Differential regulation of urease activity in *Helicobacter hepaticus* and *Helicobacter pylori*

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Helicobacter hepaticus is a pathogen of rodents, which causes diverse enteric and hepatic inflammatory diseases and malignancies. The urease enzyme is an important colonization factor of gastric *Helicobacter* species like *Helicobacter pylori*, but little is known about the role and regulation of urease in enterohepatic *Helicobacter* species. Here it is reported that urease activity of *H. hepaticus* does not contribute to acid resistance, and that it is nickel-responsive at the post-translational level. *H. hepaticus* strain ATCC 51449 did not grow or survive at pH 3.0, and supplementation with urea or NiCl₂ did not abrogate this acid sensitivity. Furthermore, urease enzyme activity of *H. hepaticus* was acid-independent, which contrasts with the acid-induced urease system of *H. pylori*. Nickel supplementation of Brucella medium resulted in a tenfold increase in urease activity in both *H. hepaticus* and *H. pylori*, but the maximum level of urease activity in *H. hepaticus* was still three- to fivefold lower when compared to *H. pylori* in the same conditions. The increase in urease activity of *H. hepaticus* was not associated with elevation of urease mRNA or protein levels. Inhibition of protein synthesis by chloramphenicol did not affect nickel-responsive induction of urease activity in *H. hepaticus*, and confirmed that nickel induction occurs at the post-translational level, probably by activation of preformed apo-enzyme. In conclusion, both the role of the urease enzyme and the regulation of urease activity differ between the enterohepatic pathogen *H. hepaticus* and the gastric pathogen *H. pylori*.

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INTRODUCTION

Helicobacter hepaticus is a pathogen of rodents, and colonization with *H. hepaticus* may result in enteric and hepatic inflammatory diseases and malignancies (Fox *et al.*, 1994; Ward *et al.*, 1994). *H. hepaticus* has recently also been implicated as participating in the formation of cholesterol gallstones (Maurer *et al.*, 2005). Members of the genus *Helicobacter* are Gram-negative, microaerophilic bacteria. While the genus is best known for the human gastric pathogen *Helicobacter pylori*, a large number of other species are recognized (Solnick & Schauer, 2001). Based on their target organ, *Helicobacter* species are subdivided into gastric and enterohepatic lineages (Solnick & Schauer, 2001). Both enterohepatic and gastric *Helicobacter* species can colonize humans and a wide variety of animals, resulting in chronic infections and chronic inflammation. With gastric *Helicobacter* species, this can progress to gastric ulceration, atrophy, metaplasia and malignancies (Blaser & Atherton, 2004). Enterohepatic *Helicobacter* species like *H. hepaticus* have been less thoroughly studied, but depending upon the species are associated with inflammatory and proliferative bowel lesions, hepatitis, and in some cases, hepatic cancer

(Franklin *et al.*, 1998, 1999; Ward *et al.*, 1994). There is increasing evidence linking infection with *Helicobacter* species to liver cancer in humans (Nilsson *et al.*, 1999; Queiroz & Santos, 2001; Verhoef *et al.*, 2003), and *H. hepaticus* infection of mice may prove to be an excellent model for the study of hepatic carcinogenesis (Suerbaum *et al.*, 2003).

H. hepaticus expresses a nickel co-factored urease enzyme (Beckwith *et al.*, 2001). Urease is a multimeric, nickel-containing enzyme produced by many pathogenic and non-pathogenic bacteria (Burne & Chen, 2000). In *Helicobacter* species, the urease enzyme consists of UreA and UreB subunits (Beckwith *et al.*, 2001). These are encoded by the *ureA* and *ureB* genes and are located in an operon, also containing the *ureI* gene (encoding a urea channel), and the *ureEFGH* genes encoding accessory proteins involved in enzyme activation (Beckwith *et al.*, 2001; Burne & Chen, 2000; Skouloubris *et al.*, 1998). Active urease converts urea into ammonia and bicarbonate; the ammonia produced is thought to mediate protection against acidic microenvironments, but may also serve as a nitrogen source (Williams *et al.*, 1996).

In gastric *Helicobacter* species like *H. pylori* and *Helicobacter mustelae*, urease is an important virulence factor (Andrutis *et al.*, 1995; Tsuda *et al.*, 1994). The ammonia produced is thought to allow protection against acidic environments, and mutants lacking urease are unable to colonize the gastric environments in different animal models (Andrutis *et al.*, 1995; Tsuda *et al.*, 1994). In *H. pylori*, urease expression and enzyme activity are controlled by an intricate regulatory network, centred on the environmental pH and the availability of the cofactor nickel (Scott *et al.*, 2002; van Vliet *et al.*, 2004b). For *H. hepaticus* and other urease-positive enterohepatic *Helicobacter* species, the role of urease in metabolism or pathogenesis has not yet been established.

Recently, differences between the urease and nickel-transport genes of *H. hepaticus* and *H. pylori* were reported (Beckwith *et al.*, 2001; Suerbaum *et al.*, 2003), which suggested that there may be differences in regulation of urease expression and activity between these two *Helicobacter* species. Here it is demonstrated that urease activity in *H. hepaticus* is nickel-responsive, but solely at the post-translational level. In contrast to *H. pylori*, *H. hepaticus* urease activity is not induced at acidic pH, and *H. hepaticus* does not grow or survive at pH 3.0. This suggests that the urease system is not a universal acid-resistance factor of ureolytic *Helicobacter* species, and that its functions may differ between gastric and enterohepatic *Helicobacter* species.

METHODS

Bacterial strains, plasmids and growth conditions. *H. hepaticus* strain ATCC 51449 (Fox *et al.*, 1994) and its isogenic *ureB* mutant (this study), and *H. pylori* strain 26695 (Tomb *et al.*, 1997), were routinely cultured at 37 °C in microaerobic conditions (10% CO₂, 5% O₂ and 85% N₂) on Dent agar (Biotrading) (van Vliet *et al.*, 2001). Liquid growth was performed in Brucella broth (Difco) supplemented with 0.2% (w/v) β -cyclodextrins (Fluka) (BBC). Bacteria were inoculated at a starting OD₆₀₀ of 0.05. The pH of liquid media was adjusted from 7.0 to 6.0, 5.5 or 3.0 using HCl and did not change by more than 0.5 pH unit during the experiments. NiCl₂ was purchased from Sigma, filter-sterilized, and used at a final concentration of 100 μ M. *Escherichia coli* ER1793 was grown aerobically in Luria-Bertani medium (Sambrook *et al.*, 1989) at 37 °C. When indicated, growth media were supplemented with chloramphenicol to a final concentration of 20 μ g ml⁻¹.

Acid shock survival. *H. hepaticus* was grown for 24 h in BBC at pH 7.0 at a starting OD₆₀₀ of 0.05. Bacteria were then harvested by centrifugation for 10 min at 4000 g and resuspended to a final OD₆₀₀ of 0.2 in PBS adjusted to pH 7.0, 5.5 or 3.0. When indicated, PBS was supplemented with NiCl₂ or urea to a final concentration of 100 μ M and 0.5 mM, respectively. Cells were incubated for 30 min at 37 °C in microaerobic conditions. Subsequently 5 μ l of tenfold dilutions were spotted on Dent agar. Plates were incubated for 2 days at 37 °C in microaerobic conditions and the presence or absence of growth at the spots was assessed by visual inspection (Baillon *et al.*, 1999). Differences in the highest dilution still containing growth between different conditions indicated differences in survival rates.

Urease enzyme assay. Urease activity was determined in freshly sonicated lysates by measuring ammonia production from hydrolysis of urea, as described previously (van Vliet *et al.*, 2001). The

concentration of ammonia in the samples was inferred from a standard NH₄Cl concentration curve. Enzyme activity was expressed as μ mol urea substrate hydrolysed min⁻¹ (mg protein)⁻¹.

RNA analysis. RNA was isolated from *H. hepaticus* using Trizol reagent (Invitrogen), according to the manufacturer's instructions. Gel electrophoresis of RNA, transfer to positively charged nylon membranes (Roche), cross-linking, hybridization to DIG-labelled specific RNA probes and detection of bound probe were performed as described previously (van Vliet *et al.*, 2001). Probes specific for *H. hepaticus ureA* and *ureB* were synthesized by *in vitro* transcription using T7 RNA polymerase (Roche) and PCR products obtained with primers HhUreA-F1 (5'-TGCATTATGCTGGGGCACTA-3') and HhUreA-R1-T7 (5'-ctaatacgactcactataggagaATAGGTCTATCGCCCTTATG-3'), and HhUreB-F1 (5'-TGGTAAAGCGGGAAATCCAG-3') and HhUreB-R1 T7 (5'-ctaatacgactcactataggagaGTGTGCAGGTAGTAGCGTTTG-3'). Lower-case letters indicate the T7 promoter used for *in vitro* transcription.

Construction of an *H. hepaticus ureB* mutant. The *ureB* gene of *H. hepaticus* ATCC 51449 was amplified using primers HhUreB-mutF1 (5'-TCCTGTGCCTCCACCAAT-3') and HhUreB-mutR1 (5'-GCATTATGCTGGGGCACT-3'), and cloned in pGEM-T_{easy} (Promega), resulting in plasmid pJS10. The *ureB* gene was subsequently interrupted by insertion of the chloramphenicol-resistance gene from pAV35 (van Vliet *et al.*, 1998) in the unique *BsmI* site, resulting in plasmid pJS11. This plasmid was introduced into *E. coli* ER1793 and subsequently used for natural transformation of *H. hepaticus* ATCC 51449. Chloramphenicol-resistant colonies isolated were designated 51449*ureB*. Two colonies derived from independent transformations were tested, and both colonies gave identical results in all experiments. Correct allelic replacement of the wild-type *ureB* gene with the interrupted version was confirmed by PCR.

Protein analysis. *H. hepaticus* wild-type and *ureB* mutant cells were grown for 24 h in unsupplemented BBC medium or BBC medium supplemented with 100 μ M NiCl₂, centrifuged at 4000 g for 10 min at room temperature and resuspended in PBS pH 7.4 to a final OD₆₀₀ of 10. The cells were subsequently lysed by sonication for 15 s on ice, using an MSE Soniprep 150 at amplitude 6. The protein concentration of lysates was determined using the bicinchoninic acid method (Pierce) using bovine serum albumin as standard. Proteins were separated by SDS-PAGE on a 10% (w/v) polyacrylamide gel and stained with Coomassie brilliant blue. Western blotting was performed by electrotransfer of proteins onto nitrocellulose membrane (Roche). The blot was probed with antibodies raised in rabbits to *Helicobacter felis* UreA or UreB (Intervet International). Bound antibodies were visualized with swine anti-rabbit antibodies labelled with alkaline phosphatase (Promega), using 5-bromo-4-chloro-3-indolyl phosphate/nitro-blue tetrazolium (BCIP/ NBT) (Promega) as substrate.

RESULTS

H. hepaticus is acid-sensitive and does not grow at acidic pH

In gastric *Helicobacter* species, urease activity is required for growth in acidic conditions and survival of acid shock. To determine whether *H. hepaticus* is able to grow under acidic conditions, cells were resuspended in BBC of pH 7.0, 5.5 and 3.0 in the presence or absence of 100 μ M NiCl₂ (Fig. 1). There was no increase in OD₆₀₀ at pH 3.0, indicating absence of growth (Fig. 1). *H. hepaticus* was able to grow at pH 5.5, although the final OD₆₀₀ values were slightly lower

Fig. 1. *H. hepaticus* is acid-sensitive and does not grow at pH 3.0. *H. hepaticus* ATCC 51449 was grown for 24 h in BBC with a pH of 7.0, 5.5 or 3.0 in the absence (white bars) or presence (black bars) of 100 μM NiCl₂. Data points shown are the mean of three independent experiments; error bars denote SD.

than at pH 7.0. Supplementation of media with NiCl₂ did not affect growth at pH 7.0 or 5.5, nor did it allow growth at pH 3.0 (Fig. 1).

To determine whether *H. hepaticus* is able to survive acid shock, cells were resuspended in PBS of pH 7.0, 5.5 and 3.0 in the presence or absence of either 100 μM NiCl₂ or 0.5 mM urea, and incubated for 30 min under micro-aerobic conditions. Viability was not affected by incubation at pH 5.5 (Fig. S1). *H. hepaticus* was not able to survive incubation at pH 3.0, even in the presence of either 100 μM NiCl₂ or 0.5 mM urea (Fig. S1), suggesting that this species is acid-sensitive, and that urease is not involved in its acid resistance.

H. hepaticus urease activity is acid-independent, but nickel-induced

To determine the effect of medium pH on growth and urease activity, *H. hepaticus* was grown for 24 h in BBC medium adjusted to pH 5.5, 6.0 and 7.0. These pH values were selected on the basis of their use in previous studies with *H. pylori*, as pH 5.5 and 6.0 are thought to resemble the

pH of the mucus layer (van Vliet *et al.*, 2004b). pH 5.5 was the lowest pH value allowing growth of *H. hepaticus* (Fig. 1). The pH of the medium did not change significantly during 24 h growth. Urease activity in *H. hepaticus* was not significantly affected by the pH of the medium (Fig. 2a), while urease activity in the positive control *H. pylori* increased at pH 6.0 and pH 5.5 (Fig. 2b), consistent with earlier data (Scott *et al.*, 2002; van Vliet *et al.*, 2004b).

To determine the effect of nickel supplementation of BBC medium on *H. hepaticus* urease activity, cells were grown for 24 h in BBC medium of pH 7.0 supplemented with NiCl₂ to a final concentration of 100 μM. Urease activity of *H. hepaticus* increased tenfold in nickel-supplemented BBC medium when compared to unsupplemented BBC medium [from 1.35 ± 0.56 to 17.07 ± 6.21 μmol urea min⁻¹ (mg protein)⁻¹; mean $\pm$ SD, $n=3$] (Fig. S2a). Likewise, urease activity of *H. pylori* grown with nickel supplementation was also increased tenfold [from 6.04 ± 4.47 to 58.63 ± 16.14 μmol urea min⁻¹ (mg protein)⁻¹; mean $\pm$ SD, $n=3$] (Fig. S2b), as was reported previously (van Vliet *et al.*, 2001). Overall, *H. hepaticus* urease activity was three- to fivefold lower than that of *H. pylori* in comparable conditions.

Nickel-responsive induction of urease activity in *H. hepaticus* is mediated at the post-translational level

To identify at which level the nickel-responsive induction of urease activity in *H. hepaticus* was mediated, the effect of nickel on transcription of *H. hepaticus* urease genes and expression of urease protein was determined (Fig. 3). Nickel supplementation of BBC medium did not affect transcription of either the *ureA* or *ureB* genes (Fig. 3a). Likewise, expression of the UreA and UreB proteins was not altered by nickel supplementation, as was shown for both UreA and UreB by Western immunoblotting using antibodies to *H. felis* UreA and UreB protein (Fig. 3b). The Western blot data were independently confirmed using an isogenic *ureB* mutant of *H. hepaticus* ATCC 51449 (Fig. 3c). This *ureB* mutant showed an absence of UreB expression, and did not have any detectable urease activity; it did not show any

Fig. 2. Urease activity is acid-independent but nickel-induced in *H. hepaticus*. Urease activity of *H. hepaticus* strain ATCC 51449 (a) and *H. pylori* strain 26695 (b) was assessed after 24 h growth in BBC medium with a pH of 7.0, pH 6.0 or pH 5.5. Note the difference in the scale of the y-axis. Each bar represents data from three to five independent experiments for each strain; error bars denote SD. Asterisks indicate a significant increase of urease activity in the test condition (pH 6.0 or 5.5) when compared to the standard growth conditions (pH 7.0) ($P \leq 0.05$, Mann-Whitney U test).

Fig. 3. Nickel-responsive induction of urease activity in *H. hepaticus* is mediated at the post-translational level. (a) Analysis of *ureA* and *ureB* transcription by Northern hybridization. Top panel, staining of total RNA with methylene blue; middle panel, visualization of *ureB* mRNA; bottom panel, visualization of *ureA* mRNA. Relevant marker sizes are indicated on the left. (b) Comparison of UreB (middle panel) and UreA (lower panel) protein expression levels using Western immunoblots with antibodies raised against *H. felis* UreA and UreB protein. The top panel displays the total protein profile of the lysates used for immunoblotting, stained with Coomassie brilliant blue. Relevant marker sizes are indicated on the left. (c) Comparison of UreB protein expression levels using SDS-PAGE followed by Coomassie brilliant blue staining. Total protein of *H. hepaticus* ATCC 51449 wild-type strain and isogenic *ureB* mutant, respectively, grown in unsupplemented BBC medium (lanes 1 and 3) and BBC medium supplemented with 100 $\mu\text{M NiCl}_2$ (lanes 2 and 4). Lanes 3 and 4 are included to facilitate identification of the UreB protein (indicated by an arrow). Relevant marker sizes are indicated on the right. (d) Effect of the protein synthesis inhibitor chloramphenicol on nickel-responsive induction of urease activity in *H. hepaticus* (left panel) and *H. pylori* (right panel). Activity of urease was assessed after 24 h growth in unsupplemented BBC medium (black bars) and BBC medium supplemented with NiCl_2 to a final concentration of 100 μM (white bars). Cells were either grown in the absence of chloramphenicol (-Cm) or in the presence of 20 $\mu\text{g ml}^{-1}$ chloramphenicol (+Cm). Data are from three to five independent experiments; error bars denote SD. Asterisks indicate a statistically significant increase in urease activity in nickel-supplemented versus unsupplemented medium ($P \leq 0.05$, Mann-Whitney U test).

significant changes in growth under the tested conditions (data not shown).

The bacteriostatic antibiotic chloramphenicol, which blocks protein synthesis (Kusters *et al.*, 1997), was used to confirm that the nickel-responsive induction of urease activity in *H. hepaticus* is indeed mediated solely at the post-translational (enzyme activity) level. Supplementation of BBC medium

with chloramphenicol to a final concentration of 20 $\mu\text{g ml}^{-1}$ resulted in cessation of growth of *H. hepaticus*, but did not significantly affect cell viability as determined by colony counts (not shown). Addition of chloramphenicol also did not affect nickel-responsive induction of urease activity of *H. hepaticus* when compared to cultures grown in BBC medium without chloramphenicol (Fig. 3d), demonstrating that this induction does not require protein synthesis. In

contrast, chloramphenicol supplementation almost completely abolished nickel-responsive induction of urease activity in *H. pylori* (Fig. 3d), consistent with the requirement for additional urease protein synthesis in nickel-responsive induction of urease activity (van Vliet *et al.*, 2001).

Nickel-responsive induction of urease activity in *H. hepaticus* is growth-phase-independent

The effect of growth phase on nickel-responsive induction of urease was determined for *H. hepaticus* and *H. pylori* (Fig. 4). Nickel supplementation did not significantly affect the growth of *H. hepaticus* or *H. pylori*, and both species reached late-exponential phase after 24 h (Fig. 4a). *H. hepaticus* already reached maximum urease activity at approximately 12 h of growth in nickel-supplemented medium (Fig. 4b). In contrast, urease activity in *H. pylori* increased steadily in nickel-supplemented cultures, and reached its maximum at approximately 18 h of growth, consistent with the requirement for transcription and translation of extra urease protein for increased urease activity during this period of time (Fig. 4b). This indicates that in *H. hepaticus* urease activity peaks during early

exponential phase, further supporting the hypothesis that nickel induction of urease activity is mediated by enzyme activation rather than increased expression of urease enzyme.

DISCUSSION

The nickel-containing enzyme urease is an important virulence factor of many bacterial pathogens (Burne & Chen, 2000). All gastric *Helicobacter* species known to date are urease-positive, and this urease activity is thought to be essential for their ability to colonize the gastric environment. In contrast, only a third of enterohepatic *Helicobacter* species are urease-positive, but are still unable to colonize the gastric environment (Solnick & Schauer, 2001), with the exception of *Helicobacter muridarum* (Lee *et al.*, 1993). Thus it is likely that not just the presence of urease activity, but also the absolute level of urease activity or its regulation may be required to allow gastric colonization. While the operon encoding the *H. hepaticus* urease enzyme was previously described (Beckwith *et al.*, 2001), to our knowledge the role of urease in *H. hepaticus* and other enterohepatic *Helicobacter* species has not yet been reported.

In this study it has been demonstrated that *H. hepaticus* is acid-sensitive, and that addition of urea does not increase its survival at pH 3.0 (Fig. S1). In contrast, addition of urea does allow gastric *Helicobacter* species like *H. pylori* to survive such acidic conditions. Taken together, these findings suggest that the urease enzyme of *H. hepaticus* is not involved in acid resistance. Consistent with this was the lack of acid-responsive induction of urease activity in *H. hepaticus* (Fig. 2a). However, urease activity in *H. hepaticus* is nickel-responsive, but this regulation is mediated solely at the post-translational level, probably through the activation of preformed apo-enzyme (Figs 3 and 4). During revision of this paper, nickel-responsive induction of *H. hepaticus* urease activity was confirmed in an independent study (Mehta *et al.*, 2005), indicating that the observed nickel induction of *H. hepaticus* urease activity is not due to the conditions employed in our study.

Despite overall similarities of the *H. hepaticus* and *H. pylori* urease gene clusters, there are notable differences. Both urease gene clusters consist of two structural genes (*ureAB*) and five accessory genes (*ureIEFGH*) (Beckwith *et al.*, 2001), but the *ureB*–*ureI* intergenic distance of *H. hepaticus* is much shorter (9 bp) than that of *H. pylori* (200 bp). This indicates that in *H. hepaticus* there is probably no promoter directly upstream of the *ureI* gene, thus limiting the possibilities for transcriptional and post-transcriptional regulation (Akada *et al.*, 2000; van Vliet *et al.*, 2001). Furthermore, the overall amino acid sequence of UreI in both species is well conserved, but several amino acid residues which were shown to be required for acid activation of the *H. pylori* UreI urea channel are absent in the *H. hepaticus* UreI protein (Weeks *et al.*, 2004). This is consistent with the extragastric lifestyle of *H. hepaticus* and its inability to grow or survive at

Fig. 4. Effect of growth phase on nickel-responsive induction of urease activity in *H. hepaticus* and *H. pylori*. (a) Growth curves of *H. hepaticus* and *H. pylori* in unsupplemented and nickel-supplemented BBC medium monitored over 24 h. *H. hepaticus* (triangles) and *H. pylori* (squares) were grown in unsupplemented BBC medium (solid symbols) and in BBC medium with 100 μ M NiCl_2 (open symbols). (b) Urease activity of *H. hepaticus* and *H. pylori* grown in unsupplemented and nickel-supplemented BBC medium monitored over 24 h. *H. hepaticus* (triangles) and *H. pylori* (squares) were grown in unsupplemented BBC medium (solid symbols) and in BBC medium with 100 μ M NiCl_2 (open symbols). Data points shown are the mean of two to eight independent experiments per time point.

pH 3.0 (Figs 1 and S1). Whether this phenotype is due to the lack of acid-responsive regulation of urease activity, absence of a *ureI* promoter or absence of the acid activation of the UreI urea channel remains to be established.

Enzymic degradation of urea by urease results in the production of ammonia and bicarbonate, and both reaction products are thought to play an important part in bacterial pathogenesis. Ammonia serves as nitrogen source and may function in acid resistance (Burne & Chen, 2000; Scott *et al.*, 2002; Williams *et al.*, 1996), whereas bicarbonate may also function in acid resistance as well as in modulating the immune system of the host (Kuwahara *et al.*, 2000; Marcus *et al.*, 2005). Expression of urease in bacteria is controlled by different stimuli, such as urea availability, environmental pH, nitrogen status of the cell, or growth phase (Burne & Chen, 2000).

In this study it has been demonstrated that urease activity in *H. hepaticus* is nickel-responsive. This form of urease regulation has to date only been identified for two other urease systems, of *H. pylori* (van Vliet *et al.*, 2001) and *Streptococcus salivarius* (Chen & Burne, 2003). The enzymic activity of *H. hepaticus* urease is induced by nickel supplementation of the growth medium, but this induction is mediated solely at the post-translational level (Fig. 3), probably by activation of urease apo-enzyme, as was previously described for *S. salivarius* (Chen & Burne, 2003). In contrast, the *H. pylori* urease system is nickel-induced at the enzyme activity and transcriptional level (van Vliet *et al.*, 2001). The nickel- and acid-responsive transcriptional regulation of urease observed in *H. pylori* may be a specific adaptation to the gastric lifestyle. This suggests that the proposed link in *H. pylori* between intracellular nickel availability and environmental pH (van Vliet *et al.*, 2004a) may not be universal in the genus *Helicobacter*.

In *H. pylori*, regulation of urease activity is dependent on nickel and the NikR regulatory protein (van Vliet *et al.*, 2002). Mutants lacking the NikR regulators are unable to colonize the murine gastric mucosa (Bury-Mone *et al.*, 2004). A NikR homologue is also present in *H. hepaticus* (Suerbaum *et al.*, 2003), and it can be envisaged that NikR may indirectly affect urease activity via regulation of nickel uptake. In *H. pylori*, NikR regulates NixA-mediated nickel uptake (Ernst *et al.*, 2005), and while the *H. hepaticus* genome sequence does not contain a *nixA* orthologue (Suerbaum *et al.*, 2003), it does contain a putative nickel-uptake ABC transporter located adjacent to the urease operon (Beckwith *et al.*, 2001). However, up to now the *H. hepaticus* *nikR* gene has proven refractory to insertional mutagenesis (C. Belzer & A. H. M. van Vliet, unpublished results), and thus a role of NikR in regulation of urease expression of *H. hepaticus* cannot be established yet.

In conclusion, *H. hepaticus* is acid-sensitive and lacks regulatory mechanisms mediating acid- and nickel-responsive regulation of urease expression. These characteristics may reflect its extragastric niche, and may be directly linked with

the animal host colonized and/or the respective target organ. We hypothesize that enterohepatic *Helicobacter* species do not require high levels of urease activity in the rodent gut. The high levels of urease activity observed in gastric *Helicobacter* species (Bury-Mone *et al.*, 2003; Scott *et al.*, 2000) are likely to be an adaptation that allows them to thrive in the gastric environment, albeit at a high metabolic cost. Regulation of urease expression and activity will allow them to adapt to changes in acidity observed during fasting or feeding. In contrast, urease-positive enterohepatic *Helicobacter* species might use their urease system for nitrogen metabolism, securing a constant supply of ammonia. The diversity in urease function and regulatory responses in *Helicobacter* species is a prime example of the adaptation required for chronic colonization of host tissues.

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