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Review

Helicobacter pylori: characteristics, pathogenicity, detection methods and mode of transmission implicating foods and water

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Abstract

Helicobacter pylori is an organism involved in the pathogenesis of human active chronic gastritis, peptic and duodenal ulcer diseases and gastric cancer. This review article covers this emerging human pathogen in terms of its phenotypic and genotypic characteristics, methods for culturing, its role in gastric pathogenicity, evidence involving its mode of transmission, difficulty in its isolation and detection methodology. In terms of transmission, both foodborne and waterborne pathways have been speculated as the mode of transmission for *H. pylori* as the patterns of the infection are consistent with those from fecal–oral and oral–oral transmission. Therefore, it is important to also evaluate methods for the detection of *H. pylori* from specifically food products and water. The detection of this pathogen has proved difficult since changes in cell morphology, metabolism and growth patterns occur when *H. pylori* is exposed to different environmental stimuli. The development of a viable but non-culturable coccoid (VNC) form is observed. These VNC forms do not undergo cellular division and cannot be cultured by traditional methods, increasing the difficulty in their detection. Since both viability and virulence in the VNC form of *H. pylori* are retained, the examination of food products and water for these forms is critical. Current methods include filtration, immuno-separation (IMS), polymerase chain reaction (PCR), probe hybridization, immuno-staining, autoradiography and ATP bioluminescence.

Keywords: *Helicobacter pylori*; Pathogen; Transmission; Pathogenicity; Detection methods; Foods; Water

1. Introduction

In 1989, the human pathogen formerly known as *Campylobacter pylori* was transferred to a new genus, *Helicobacter pylori* (Goodwin et al., 1989). The bacterium was originally isolated in 1982 from endoscopic biopsy specimens of human gastric mucosa. Based on DNA hybridization and base composition, ultrastructural studies, cellular fatty acid and respiratory quinones analyses, enzyme profiles and growth requirements, the bacterium was transferred from the former classification (Goodwin et al., 1989). The name *Helicobacter pylori* is latin for 'spiral rod of the lower part of the stomach' and from its reservoir the pathogen is involved in the pathogenesis of human active chronic gastritis, pep-

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tic and duodenal ulcer diseases (Dubois et al., 1994; Cohen, 1996). *H. pylori* has also been recently indicated as the etiologic agent in gastric cancer (Dunn et al., 1997).

This review article covers this emerging human pathogen in terms of: (1) its unique phenotypic and genotypic characteristics; (2) methods for in vitro culturing so as to overcome its fastidious nature; (3) evidence of its role in gastric pathogenicity; (4) proposed evidence involving its mode of transmission involving foods and water and; (5) detection methodology from food products and water.

The revision of the latter is important since the majority of the literature on this pathogen includes only clinical review articles that have evaluated invasive and noninvasive methods for the detection of *H. pylori* in individuals suspected to be infected with the pathogen or for the evaluation of the effectiveness of a clinical treatment in diagnosed patients (Cutler, 1996; Bazzoli et al., 1997; Dunn et al., 1997; Goddard and Logan, 1997). Briefly, some of the clinical noninvasive methods evaluated are: (1) urea breath test using labeled non-radioactive isotopes (Cutler, 1996; Bazzoli et al., 1997; Goddard and Logan, 1997); (2) blood serology; (3) gastric juice polymerase chain reaction (PCR) and (4) urinary excretion of labeled ammonia (Dunn et al., 1997). Gastric tissues are collected using clinical invasive methods. These are then examined by methods such as: (1) urease test; (2) histological examination; (3) culturing; (4) DNA probes and PCR analysis (Cutler, 1996; Dunn et al., 1997).

However, there are no review articles evaluating the screening methods for *H. pylori* in food products or water. Even though the precise mode of transmission for this human pathogen is still unknown, foodborne and waterborne pathways have been speculated (Cohen, 1996; Hultén et al., 1996; Dunn et al., 1997). Furthermore, a recent review also recognizes food as a potential source of dissemination (Meng and Doyle, 1997). It is therefore important to evaluate methods for the detection of this pathogen in food products and water.

Some of the reported approaches used for the detection of *H. pylori* (in particular its viable nonculturable (VNC) form) from water sources include filtration followed by immuno-separation (IMS), polymerase chain reaction (PCR) and probe hybridization (Enroth and Engstrand, 1995; Hultén et al., 1996, 1998), incorporation of [^{3}H]thymidine followed by autoradiography (Shahamat et al., 1993) and adenosine 5′-triphosphate (ATP) measurement by generating a bioluminescence reaction (Sörberg et al., 1996, 1997). IMS and PCR have also been used to detect the pathogen from food products (Cohen, 1996). Methods for recovering only the vegetative rod form from foods include homogenization, concentration by centrifugation and selective plating (Poms, 1997).

2. Overview of the human pathogen

2.1. Phenotypic characteristics

H. pylori are gram-negative and microaerophilic organisms. They are motile by means of multiple unipolar-sheathed flagella. Cells grow at 30 and 37°C but not at 25°C and the growth at 42°C is variable. The bacterium can be characterized in the laboratory by using its oxidase, catalase and strong urease positive activity, nitrate reduction, H_2S production in triple sugar iron agar and resistance to nalidixic acid and susceptibility to cephalothin and metronidazole (Goodwin et al., 1989). Also the bacterium shows a negative reaction for hippurate hydrolysis (Goodwin et al., 1989). Hippurate is a compound excreted by the human body as a detoxification mechanism of benzoic acid which, when conjugated with glycine, forms hippuric acid (Lindsay, 1985).

Some differences between *H. pylori* and the closely related genus *Campylobacter* are the strong urease activity as well as the presence of a flagellar sheath and bulb in *H. pylori*. Glycocalyx formation and smooth cell wall membrane are observed when cultures of *H. pylori* are grown in vitro by shaking. Glycocalyx formation under in vitro conditions is a characteristic feature of helicobacters since these external layers of polysaccharides are formed in bacteria in their natural habitats but often they are lost when bacteria are cultivated in the laboratory (White, 1995).

Helicobacter and *Campylobacter* also show opposite antimicrobial susceptibilities. The presence of non-protein substances known as quinones that function as proton carriers in the electron transport chain, is another separating feature between the two genera. *H. pylori* only possesses MK-6 quinones and lacks

the methylated MK-6 quinones. The methylated forms of these quinones are characteristically found in all campylobacters.

Another unique characteristic of *H. pylori* that has been recently found and separates it from the genus *Campylobacter* is its carbohydrate profiles. Previous reports based solely on carbohydrate fermentation patterns, had reported that *H. pylori* could not metabolize common saccharides (Tee et al., 1991). But Mendz and Hazell (1993), by using nuclear magnetic resonance spectroscopy, demonstrated the presence of monosaccharide kinases and thus, the potential ability to catabolize D-glucose. This catabolism was explained by the authors as a phosphorylation rather than a fermentation pathway when adenosine triphosphate (ATP) is used as the phosphate donor. Later, the same group of researchers (Mendz et al., 1993) demonstrated not only the catabolism of saccharides but also the transport and incorporation of D-glucose into lactate as being dependent on the strain and on the growth conditions. This is relevant because glucose is not necessary for growing *H. pylori* under in vitro conditions, however it is a substrate abundant in human hosts.

2.2. Genotypic characteristics

The identification and characterization of *H. pylori* at the genomic level is very significant when trying to elucidate the transmission pathway of diseases suspected to be caused by this microorganism (Taylor et al., 1992). In 1992, Taylor et al. demonstrated genetic diversity among *H. pylori* strains suggesting that the genome possesses genetic variability. In the study, only two *H. pylori* isolates each from two unrelated patients and three isolates from different anatomical sites of a third patient, possessed the same genomic pattern whereas isolates from all other patients each showed different genomic patterns. All genomic patterns remained constant after multiple sub-culturing under in vitro conditions. These authors proposed that the wide genomic variation among strains isolated from different patients might be due to a genomic rearrangement as an adaptation mechanism when the bacterium infects a new host. Such rearrangement can occur if a short repetitive DNA sequence moves, or if a chromosomal DNA sequence is amplified and the adjacent sequence is deleted.

Other possible mechanisms for the genomic rearrangement are if a nucleotide sequence not associated with phenotypic changes is modified or if exogenous DNA is added by natural transformation (Taylor et al., 1992). The latter mechanism, that of natural transformation, has the potential to occur since *H. pylori* are known to be naturally competent for transformation as well as potentially capable of bacteriophage transduction (Taylor, 1992).

2.3. Growth requirements

H. pylori is a fastidious microorganism that requires nutrient rich media, an atmosphere enriched in CO_2, high humidity (96–100%) and a pH near 7.0. Media are usually supplemented with blood as a reducing agent rather than as a nutrient (Cohen, 1996) and shaking is needed in liquid systems to provide good dispersion of gases throughout the liquid (Sjöström and Larsson, 1996). The evaluation of several media for the growth of *H. pylori* has been done by various authors (Tee et al., 1991; Xia et al., 1993, 1994; Hachem et al., 1995; Poms, 1997). Tee et al. (1991) reported the need to use a combination of more than one selective media for maximum recovery of *H. pylori* from biopsy samples.

Brain heart infusion broth (BHIB) supplemented with 10% horse serum was reported by Xia et al. (1993) as the best liquid medium to maintain the viability of *H. pylori* for 3 days from -4°C to 21°C. They also reported chocolate agar slants supplemented with 7% horse blood as the best medium for transport of cultures. The same authors (Xia et al., 1994) later reported that chocolate agar slants were not only the most suitable system to transport samples but also to delay the development of the viable non-culturable (VNC) forms at ambient temperature. The concept of the VNC form is discussed later in this review (Sections 3 and 4).

Other authors have reported BHIA supplemented with 7% fresh whole defibrinated horse blood as the most suitable medium for primary isolation of *H. pylori* from gastric mucosal biopsy samples (Hachem et al., 1995). These authors (Hachem et al., 1995) concluded that the use of fresh media and maintenance of adequate humidity throughout incubation are the two main requirements for a successful isolation. The evaluation of several media for the recovery of *H. pylori* from foods was assessed in a

recent study by Poms (1997). The author evaluated solid media including BHI, Brucella, Columbia, DNAse, Muller Hinton, Tryptic soy (TSA) and Wilkins–Chalgren agar supplemented with 5% horse blood on two different strains (*H. pylori* NCTC 11637 and 11638).

Wilkins–Chalgren agar performed the best and was further evaluated using various supplements including 5% of whole horse blood, horse serum, lysed horse blood, whole horse blood and 40 mg/l of 2,3,5-triphenyltetrazolium chloride (TTC). The study concluded that supplementation with 5% horse serum demonstrated similar recovery to that of blood supplementation, however the horse serum presented the advantages of having a longer shelf-life and a lower cost when compared to the blood and therefore it was recommended to supplement Wilkins–Chalgren agar (Poms, 1997).

2.4. Role of H. pylori in gastric pathogenicity

It has been suggested that *H. pylori* is involved in the pathogenesis of human active chronic gastritis, peptic and duodenal ulcer disease and recently in that of adenocarcinoma and lymphoma of the stomach (gastric cancer) (Dubois et al., 1994; Cohen, 1996; Bazzoli et al., 1997; Dunn et al., 1997). A high percentage of adults around the world harbor the bacterium. It is estimated that in the US, 10% of the adult population is infected with the bacterium (Cohen, 1996). In contrast, in developing countries, the occurrence of the pathogen has been estimated at between 70 to 90% of the total population (Dunn et al., 1997). However, it is believed that a different degree of virulent factors or the involvement of cofactors such as from the host or other bacteria in the host, explain the fact that most of these infected individuals are only carriers of *H. pylori* and do not suffer or manifest any clinical symptoms (Dubois, 1995).

Humans are not the only potential host for *H. pylori* infection. The bacterium has been found as a natural infection in various animals such as the pigtail macaque and other primates (Drazek et al., 1994). Rhesus monkeys have also been successfully used as a model for infection in humans (Dubois et al., 1994). This last study conducted with rhesus monkeys, demonstrated that the degree in gastritis presented by these animals decreased when *H. pylori* was eradicated, thus relating the bacterium to the physiological symptom (Dubois et al., 1994).

The mechanisms that cause the inflammatory reaction or gastritis due to infection with *H. pylori* are unknown, although several hypotheses have been postulated. The pathogen is capable of adaptation to the mucus habitat and of association with the intracellular junctions of the gastric epithelium. Also, both its spiral shape and high motility allow this bacterium to resist peristaltic flushing (Dubois, 1995). High levels of ammonium ions are produced by the characteristic strong urease activity of *H. pylori*. These levels can be toxic to the gastric superficial epithelial cells. However, because it has been observed that animals infected with other spiral gastric bacteria that are urease positive do not manifest such damage, it has been suggested that urease activity is a virulence factor but not necessarily the cause of the inflammatory response (Dubois et al., 1994). Also, *H. pylori* urease negative strains have been isolated (Dubois, 1995).

H. pylori also produces a vacuolating cytotoxin (VacA) responsible for massive vacuolization in mammalian cell lines as well as in gastric epithelia of patients with chronic gastritis (Marchini et al., 1994). This virulence factor has been observed in both humans and rhesus monkeys (Dubois et al., 1994). The vacuolating cytotoxin factor is being supported as a potential mechanism for gastritis after clinical reports have indicated that patients suffering from duodenal ulcers or gastric cancer show a much higher probability of harboring cytotoxin producer strains as compared to only a fraction of those patients suffering only from inflammatory symptoms (Dubois, 1995). An antigen-mediated immunopathologic effect has also been suggested as a mechanism for the gastritis (Dubois et al., 1994).

Ge and Taylor (1996) suggested another virulence factor involving an Fts membrane protein. It was shown that when the *fts* gene was disrupted, a loss in viability was observed thus indicating a requirement for the presence of this protein. The authors proposed multiple roles involving this Fts factor such as protein assembly, secretory protein export and proteolytic activity. This same protein has been shown to be involved in the export of extracellular virulence factors in *Escherichia coli* (Ge and Taylor, 1996).

The correlation between *H. pylori* and gastric cancer was first reported by Recavarren-Arce et al. (1991). These researchers presented evidence suggesting the relationship between *H. pylori* infection and chronic atrophic gastritis which is a pre-malignant condition that if progresses can potentially lead to gastric cancer. The mechanism proposed involves an increase in the pH above 4.5 that allows bacterial overgrowth. An increase in bacterial metabolites can induce the reduction of nitrate to nitrites at high levels which can combine with amines and amides. Amines and amides can be present in the body from various food and drugs and by combining with nitrites, mutagenic and carcinogenic N-nitroso compounds can be formed.

In 1994, a review article by Hwang et al. proposed a multiple-factors phenomenon when considering gastric cancer. Diet containing promoters or inhibitors of oxidation, environmental factors and *H. pylori* infection were all proposed to be involved in the development of gastric cancer so as to explain different clinical observations among people harboring the bacterium (Hwang et al., 1994).

2.5. Mode of transmission

The mode of transmission of *H. pylori* is still unknown, but foodborne and waterborne pathways have been speculated as the epidemiological patterns of the infection are consistent with those from fecal–oral and oral–oral transmission (Cohen, 1996; Hultén et al., 1996; Dunn et al., 1997). Fecal–oral transmission is highly probable since the pathogen has been detected in human feces (Enroth and Engstrand, 1995; Aleljung et al., 1996; Nilsson et al., 1996).

It is now recognized that the presence of *H. pylori* in the human stomach is highly frequent, especially among people living under poor hygienic conditions (Dubois, 1995). However, there has been a tendency in the literature to attribute a direct correlation between the infection and socioeconomic status as indicated in reviews such as that of Dubois (1995) and Hultén et al. (1996). This correlation might possibly create a stigma involving the disease. A study conducted in 1991 by Klein et al., evaluated the risk factors for *H. pylori* prevalence among Peruvian families. They concluded that those families consuming water from external sources, thus having no internal plumbing, were three times more likely to be infected with *H. pylori* but the risk was rather due to the source of water and not the socioeconomic status (Klein et al., 1991).

Another study by Hopkins et al. (1993) using seroprevalence, demonstrated a seropositivity of infection to be highly correlated not only with age and low socioeconomic status, but also with the consumption of uncooked vegetables. These authors concluded that a diet including uncooked vegetables potentially contaminated via irrigation of polluted water represented a potential pathway, thus supporting, once more, a water and foodborne route. Although for some families not having internal plumbing (thus being unable to consume potable water) is a consequence of their socioeconomic status, the literature should more clearly address other factors such as lack of personal hygienic practices, food handling practices etc., as the potential causes for the transmission of the infection. Finally, a zoonosis transmission pathway involving domestic cats has also been proposed (Handt et al., 1994). These researchers isolated *H. pylori* from gastric tissues harvested from domestic cats. These animals have been previously reported to harbor other gastric spiral organisms such as *Helicobacter felis*.

Goodman et al. (1996) conducted a well defined and comprehensive epidemiological study of *H. pylori* transmission pathway in a rural community in Colombia. Person-to-person, waterborne, foodborne and zoonotic transmission pathways were evaluated in the study as possible routes. The results demonstrated no single mode of transmission but rather demonstrated a multiple pathway-phenomenon. However, some interesting findings of the study showed that residential crowding, especially the number of children, was strongly correlated with the infection. This finding agreed with previous observations associating residential crowding, especially during childhood, with the disease (Goodman and Correa, 1995). Also, when evaluating the waterborne transmission, the number of raw vegetables, in particular lettuce, consumed per day showed a positive dose–response effect with the disease (Goodman et al., 1996).

Previous reports in the literature that evaluated *H. pylori* survival under in vitro conditions in water, milk and saline (Bode et al., 1993; Shahamat et al.,

1993) and those that indicated its isolation from the feces of patients with gastritis (Enroth and Engstrand, 1995; Aleljung et al., 1996; Nilsson et al., 1996), saliva and dental plaque (Hultén et al., 1996) and animals (Handt et al., 1994), support the multiple pathway route theory proposed by Goodman et al. (1996).

3. Difficulty in its isolation from the environment, water and food

Isolation of *H. pylori* from samples other than gastric tissues has been extremely difficult to accomplish (Goodman and Correa, 1995). There has not been an in vivo isolation of *H. pylori* from the environment such as water systems using traditional culture techniques. Its presence in environmental samples such as water and stool specimens has been only shown using sophisticated and expensive techniques such as filtration, immuno-concentration, polymerase chain reaction (PCR) and hybridization with specific probes (Enroth and Engstrand, 1995; Hultén et al., 1996; Nilsson et al., 1996; Hultén et al., 1998). This lack of success is due to the fact that when *H. pylori* is exposed to variable environmental conditions, changes in morphology, metabolism and growth patterns are observed (Bode et al., 1993). As well, faster-growing commensal organisms from the environmental sample affect the screening (Hultén et al., 1996).

All these adverse environmental factors trigger the development of a viable but non-culturable coccoid (VNC) state, which lowers the metabolic activity of cells. This phenomenon is observed in a variety of bacterial genera from natural environments and it represents a concern in detecting the presence of major pathogens such as *Vibrio cholera* and *Vibrio vulnificus* from environmental samples and food (Duncan et al., 1994), and *E. coli* O157:H7 in water and food products (Rigsbee et al., 1997). Cells from these genera in the VNC state have been shown to be active metabolically. However, cells in this state do not undergo cellular division, thus colonies on plates are not developed. VNC cells from these genera have also been shown to retain their virulence. Therefore, the examination of food and water for the presence of these VNC cells has been recommended (Rigsbee et al., 1997).

4. Viability and retained virulence of the *H. pylori* VNC state

In the case of *H. pylori*, it has been reported (Shahamat et al., 1993) that the VNC forms in water are still viable by a slow uptake of [^{3}H]thymidine, thus supporting the theory of viability of the *H. pylori* VNC forms. In that study, it was also found that temperature was the main environmental factor associated with the development of VNC forms. Water samples incubated at 4 and 15°C contained VNC forms, whereas those incubated at higher temperatures (22 and 37°C) resulted in loss of complete metabolic activity (Shahamat et al., 1993). Other studies with *E. coli* O157:H7 in water have also shown that temperature is the signal for entry into the VNC state (Rigsbee et al., 1997). Other researchers have attempted to demonstrate the viability of the *H. pylori* VNC state by detecting some form of metabolism. Gribbon and Barer (1995) demonstrated, by using tetrazolium salts as cytochemical indicators of oxidative metabolism, that the *H. pylori* VNC forms retained significant levels of activity at 4°C. Viability of the VNC form at 4°C has also been demonstrated by other methods such as immuno-staining (Bode et al., 1993) and autoradiography (Shahamat et al., 1993).

Finally, viability of the *H. pylori* VNC state has also been shown by demonstrating that the VNC state maintains basal respiration (Bode et al. 1993). The authors induced the VNC state using bactericidal substances and storage under low temperature. They detected DNA synthesis in 3-month-old VNC forms when using immuno-staining techniques. After 3 months the VNC state was also shown to retain the complete set of flagella (thus motility) as well as ultrastructures and polyphosphate granules. These findings support the viability of the VNC coccoid state observed in *H. pylori*.

Virulence of the *H. pylori* VNC state or germination into the vegetative rod state have also been speculated since clinical observations have indicated that recuperated patients suffering from duodenal ulcers, re-obtain the infection by the same strain after chemical therapy (Bode et al., 1993; Gribbon and Barer, 1995). This suggests that the VNC forms triggered when exposed to chemical stress such as that from antimicrobial therapy, may be able to germinate once the treatment (stress) is withdrawn or that the *H. pylori* VNC forms are virulent enough to

induce the clinical symptoms. Recent reports support the latter alternative (Aleljung et al., 1996; Wang et al., 1997).

Aleljung et al. conducted a well-controlled study where low levels (10^2 colony forming units, cfu) of the coccoid *H. pylori* VNC forms were enough to colonize and produce an acute inflammatory reaction in the stomach mucosa of mice. Higher levels (10^4–10^6 cfu) of the vegetative rod form were needed to induce a similar reaction (Aleljung et al., 1996). Furthermore, it was demonstrated that both coccoid VNC and vegetative rod forms of *H. pylori* significantly increased inflammatory cells in the stomach mucosa of mice (Wang et al., 1997).

Finally, clinical cases involving failure in eradicating *H. pylori* (therefore leading to re-infection) may also be due to an acquired resistance to various drugs after prolonged treatment (Glupczynski et al., 1991), to genetic natural transformation (Taylor, 1992) or to drug over-prescription (Vasquez et al., 1996). However, Vasquez et al. (1996) noticed that the development of drug resistant *H. pylori* strains isolated from individuals living in third world countries was not due to over-prescription alone. This conclusion was reached when they found strains not to be tetracycline-resistant, a commonly over-prescribed drug in these countries (Vasquez et al., 1996).

5. Methodology for the detection of *H. pylori* in food products and water

After establishing the retained viability and virulence of the *H. pylori* coccoid VNC form and its difficulty in isolation and detection, the selection of the most suitable detection method is critical. Several methods have been reported to be successful in detecting this pathogen from environmental, food and water sources. Microbiological methods are briefly discussed as they cannot detect the VNC coccoid form. Other discussed methods include immuno-separation (IMS), molecular techniques, autoradiography and ATP bioluminescence, which have proven successful in detecting both *H. pylori* forms.

5.1. Microbiological methods

Classical microbiological methods to establish survival and recovery models of the pathogen from foods have been utilized. Poms (1997) conducted such models in a variety of foods (skinless chicken and fresh lettuce) using four different inoculum levels. The recovery of *H. pylori* from foods was accomplished by two centrifuging cycles, followed by plating the samples onto Wilkins–Chalgren supplemented with 5% defibrinated blood and antibiotics (colistin methanesulfonate 30 mg; cycloheximide 100 mg; nalidixic acid 30 mg; trimethoprim 30 mg; vancomycin 10 mg/l) as selective medium. This classical approach resulted only in a 10% recovery. This classical method is expected to underestimate the survival of the pathogen in the food systems as it does not account for the VNC coccoid form, although this factor was not further evaluated by the author (Poms, 1997).

5.2. Immuno-separation (IMS) and molecular techniques

Several investigators have successfully used an immuno-separation (IMS) step followed by molecular techniques. Cohen (1996) reported a protocol involving an initial concentration of the pathogen from foods using IMS followed by a PCR method which amplified a fragment of the 16S rRNA of *H. pylori* from both vegetative and coccoid forms. Nilsson et al. (1996) developed an optimized protocol using the paramagnetic binding beads and viscous samples by diluting the samples prior to the IMS step.

Other researchers have also used IMS with *H. pylori*-genus specific paramagnetic binding (for both forms of the pathogen) followed by a PCR and hybridization steps with labeled probes. This approach has been successful in detecting the pathogen in water systems (Enroth and Engstrand, 1995; Hultén et al., 1996, 1998). These researchers have reported that the advantage of using this protocol is that it offers excellent specificity using the IMS concentration step, followed by high sensitivity of the molecular methods.

5.3. Autoradiography

Viability models in water systems have been developed with the use of autoradiography detection. Shahamat et al. (1993) followed the incorporation of [^{3}H]thymidine into cells to assess the viability when exposed to a variety of parameters such as tempera-

ture and water source, followed by a visual observation using autoradiography. The approach proved to be useful for the assessment of viability but required a long exposure time (24–72 h) for the [^{3}H]thymidine uptake into cells.

5.4. ATP bioluminescence

The assessment of viability in a cell can also be conducted by detecting adenosine 5′-triphosphate (ATP) levels by the luciferin–luciferase bioluminescence reaction. This particular reaction involving the firefly luciferin–luciferase (LL), is analytically significant in that it is solely thermodynamically driven by energy derived from the intracellular ATP (Stanley, 1989; Lehninger et al., 1993). This technique can be used in a pure culture system, however, when used in a complex system such as food and water, it will not distinguish between ATP from different cell sources (Schram, 1991). Nevertheless, some researchers have used ATP bioluminescence as an indication of viability in *H. pylori* pure culture models (Sörberg et al., 1996, 1997). Sörberg et al. (1996) studied the morphologic conversion of *H. pylori* from rods to the VNC coccoid form by using light microscopy, viable counts and ATP bioluminescence. They found that viable counts and ATP bioluminescence levels decreased during the morphologic conversion. When compared to the rod form, the VNC coccoid form was indicated to have 1000-fold lower ATP per cell (Sörberg et al., 1996). The detection of ATP levels offers an alternative method not only much simpler and economical than the currently used methods (PCR, DNA probing etc.) but also more rapid. A result obtained as a light output in relative light units (RLU) is obtained after 15 s once the sample is extracted.

6. Conclusion

Several emerging foodborne pathogens have been recognized in the past two decades. *E. coli* serotype O157:H7, *Listeria monocytogenes*, *Campylobacter jejuni* and *Vibrio cholera* are only examples of the bacterial strains included among those recently identified (Knabel, 1995). *H. pylori* is not classified as a foodborne or waterborne pathogen since its mode of transmission is still to be established. Nevertheless, it has been recognized as an important human pathogen (Bazzoli et al., 1997; Dunn et al., 1997) and food products have been recognized as a potential source of dissemination (Meng and Doyle, 1997).

The food industry faces the tremendous challenge of assuring that foods are free of such pathogens since these are ubiquitous in the environment and have been shown to evolve and adapt rapidly when exposed to different environments (Knabel, 1995). Therefore, more sensitive and selective approaches as well as model systems need to be evaluated for the detection of these pathogens in food products, water and environmental samples.

In the case of *H. pylori*, the ability to survive in a coccoid VNC form and its correlation with water and raw vegetables irrigated with such water, represent a concern to the food industry especially with minimally processed foods, fruits and vegetables if eaten uncooked. This review article covered the most important aspects of this human pathogen in terms of its characteristics, role in gastric pathogenicity and mode of transmission implicating foods and water. Also, it reviewed several reported methods used to overcome the difficulty in detecting this pathogen, especially from foods and water.

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