

P. aeruginosa strain PAO1 demonstrated statistically significant (compared with CB controls), positive chemotaxis towards capillaries containing desmosine and isodesmosine [according to Tukey’s honest significant difference test ($P \leq 0.05$) for isodesmosine and desmosine at concentrations of 10 mM, $P=0.002$ and $P=0.0025$, respectively].

Fig. 1 shows the chemotaxis response increases as the attractant concentration increases. The optimal concentration of chemoattractant was unknown, as these experiments were limited by the compounds’ solubilities in CB. For relative comparison, the chemotactic response of lysine is also shown in Fig. 1.

HPLC analyses showed the presence of a low percentage of lysine in the isodesmosine and desmosine samples used in this study (Table S1, available in the online Supplementary Material). Statistical analysis showed that the chemotactic response of PAO1 to isodesmosine was not a consequence...
of lysine contamination [according to ANOVA test ($P \leq 0.05$), $P = 0.0146$]. However, desmosine chemotaxis results were likely influenced by lysine contamination [according to ANOVA test ($P \leq 0.05$), $P = 0.4127$].

There are three amino acid receptors in $P. aeruginosa$ strain PAO1: PctA, PctB and PctC (Kuroda et al., 1995; Taguchi et al., 1997). Isodesmosine contains lysine residues and chemotaxis towards lysine is directly linked to PctA and PctB, and therefore we hypothesized that the chemotactic response of $P. aeruginosa$ PAO1 is mediated by some of these receptors. To test this hypothesis, PAO1 ΔpctA and its complement were monitored microscopically using capillaries containing isodesmosine. Fig. 2 shows the lack of chemotactic response in ΔpctA to isodesmosine, with the flanking images showing chemotraction in the wild-type and the complemented mutant ΔpctA pMA18-1(pctA). The negative control (CB), positive chemotaxis controls (succinate and Casamino acids) and the pctA-based amino acid control (leucine) all displayed the appropriate phenotype (Fig. 2). None of the other 24 mcp mutants showed a response to isodesmosine (data not shown). We also measured the chemotactic response of PAO1, ΔpctA and its complemented mutant quantitatively. Fig. 3 shows the restoration of the chemotraction phenotype for isodesmosine (at 10 mM isodesmosine) in the complemented mutant. As expected, the background accumulation of cells in capillaries containing isodesmosine was statistically indistinguishable for the wild-type and the complemented strain ($3.40 \times 10^3 \pm 0.9 \times 10^3$ versus $3.19 \times 10^3 \pm 0.8 \times 10^3$) while the mutant, ΔpctA, showed an accumulation of approximately zero under both experimental conditions. Taken together with the qualitative capillary assays, these results demonstrated that pctA is the receptor for isodesmosine.

**Chemoattraction to isodesmosine is found in a variety of $P. aeruginosa$ clinical isolates**

In a qualitative assessment of 15 highly diverse $P. aeruginosa$ clinical isolates, 12 strains exhibited putative chemotraction to isodesmosine (Fig. 4). Based on qualitative assay, the strongest chemotaxis response for this compound was observed for the strains isolated from urine and ear infections. The three strains that did not show any chemotraction were isolated from acute infections associated with the foot, abdominal fluid and tracheal aspirate. Quantitative chemotaxis studies with some of these strains confirmed these results (Table S2).

**DISCUSSION**

Kuroda et al. (1995) and Taguchi et al. (1997) identified the chemoreceptors PctA, PtcB and PctC associated with PAO1 chemoattraction towards 20 natural amino acids. Recently, Rico-Jiménez et al. (2013) demonstrated that the recombinant ligand-binding region of PctC binds the non-proteinogenic amino acid GABA. An assessment of other non-proteinogenic amino acids has not been conducted, even though there is substantial diversity in structure and side-chain groups in this chemical class and they are reasonably abundant in animals and plants. Desmosine and isodesmosine, for example, are generated by the degradation of the lysine-rich, connective tissue protein elastin during various acute and chronic disease processes. Therefore, we hypothesized that desmosine and isodesmosine would be cheemoattractants for strains of $P. aeruginosa$ and that this process is mediated by the receptor PctA.

In this study, PctA was demonstrated to be responsible for the chemoattraction response of PAO1 to isodesmosine, and possibly, by extension, other $P. aeruginosa$ isolates. Previous studies have shown that PAO1 responds to amino acids in the range 1 to 10 mM (Jeong et al., 2010), which is the response range used in this study. A comparison to L-lysine (Fig. 1), confirmed that the response of PAO1 was as expected (Taguchi et al., 1997), albeit at a higher level of chemoattraction than elicited by isodesmosine. Structurally, isodesmosine has the same chiral stereochemistry as L-lysine; however, this amino acid contains three allysyl side chains plus one unaltered lysyl side chain. The difference between this molecule and L-lysine probably influenced their interaction with PctA, resulting in the different responses reported here.

Most studies on *Pseudomonas* swimming chemotaxis have primarily used *P. aeruginosa* strain PA01, and although other strains, such as RM46, PAO2, J-20, M2, WR5, 103, MB720, PFB360 and PFB362 (Barber et al., 1979; Craven...
& Montie, 1981, 1985; Hamilton & Shelley, 1971; Sly et al., 1993; Stinson et al., 1977), have also been investigated in regard to sugars and amino acids, the use of clinical isolates in chemotaxis studies is rare (Craven & Montie, 1981). Here, we showed the chemotactic response in 15 diverse (in terms of source of isolation) clinical isolates, suggesting that the chemotactic response to isodesmosine could be a conserved feature of acute infection isolates. Therefore, further investigation into the extent of this phenotype, as well as the potential chemotactic response towards other non-natural amino acids and their influence on infection pathogenesis, is warranted.

**Fig. 4.** Qualitative capillary chemotaxis assays comparing responses of 15 acute infection *P. aeruginosa* isolates to isodesmosine (10 mM). The negative control (CB) is shown for contrast. Dark-field images of cells gathered at the mouth of capillaries containing attractants. All photographs are taken after 5 min.
ACKNOWLEDGEMENTS

We thank Pilar Linan Arantave for helping us improve the quality of the images. We thank the MSU Mass Spectrometry Core for performing the HPLC analyses.

REFERENCES


Edited by: K. Ottemann