Survival and Chemotactic Behavior of *H. pylori* at Different Media pH

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Abstract

**Background:** *H. pylori* is a human gastric pathogen. Chemotaxis is an essential factor in colonization of *H. pylori*, but very little is known about its chemotactic responses at different pH conditions, especially in acidic environment of stomach as its natural habitant.

**Methods:** We first determined survival of *H. pylori* under various pH conditions in the presence and absence of urea. Chemotaxis was then assayed in three strains of *H. pylori* by modified Adler’s procedure, in which potassium bicarbonate was used as an attractant for estimating the chemotactic activity which was compared with phosphate buffer as an inert reagent.

**Results:** *H. pylori* cannot withstand pH 1, but in the presence of urea, it survives in solutions with pH values of 2 to 9 and remains viable at pH of 3 to 9, irrespective of presence of urea. Maximum chemotactic activity occurred at pH values of 5.5 to 6.5, whereas no chemotaxis was found in solutions with pH 3. Chemotactic activities are increased at pH 3 to 5.5 and reduced at pH 6 to 9, beyond which no chemotaxis was observed.

**Conclusion:** In view of the fact that chemotaxis is severely affected by media pH, it is concluded that the antrum of the stomach is most suitable for colonization of *H. pylori* for which maximum colonization occurs at neutral pH.


**Keywords** • *H. pylori* • motility • chemotaxis • pH

Introduction

*H. pylori* a gram-negative microaerophilic bacterium, was first isolated in 1983 by Warren and Marshall from human gastric biopsy specimen.¹ It is believed to cause different gastrointestinal diseases including peptic ulcer, gastritis²,³ and gastric lymphoma,⁴ as well as gastric adenocarcinoma.⁵,⁶,⁷ Motility by flagella and chemotactic behavior are essential virulence factors for *H. pylori* and their role in colonization of the stomach has firmly been established.⁸,⁹,¹⁰ Several studies have shown that non-motile mutants of the bacterium are unable to colonize in animal models¹¹,¹²,¹³ Chemotactic activities of *H. pylori* are reported to increase in viscous solution.¹⁴,¹⁵ *H. pylori* live in acidic environment of human stomach to survive at low pH of the gastric mucosa, this bacterium produces high
levels of urease, a hydrolase enzyme, that split urea into ammonia and carbon dioxide. Acid is neutralized with the ammonium ions formed from reaction of ammonia with water. \(^{16,17,5,18}\)

Urease activity was regarded to be toxic to human gastric epithelial cells. \(^{19}\) \(H\) pylori is viable at pH 4 to 8, irrespective of the presence of urea and multiplies only at pH of 6 to 8, with its optimal growth occurring at pH 6. \(^{19,20,21}\) It can even survive for several hours at pH 1 in the presence of urea. \(^{20,22,23}\) It is motile at pH 5 to 8 and its optimum motility is at pH 5. \(^{21}\)

It is found that some chemicals such as urea, and sodium and potassium bicarbonate that are continuously secreted from the gastric epithelial cells of to the lumen serve as potent attractants for \(H\) pylori at neutral pH. \(^{22,24,25}\) Despite these data, little is known about the chemotactic behavior of \(H\) pylori at various pH of the stomach, ranging from highly acidic to nearly neutral pH, which is the ecological niche of this bacterium.

In this study, we investigated the chemotactic responses of three different strains of \(H\) pylori to potassium bicarbonate, a potent attractant, in solutions of phosphate buffer at different pH.

### Materials and Methods

Bacterial strains and growth conditions: Three motile and urease positive strains of \(H\) pylori, \(K_15\), \(K_{22}\), and \(K_{46}\), which were previously isolated from human gastric biopsies of symptomatic patients referred to gastroscopy unit at Kerman University Hospital, were used in all experiments. They were grown in brucella broth (Merck), supplemented with 7% horse serum under microaerobic condition using Anaerocult C (Merck) at 37°C for 3-5 days.

Motility assay. Bacterial strains grown in broth culture were introduced into the motility agar plates containing 0.35 % refined agar (Merck) in brucella broth supplemented with 7 % horse serum and incubated for 3 days at 37°C. Motility of these bacteria was also observed, using phase contrast microscope.

**Bacterial growth under different pH.**

The medium for growing bacteria included an acid-precipitated medium, prepared by supplementing brucella broth with 7% horse serum and adjusted with HCl and KOH to pH of 1 to 9. Precipitated proteins were then removed and urea was added at a concentration of 40mM. Cultures were incubated at 37°C for 24 to 48 h under microaerobic condition. The pH of the medium was adjusted with HCl and KOH. Subcultures were then made from bacterial strains in brucella-serum agar plates and incubated under similar condition. Colonies were counted after 3 days.

**Chemotaxis assay**

Chemotaxis was assayed by a modified method described by Adler (26). In brief, a small chamber was made from a V-shape sealed capillary tube glued on a glass slide, and covered with a cover slip. The chamber was filled with 200µl of washed bacterial cell suspension containing about \(3\times10^8\) cells per ml in 20mM phosphate buffer (pH 7.2). Three 75mm/75µl capillary tubes were first flame-sealed at one end and then filled with the testing mixtures made from potassium bicarbonate (10 mM) dissolved in phosphate buffer at the desired pH (3 to 9). These tubes were inserted gently into the chemotaxis chamber containing the bacterial cell suspension. After 60 min the capillary tubes were removed from the chamber and the sealed end of tubes were gently broken and the contents of each tube were spread over a known area on a glass slide for enumeration of bacteria. A comparison was made between the test and controls.

**Statistical analysis**

Data are presented as mean±SD. The results obtained from duplicate testing each one of the three strains compared with each other. There were no significant differences between the results obtained from three strains. Mean numbers of bacterial cells obtained from chemotaxis assay at different pH conditions were compared by one way analysis of variance (ANOVA) and the differences were considered significant at P<0.05.

**Results**

All three strains of bacteria showed efficient swarming in brucella-serum motility agar and active swimming in phosphate buffer under phase-contrast microscope. There was no viable bacterium in subcultures at pH 1, irrespective of the presence or absence of urea. In the absence of urea, no viable bacterium was found on exposure to pH 2.

In the presence of urea, few colony forming units (CFU) of bacteria were found in the subcultures at pH 2, whereas all bacterial strains survived well when grown in pH media of 3 to 9. Maximum CFU was observed at pH values of 6 to 7.5 (data not shown). In the absence of urea, all three strains of bacteria survived in solutions with pH of 4 to 9.

The chemotactic responses of the strains were determined by comparing the average numbers of bacteria attracted to the test capillary tubes with those entering control tubes.
**Discussion**

Our results showed that *H pylori* grew best at neutral pH, but as described in previous studies it can tolerate acidic pH as low as 2.0. It is postulated that *H pylori* can survive at even much lower and higher pHs than have previously been described. The growth of *H pylori* at very low pH has also been reported in other studies.

*H pylori* is one of few bacteria capable of surviving in highly acidic environment of the stomach. In order to access localized nutrients; it is believed that, the bacterium must move very quickly to more neutral regions of the mucus layer of stomach or nutrient sources. Random motility is not sufficient for this requirement, for which *H pylori* probably uses chemotactic motility.

It is believed that the environmental conditions, such as pH, plays an important role in the regulation of such chemotactic motility, a process studied extensively in many flagellated bacteria. Also several studies have found that chemotaxis mutants of *H pylori* were unable to or weakly infect animal models. To understand how chemotactic response is affected by pH, we used potassium bicarbonate as a potent attractant for *H pylori* and observed positive chemotaxis at different pH values.

Chemotactic activities of urease negative *H pylori* were lower than those of urease positive strains. Other studies have found that urease mutants of *H pylori* did not colonize the stomach of piglets, indicating that the bacterium used urease for neutralization of the acid in the stomach. It is speculated that the disability of urease negative *H pylori* in colonization is due to the fact that low gastric pH exerts an inhibitory effect on chemotaxis. Our finding supported this conclusion, because effective chemotaxis occurred in a nearly neutral pH. In addition, self-infection studies have established that infection did not occur during fasting, without antacids, when the intragastric pH was about 1.5. Therefore, infection more likely occurs after meals, due to the buffering effects of the meal.

It is suggested that the use of antacids after meals, or proton pump inhibitors, without antibiotics, may provide a predisposing condition to colonization *H pylori* of the stomach and development of infection. We found an increase in motility of *H pylori* after resumption of urease activity which counteracted acidity, promoted chemotactic responses and caused colonization of the gastric mucus layers as reported in other studies.

Studies on mice showed that more effective colonization of *H pylori* occur in the absence of acid-secreting parietal cells of the stomach. We think that variation in colonization of *H pylori*, in different parts of the human stomach was due to variable chemotactic responses of *H pylori* to different acidity of the stomach.

In conclusion, chemotactic responses of *H pylori* were severely affected by pH condition of the media. Positive chemotaxis was found at pH values of 4.5 to 7.5. With regard to the absence of parietal cells in the antrum and thus,
its lower acidity than other parts of the stomach, it is suggested that probably further chemotactic motility, at higher pH of the antrum, is an important factor in maximum colonization of H pylori in this region.

References


