ELSEVIER

FEMS Microbiology Letters 224 (2003) 175–181

FEMS MICROBIOLOGY Letters

www.fems-microbiology.org

Helicobacter pylori strain ATCC700392 encodes a methyl-accepting chemotaxis receptor protein (MCP) for arginine and sodium bicarbonate

Oscar Cerda, Ana Rivas, Héctor Toledo *

Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile, Casilla 70086, Santiago-7, Chile

Received 14 January 2003; received in revised form 7 May 2003; accepted 9 May 2003

First published online 25 June 2003

Abstract

Helicobacter pylori ATCC43504 responds chemotactically to aspartic acid and serine, but not to arginine, nor to sodium bicarbonate. In contrast, *H. pylori* ATCC700392 (strain 26695) shows chemotaxis to all four attractants. Open reading frame HP0099 from *H. pylori* 26695 is predicted to encode one of three methyl-accepting chemotaxis receptor proteins (MCPs). When *Escherichia coli* is transformed with a plasmid carrying HP0099 from strain 26695, the recombinants acquire chemotaxis to arginine, bicarbonate, and urea. In *H. pylori* 43504, the HP0099 gene is interrupted with a mini-IS*605* insertion, which accounts for its inability to recognize arginine and bicarbonate as attractants. Together, these results argue that the *H. pylori* HP0099 gene encodes an MCP for arginine and bicarbonate.

Keywords: Chemotaxis; Signal transduction; HP0099; IS*605*; *Helicobacter pylori*

1. Introduction

The colonization of the human host by many bacterial pathogens is dependent on flagella. Flagellated bacteria swim toward chemical attractants and away from repellents by a mechanism known as chemotaxis. As yet, we know little about how these microorganisms use the ability to swim to their advantage, to colonize their human host. Chemotaxis is an adaptive response to microenvironmental changes, and is regulated by a complex signal transduction mechanism [1]. Flagellated bacteria express four sets of genes necessary for chemotaxis. These include the *fla* and other genes involved in the assembly of flagella, the *mot* genes encoding components of the flagellar motor, the *che* genes involved in the regulation of chemotactic responses, and the *mcp* genes encoding the methyl-accepting chemotaxis proteins (MCPs). MCPs are membrane-spanning proteins that interact with specific ligands, and transduce information across the cell membrane to the intracellular Che proteins. The Che proteins relay signals from the MCPs to the flagellar motor, which drives flagellar rotation.

Helicobacter pylori is a microaerophilic Gram-negative pathogen, and has a bundle of unipolar flagella [2]. *H. pylori* can penetrate the gastric mucous layer, and causes gastric ulcers, adenocarcinomas, and lymphomas [2–4]. The colonization of the gastric mucosa by *H. pylori* depends on its ability to make urease, as well as on its flagella, and chemotaxis [5–7]. *H. pylori* produces large amounts of cytoplasmic urease constitutively [5], and releases urease due to autolysis [8]. Presumably, the main role of urease is to neutralize the acidic microenvironments colonized by *H. pylori*, by producing ammonia and carbon dioxide from urea. *H. pylori* flagella are a mosaic of two flagellins, FlaA and FlaB [9]. Both flagellin subunits are necessary for full motility on soft agar plates and for full colonization of gnotobiotic piglets. An aflagellated strain of *H. pylori* colonizes the stomach less frequently [6].

In the stomach of infected patients, *H. pylori* resides mainly in the surface mucous layer [10]. Because this gelatinous layer has a rapid turnover [11], bacteria proliferating in this layer must retain the ability to move toward the stomach epithelial cell surface, and against the mucous flow toward the duodenum. For this reason, the motility

* Corresponding author. Tel.: +56 (2) 678 6053; Fax: +56 (2) 735 5580.
E-mail address: htoledo@machi.med.uchile.cl (H. Toledo).

0378-1097/03/$22.00
doi:10.1016/S0378-1097(03)00423-3

of *H. pylori* should be important for bacterial growth, colonization, and persistent infection.

The complete genome sequences of two *H. pylori* strains have been determined, strains 26695 [12], and J99 [13]. Three *H. pylori* open reading frames (ORFs), HP0082, HP0099, and HP0103, in the genome sequence of strain 26695 (ATCC700392), are predicted to encode MCPs, with homology to those of other bacteria. To understand how the motility of *H. pylori* contributes to its virulence, we have identified a subset of the attractants to which *H. pylori* responds, and the MCP responsible for a subset of these chemotactic responses. Our results show that the *H. pylori* HP0099 gene encodes an MCP for the attractants arginine and sodium bicarbonate. This is the first report in which the substrate specificity of an MCP from *H. pylori* has been identified.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Bacterial strains and plasmids used in this work are listed in Table 1. Frozen stocks of *H. pylori* were incubated in biphasic cultures on *H. pylori* selective supplement (Dent from Oxoid) trypticase soy agar supplemented with 5% horse serum under microaerobic conditions using a Gaspack jar and a Campygen atmosphere generator pack (Oxoid), at 37°C for 24 h. Bacteria were then transferred to plates and incubated for 24–48 h in an incubator with 5.5% CO_2 and 80% humidity [14]. *Escherichia coli* strains were grown in Luria–Bertani (LB) medium supplemented with ampicillin (Amp; 100 μg ml^{-1}) and/or kanamycin sulfate (Kan; 20 μg ml^{-1}), when appropriate. Media were from Difco and Becton-Dickinson.

2.2. Motility assays

Bacterial cells grown microaerobically at 37°C for 2 days on *H. pylori* selective supplement (Dent from Oxoid) trypticase soy agar plates were stabbed with toothpicks into 0.3% agar plates containing trypticase soy broth supplemented with 5% horse serum.

2.3. Chemotaxis assays

Chemotaxis assays were carried out by the method of Mazunder et al. [15]. Cells were scraped from plates, suspended in chemotaxis buffer (10 mM potassium phosphate, pH 7; 3% w/v polyvinylpyrrolidone) to a concentration of 3×10^8 cells ml^{-1}, and 100 μl of the bacterial suspension was drawn into a disposable 200 μl pipet tip. Solutions of compounds to be tested (10 mM, unless otherwise noted) for a chemotactic response (100 μl in chemotaxis buffer) were drawn up through a 25-gauge needle into a 1-ml syringe, and the point of the syringe–needle combination was submerged into the bacterial suspension. After incubation of the test apparatus horizontally for 45 min at 30°C, the syringe was removed from the bacterial suspension, its contents were diluted serially in chemotaxis buffer, and the dilutions were plated onto agar serum plates. The titers of cells that had accumulated in the syringe were measured as colony forming units. Syringes containing chemotaxis buffer alone were included in each experiment as controls. Test compounds were identified as attractants if the relative chemotaxis response (RCR), cal-

Table 1
Bacterial strains and plasmids

Strain/plasmid	Characteristics	Source/ reference
H. pylori		
ATCC700392	strain 26695	Tomb [12]
ATCC43504		
E. coli		
RP437	{*thr*(Am)-1 *leuB*-6 *his*-4 *metF*(Am)-59 *eda*-50 *rpsL*136 [*thi*-1 *ara*-14 *lacY*-1 *mtl*-1 *xyl*-5 *tonA*-31 *tsx*-78]}	Parkinson
RP4372	{*thr*(Am)-1 *leuB*-6 *his*-4 *metF*(Am)-59 *eda*-50 *rpsL*136 [*thi*-1 *ara*-14 *lacY*-1 *mtl*-1 *xyl*-5 *tonA*-31 *tsx*-78]} Δ(*tar-tap*)	Parkinson
RP5700	{*thr*(Am)-1 *leuB*-6 *his*-4 *metF*(Am)-59 *eda*-50 *rpsL*136 [*thi*-1 *ara*-14 *lacY*-1 *mtl*-1 *xyl*-5 *tonA*-31 *tsx*-78]} Δ(*tsr*)	Parkinson
RP2361	{*thr*(Am)-1 *leuB*-6 *his*-4 *metF*(Am)-59 *eda*-50 *rpsL*136 [*thi*-1 *ara*-14 *lacY*-1 *mtl*-1 *xyl*-5 *tonA*-31 *tsx*-78]} Δ(*tar*)	Parkinson
RP4372/pHA99	RP4372 transformed with pGP1-2 and pHA99	This work
RP 4372/pHA699	RP4372 transformed with pGP1-2 and pHA699	This work
RP 5700/pHA99	RP5700 transformed with pGP1-2 and pHA99	This work
RP 2361/pHA99	RP2361 transformed with pGP1-2 and pHA99	This work
Plasmids		
pUC19	Amp^R	Gibco BRL
pET21a	Amp^R T7lac f1-*ori* His-Tag	Novagen
pGP1-2	Kan^R T7 RNApol P_{lac} *cI*-857	Tabor [20]
pHP99	HP0099 ORF from ATCC43504 ligated to pUC19	This work
pHP9915	HP0099 ORF from ATCC700392 ligated to pUC19	This work
pHA699	HP0099 ORF from ATCC43504 ligated to pET21a	This work
pHA99	HP0099 ORF from ATCC700392 ligated to pET21a	This work

culated as the ratio of the bacteria that entered the test needle–syringes to those in the control needle–syringes, was ≥ 2 [15–17]. Test compounds with an RCR ≤ 0.5 were identified as repellents. Results are expressed as the means of at least five independent experiments involving duplicate test and control assays. Data were considered to be statistical significant if the differences between control and test titers had P values of < 0.05, as determined by the Student's t-test.

2.4. DNA manipulations and genetic techniques

Primers (CGATTGGACGTCTTTTTAATCC and CCCGCAAAAGCTTCTTTAGC) were designed to amplify HP0099, one of the three genes predicted to encode MCPs, using the genome sequence of *H. pylori* strain 26695 [12] as our starting sequence. Conditions used for PCR were those of the Expand™ Long Template PCR system (Boehringer Mannheim), with chromosomal DNA prepared from *H. pylori* strains 43504 and 26695 as templates [18]. PCR products were subcloned into pUC19 to make plasmids pHP99 from *H. pylori* 43504 and pHP9915 from *H. pylori* 26695 (Table 1). Additional primers (CCCCATATGTTGTCTAAAGGTTTGAGTATCGG and CCCCTCGAGTAATCGCCCTCGAGCAATTC) were used to amplify these inserts, the products were cleaved with *Nde*I and *Xho*I, then ligated to plasmid pET21a (Novagen), to make plasmids pHA99 and pHA699, respectively. Inserts were sequenced by the method of Sanger et al. [19], to confirm that PCR amplification had not introduced additional mutations in the HP0099 sequences.

2.5. Expression of HP0099 in E. coli

To construct derivatives of *E. coli* expressing HP0099, *E. coli* was first electroporated with pGP1-2 [20], then with plasmid subclones of the gene in vector pET21a (Table 1). To express HP0099, electroporants were grown to exponential density in LB Amp Kan medium at 30°C, shifted to 42°C for 30 min to induce the expression of T7 RNA polymerase, and incubated at 37°C for 30 min. Cells were collected by centrifugation, resuspended in chemotaxis buffer and sonicated, and intracellular proteins were analyzed by polyacrylamide gel electrophoresis [21]. Cells were also assayed for their chemotactic responses to a variety of substrates before and after heat induction, using the method of Mazunder et al. [15].

3. Results

3.1. Strains of H. pylori show different chemotactic responses to arginine, bicarbonate, and urea

To characterize the chemotactic responses of *H. pylori*, we first determined whether the two type strains of *H. pylori* with which we work, 26695 and 43504, are motile. Both strains spread extensively when stabbed into soft agar plates, behavior characteristic of flagellated bacteria with chemotactic responses.

Second, we designed a quantitative assay to measure the chemotactic responses of these strains. In this assay, chemotaxis is measured as the ability of resuspended cells to swim up a needle and into a syringe carrying a solution of attractant. We tested several potential attractants, including urea and sodium bicarbonate, which the bacteria produce to neutralize the acidic microenvironment, Ni^{2+} ion, a urease cofactor, and the amino acids required for the growth of *H. pylori*. As shown in Fig. 1, *H. pylori* strains 26695 and 43504 both respond to aspartate and serine as attractants, and to urea and Ni^{2+} as repellents. Neither strain responds to alanine, cysteine, glutamate, histidine, leucine, methionine, or tryptophan, additional auxotro-

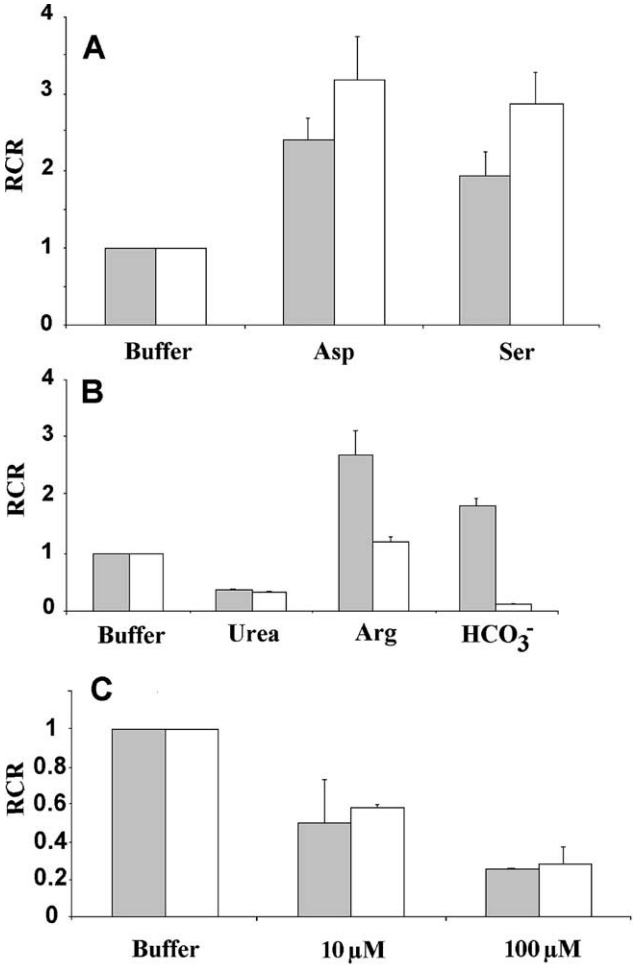

Fig. 1. *H. pylori* responds chemotactically to aspartate, serine, arginine, bicarbonate and Ni^{2+}. Open bars correspond to *H. pylori* 43504 and filled bars to *H. pylori* 26695. The chemotactic response is expressed as RCR, as described in Section 2. Each bar represents the average data from five or more independent experiments. A: Both strains recognize aspartate and serine as attractants. B: Whereas strain 26695 recognizes arginine and bicarbonate as attractants, strain 43504 does not. C: Both strains recognize nickel chloride as a repellent.

phic requirements of *H. pylori* (not shown). These two strains differ in their responses to arginine and bicarbonate. Strain 26695 responds to both as attractants, whereas strain 43504 shows no significant response to arginine, and may recognize bicarbonate as a repellent.

To understand the differences in the chemotactic responses to arginine and bicarbonate between these two *H. pylori* strains, we explored the possibility that a genetic difference between their MCP-encoding genes might account for these differences.

3.2. *The MCP-encoding gene, HP0099, is interrupted by an insertion sequence in* H. pylori *strain 43504, but not in strain 26695*

To characterize the MCP-encoding genes in *H. pylori* strains 26695 and 43504, we amplified, subcloned and sequenced the HP0099 gene from each strain, as described in Section 2. As expected, we found that the sequence of the HP0099 gene from *H. pylori* strain 26695 is identical to that in its complete genome sequence. In contrast, when we compared the sequence of HP0099 from 43504 with that from 26695, we found a surprising difference. The strain 43504 HP0099 gene has an insertion 371 bp long at nucleotide bp 318 within this ORF, with respect to the 26695 sequence. This insertion is that of a mini-IS*605* element, a small IS specific to *H. pylori*, and is flanked by 49-bp and 41-bp ends. The left end of the mini-IS*605* element in HP0099 is identical to that of full-length IS*605* (GenBank AC000108) [22], and the right end shares 83% identity with that of a mini-IS*605* element reported by Censini et al. [23] (Fig. 2). BLAST analysis of this mini-IS*605* element shows that it shares significant identity with internal sequences of *rdxA* (100%), encoding an NAD(P)H nitroreductase that catalyzes the conversion of metronidazole from a prodrug to an antibiotic; *vacA* (94%), encoding a vacuolating cytotoxin; and *cagI* (93%), encoding a pathogenicity determinant (Fig. 2).

Together, these results argue in support of the hypothesis that HP0099 encodes an MCP that recognizes the attractants arginine and bicarbonate, and the failure of strain 43504 to respond to these attractants is due to the inactivation of the HP0099 gene by a mini-IS*605* insertion.

3.3. *Expression of* H. pylori *gene HP0099 in* E. coli *confers the ability to respond to arginine, sodium bicarbonate, and urea as chemoattractants*

E. coli produces two major MCPs, Tar, which responds to aspartic acid as an attractant and Ni^{2+} as a repellent, and Tsr, which recognizes serine as an attractant [24]. *E. coli* does not respond to arginine or bicarbonate as attractants. To test the hypothesis that the *H. pylori* HP0099 gene encodes an MCP that responds to arginine

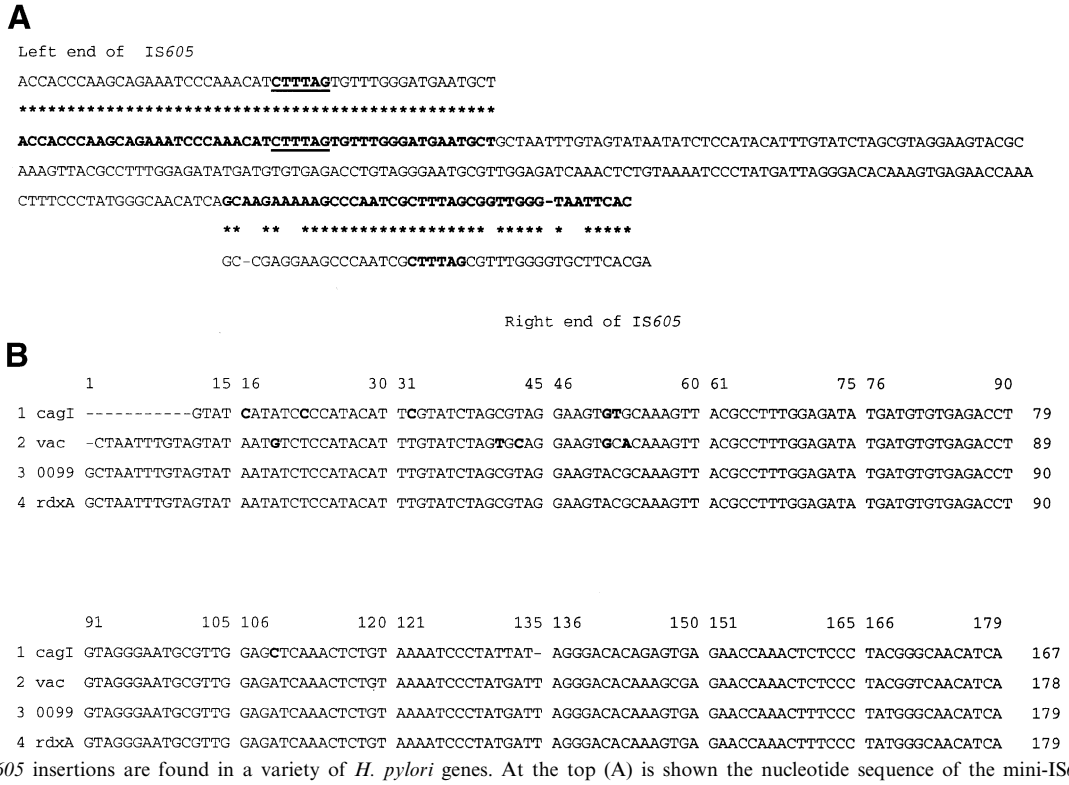

Fig. 2. Mini-IS*605* insertions are found in a variety of *H. pylori* genes. At the top (A) is shown the nucleotide sequence of the mini-IS*605* insertion in ORF HP0099 of *H. pylori* strain 43504 aligned with the sequences of the left and right ends of the full-length IS*605* element. Asterisks indicate identity between the sequences of full-length IS*605* and mini-IS*605* from HP0099 of *H. pylori* 43504. B: Alignment of the internal sequence of the mini-IS*605* element in HP0099 with those found in the *cagI*, *vacA* and *rdxA* genes; differences between these latter sequences and the element in HP0099 are indicated in boldface.

and bicarbonate as attractants, we expressed this gene in mutant derivatives of *E. coli* missing one or more MCPs, and tested whether these recombinant strains have acquired the ability to recognize arginine and bicarbonate as attractants. Because we have shown that *H. pylori* also responds to aspartic acid and serine as attractants (Fig. 1), we also tested the responses of the recombinant strains to these compounds.

The *E. coli* strains we used in this experiment are the otherwise isogenic derivatives of parent strain RP4372 (Δ*tar-tap*), RP5700 (Δ*tsr*) and RP2361 (Δ*tar*) (Table 1). Each strain was transformed with plasmid pGP1-2, which expresses T7 RNA polymerase from the inducible phage λ P_L promoter. Plasmid pGP1-2 also carries the gene encoding a temperature-sensitive λ repressor (*cI*-857) expressed constitutively from the *lac* promoter. In this strain, λ repressor inhibits transcription from the P_L promoter at low temperature (30°C). At high temperature (42°C), λ repressor is inactivated, resulting in the expression of T7 RNA polymerase, which can drive the expression of a target gene from the T7 early promoter present on a second plasmid.

Plasmids pHA99 and pHA699 carry the HP0099 genes from strains 26695 and 43504, respectively, and place expression of these genes under the control of the phage T7 early promoter. These plasmids were introduced into derivatives of *E. coli* RP437 with plasmid pGP1-2 by electroporation. After heat induction, strains with plasmid pHA99 (with an intact HP0099 gene), but not those with pHA699 (with HP0099 interrupted by a mini-IS*605* insertion), were found to express a new protein with an apparent molecular mass in agreement with that predicted from the HP0099 sequence (results not shown).

To assay the chemotactic responses of these recombinant strains, we compared samples of cells grown at 30°C with those prepared after heat induction, using the capillary assay [15]. All the *E. coli* strains carrying plasmid pHA99 displayed chemotaxis to arginine, bicarbonate, and urea after heat induction (Fig. 3). In contrast, *E. coli* RP4372 carrying plasmid pHA699 did not show chemotaxis to arginine, bicarbonate, or urea. *E. coli* strains with deletions of *tar* or *tsr* were not complemented by plasmid pHA99 for chemotaxis toward aspartic acid or serine as attractants (Fig. 3). Because the expression of *H. pylori*

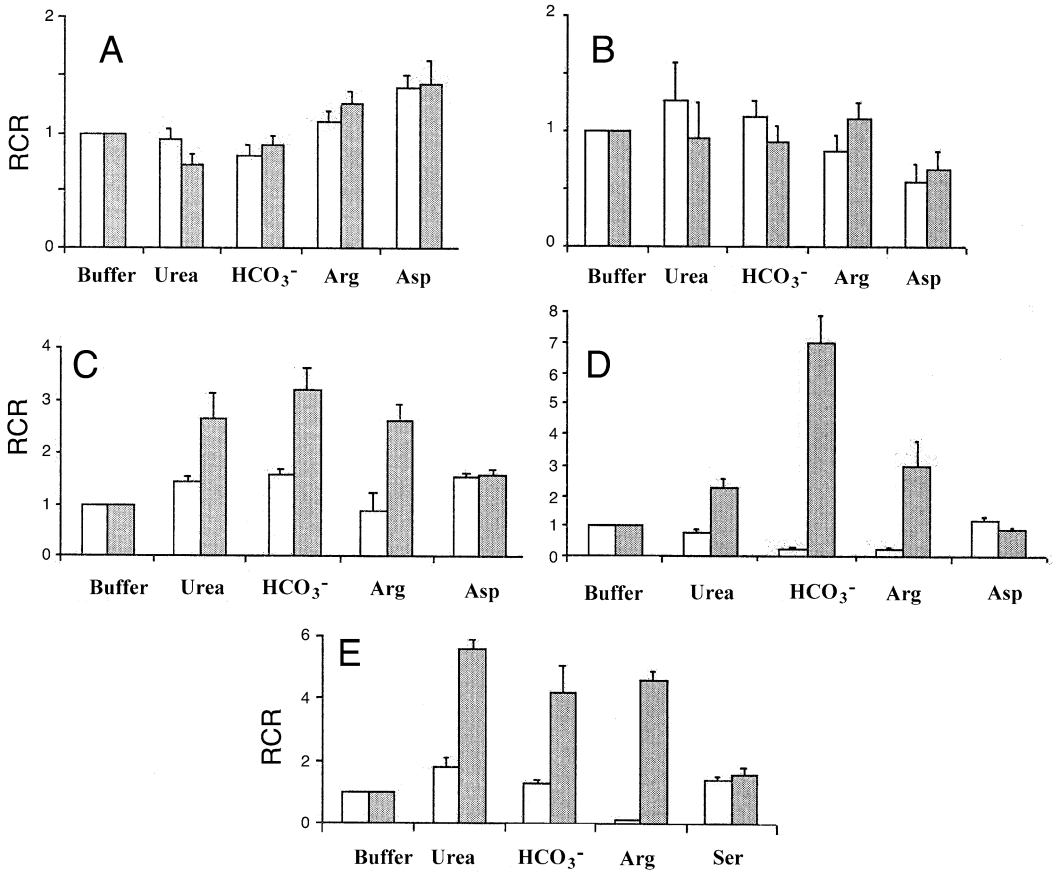

Fig. 3. Expression of *H. pylori* HP0099 in *E. coli* results in a gain of function, chemotaxis toward arginine, bicarbonate, and urea. The figure shows the RCR of strains of *E. coli* carrying plasmid pGP1-2 and a second plasmid with or without an active HP0099 gene to aspartate, serine, arginine, urea, and sodium bicarbonate. Open bars correspond to cells grown at 30°C, and filled bars to cells following heat induction. A: Control strain RP4372 (Δ*tar-tap*) with pGP1-2 and pET21a plasmids. B: Strain RP4372 (Δ*tar-tap*) with pGP1-2 and pHA699. C: Strain RP4372 (Δ*tar-tap*) with pGP1-2 and pHA99. D: Strain RP2361 Δ(*tar*) with pGP1-2 and pHA99. E: Strain RP5700 Δ(*tsr*) with pGP1-2 and pHA99.

HP0099 in *E. coli* results in a gain of function, chemotaxis toward arginine, sodium bicarbonate, and urea, we conclude that HP0099 encodes an MCP that recognizes arginine and bicarbonate as attractants in *H. pylori*.

4. Discussion

In this report, we have shown that *H. pylori* strain 26695 displays chemotaxis toward the attractants aspartic acid, serine, arginine, and sodium bicarbonate, and away from the repellents Ni^{2+} and urea. The complete genome sequence of this strain includes three ORFs predicted to encode MCPs, HP0082, HP0099, and HP0103. We have amplified, cloned, and expressed one of these three ORFs in *E. coli*, and have shown that the expression of HP0099 from a plasmid in *E. coli* results in a gain of function, the ability to respond to arginine, bicarbonate, and urea as attractants. In contrast, the expression of a mutant HP0099 gene with a mini-IS*605* insertion from an otherwise isogenic plasmid in *E. coli* does not result in this gain of function. These results show that HP0099 encodes an MCP for these substrates.

Expression of HP0099 in mutants of *E. coli* defective in MCPs necessary for its responses to aspartate and serine does not confer the ability to recognize these chemicals as chemoattractants. This result suggests that these amino acids are not substrates of HP0099, but rather are substrates of HP0082 and/or HP0103 in *H. pylori*. Consistent with this idea, we have found that *H. pylori* strain 43504, which carries an insertion of a mini-IS*605* element in its HP0099 gene, does not respond to arginine or bicarbonate as attractants, but retains chemotactic responses to aspartate and serine.

Mizote et al. [25] have shown that *H. pylori* strain CPY3401 has the ability to swim toward urea, sodium bicarbonate, and sodium ions. Under the conditions of our assay, we could not detect the significant chemotaxis of strain 26695, nor of two other clinical isolates of *H. pylori*, toward urea as an attractant. Rather, for strain 26695, urea appears to act as a repellent. Conversely, the overexpression of HP0099 from strain 26695 in *E. coli* results in a gain of function, chemotaxis toward urea, in this heterologous host. Taken together, these results show that HP0099 encodes an MCP that also recognizes urea. Whether an individual isolate of *H. pylori* recognizes urea as an attractant or repellent likely depends on the interactions of the HP0099 MCP with the ensemble of Che proteins and/or the presence of other MCPs, which appear to be functionally polymorphic among different *H. pylori* isolates.

Sequence comparisons among *H. pylori* isolates have shown them to be highly polymorphic [26]. We have found an additional factor that contributes to this extensive, intraspecific genetic variability, a family of mini-insertion sequences related to transposon IS*605*. These mini-IS*605* elements retain the unique ends of the larger IS*605* elements, but appear to have lost the functions required in *trans* for transposition. They are found in a variety of genes involved in the interaction of *H. pylori* with its human host, including *rdxA*, *vacA*, *cagI*, and HP0099.

Recently, Andermann et al. [7] have shown that mutant strains of *H. pylori* with inactive HP0099 or HP0082 genes can colonize mice as well as the wild-type strain, but are defective in competition with the wild-type strain upon coinfection of mice. They concluded that the MCPs encoded by these genes likely assist in the colonization of the stomach.

It is not surprising that chemotaxis toward bicarbonate and arginine might be important in the process of stomach colonization by *H. pylori*. Bicarbonate is secreted into the gastric mucosa by chloride–bicarbonate exchangers localized in parietal cells and Na^{+}–H^{+} exchangers distributed in mucous neck, chief, and surface mucous cells [27]. A chemotactic response to bicarbonate may contribute to the persistence of *H. pylori* in the gastric epithelia and in the gastric mucous layer, by facilitating its evasion of the acidic periphery of the mucous layer and movement towards the epithelial cell surface. A chemotactic response to arginine may play two important roles in survival and colonization. First, *H. pylori* does not synthesize L-arginine, and must obtain this amino acid from extracellular sources. Second, the chemotaxis of *H. pylori* toward arginine may help maximize substrate turnover by its arginase. *H. pylori* arginase inhibits nitric oxide (NO) production by activated macrophages at physiological concentrations of L-arginine, the common substrate for NO synthase and arginase. Mutations that inactivate the gene encoding the constitutively expressed arginase in *H. pylori* permit the high-level production of NO by macrophages, and stimulate the NO-dependent killing of *H. pylori* by macrophages [28]. Arginase also catalyzes the first step in the conversion of arginine to urea, the substrate of urease [29], a critical colonization factor.

Acknowledgements

We thank Dr. John S. Parkinson for kindly providing *E. coli* strains. We also thank Dr. Philip Youderian, for helpful comments during the preparation of the manuscript. This work was supported by Grant 1980721 from FONDECYT.

References

[1] Manson, M.D., Armitage, J.P., Hoch, J.A. and Macnab, R.M. (1998) Bacterial locomotion and signal transduction. J. Bacteriol. 180, 1009–1022.

[2] Dunn, B.E., Cohen, H. and Blaser, M.J. (1997) *Helicobacter pylori*. Clin. Microbiol. Rev. 10, 720–741.

[3] Warren, J.R. and Marshall, B. (1983) Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet i, 1273–1275.
[4] Parsonnet, J., Hasnen, S., Rodriguez, L., Gelb, A.B., Warnke, R.A., Jellum, E., Orentreich, N., Vogelman, J.H. and Friedman, G.D. (1994) *Helicobacter pylori* infection and gastric lymphoma. N. Engl. J. Med. 330, 1267–1271.
[5] Mobley, H.L.T., Island, M.D. and Hausinger, R.P. (1995) Molecular biology of microbial ureases. Microbiol. Rev. 59, 451–480.
[6] Eaton, K.A., Suerbaum, S., Josenhans, C. and Krakowka, S. (1996) Colonization of gnotobiotic piglets by *Helicobacter pylori* deficient in two flagellin genes. Infect. Immun. 64, 2445–2448.
[7] Andermann, T.M., Chen, Y.-T. and Ottemann, K.M. (2002) Two predicted chemoreceptors of Helicobacter pylori promote stomach infection. Infect. Immun. 70, 5877–5881.
[8] Phadnis, S.H., Parlow, M.H., Levy, M., Ilver, D., Caulkins, C.M., Connors, J.B. and Dunn, B.E. (1996) Surface localization of *Helicobacter pylori* urease and a heat shock protein homolog requires bacterial autolysis. Infect. Immun. 64, 905–912.
[9] Josenhans, C., Labigne, A. and Suerbaum, S. (1995) Comparative ultrastructure and functional studies of *Helicobacter pylori* and *Helicobacter mustelae* flagellin mutants: both flagellin subunits, FlaA and FlaB, are necessary for full motility in *Helicobacter* species. J. Bacteriol. 177, 3010–3020.
[10] Kirschner, D.E. and Blaser, M.J. (1995) The dynamics of *Helicobacter pylori* infection of the human stomach. J. Theor. Biol. 176, 281–290.
[11] Messier, B. and Leblond, C.P. (1960) Cell proliferation and migration as revealed by radioautography after injection of thymidine-H3 into male rats and mice. Am. J. Anat. 106, 247–265.
[12] Tomb, J.F., White, O., Kerlavage, A.R., Clayton, R.A., Sutton, G.G., Fleischmann, R.D., Ketchum, K.A., Klenk, H.P., Gill, S., Dougherty, B.A., Nelson, K., Quackenbush, J., Zhou, L., Kirkness, E.F., Peterson, S., Loftus, B., Richardson, D., Dodson, R., Khalak, H.G., Glodek, A., McKenney, K., Fitzegerald, L.M., Lee, N., Adams, M.D., Hickey, E.K., Berg, D.E., Gocayne, J.D., Utterback, T.R., Peterson, J.D., Kelley, J.M., Cotton, M.D., Weidman, J.M., Fujii, C., Bowman, C., Watthey, L., Wallin, E., Hayes, W.S., Borodovsky, M., Karp, P.D., Smith, H.O., Fraser, C.M. and Venter, J.C. (1997) The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature 388, 539–547.
[13] Alm, R.A., Ling, L.S., Moir, D.T., King, B.L., Brown, E.D., Doig, P.C., Smith, D.R., Noonan, B., Guild, B.C., de Jonge, B.L., Carmel, G., Tummino, P.J., Caruso, A., Uria-Nickelsen, M., Mills, D.M., Ives, C., Gibson, R., Merberg, D., Mills, S.D., Jiang, Q., Taylor, D.E., Vovis, G.F. and Trust, T.J. (1999) Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen Helicobacter pylori. Nature 397, 176–180.
[14] Toledo, H., Valenzuela, M., Rivas, A. and Jerez, C.A. (2002) Acid stress response in *Helicobacter pylori*. FEMS Microbiol. Lett. 213, 67–72.
[15] Mazunder, R., Phelps, T.J., Krieg, N.R. and Benoit, R.E. (1999) Determining chemotactic responses by two subsurface microaerophiles using a simplified capillary assay method. J. Microbiol. Methods 37, 255–263.
[16] Adler, J. (1973) A method for measuring chemotaxis and use the method to determine optimum conditions for chemotaxis by *Escherichia coli*. J. Gen. Microbiol. 74, 77–91.
[17] Moulton, R.C. and Montie, T.C. (1979) Chemotaxis by *Pseudomonas aeruginosa*. J. Bacteriol. 137, 274–280.
[18] Owen, R.J. and Bickley, J. (1997) Isolation of *H. pylori* genomic DNA and restriction analysis. In: Methods in Molecular Medicine: *Helicobacter pylori* Protocols (Clayton, C.L. and Mobley, H.L.T., Eds.), pp. 81–88. Humana Press Inc., Totowa, NJ.
[19] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[20] Tabor, S. and Richarson, C.C. (1987) DNA sequence analysis with a modified bacteriophage T7 DNA polymerase. Proc. Natl. Acad. Sci. USA 84, 4767–4771.
[21] Delgado, M., Toledo, H. and Jerez, C.A. (1998) Molecular cloning, sequencing, and expression of a chemoreceptor gene from *Leptospirillum ferrooxidans*. Appl. Environ. Microbiol. 64, 2380–2385.
[22] Akopyants, N.S., Clifton, S.W., Kersulyte, D., Crabtree, J.E., Youree, B.E., Reece, C.A., Bukanov, N.O., Drazek, E.S., Roe, B.A. and Berg, D.E. (1998) Analysis of the *cag* pathogenicity island of *Helicobacter pylori*. Mol. Microbiol. 28, 37–53.
[23] Censini, S., Lange, C., Xiang, Z., Crabtree, J.E., Ghiara, P., Borodovsky, M., Rappuoli, R. and Covacci, A. (1996) *cag*, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. Proc. Natl. Acad. Sci. USA 93, 14648–14653.
[24] Dahl, M., Boos, W. and Manson, M. (1989) Evolution of chemotactic-signal transducers in enteric bacteria. J. Bacteriol. 171, 2361–2371.
[25] Mizote, T., Yoshiyama, H. and Nakazawa, T. (1997) Urease-independent chemotaxis responses of *Helicobacter pylori* to urea, urease inhibitor, and sodium bicarbonate. Infect. Immun. 65, 1519–1521.
[26] Akopyanz, N., Bukanov, N.O., Westblom, T.U., Kresovich, S. and Berg, D.E. (1992) DNA diversity among clinical isolates of *Helicobacter pylori* detected by PCR-based RAPD fingerprinting. Nucleic Acids Res. 20, 5137–5142.
[27] Stuart-Tilley, A., Sardet, C., Pouyssegur, J., Schwartz, M.A., Brown, D. and Alper, S.L. (1994) Immunolocalization of anion exchanger AE2 and cation exchanger NHE-1 in distinct adjacent cells of gastric mucosa. Am. J. Physiol. 266, C559–C568.
[28] Gobert, A.P., McGee, D.J., Akhtar, M., Mendz, G.L., Newton, J.C., Cheng, Y., Mobley, H.L.T. and Wilson, K.T. (2001) *Helicobacter pylori* arginase inhibits nitric oxide production by eukaryotic cells: A strategy for bacterial survival. Proc. Natl. Acad. Sci. USA 98, 13844–13849.
[29] Mendz, G.L. and Hazell, S.L. (1996) The urea cycle of *Helicobacter pylori*. Microbiology 142, 2959–2967.