Pergamon

Wat. Sci. Tech. Vol. 38, No. 12, pp. 181–185, 1998.

0273–1223/98 $19·00 + 0·00

PII: S0273-1223(98)00822-1

BIOFILMS IN DRINKING WATER SYSTEMS – A POSSIBLE RESERVOIR FOR *HELICOBACTER PYLORI*

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ABSTRACT

Helicobacter pylori is a major bacterial pathogen involved in several gastrointestinal diseases. Transmission routes and reservoirs of *H pylori* are not well understood despite several studies. In contrast to many other infectious diseases, clinical symptoms allowing definitive diagnosis of infection are absent. Person-to-person transmission with faecal-oral and oral-oral routes have been proposed with socio-economic status and density of living as associated factors. Studies have shown that *H pylori* can survive in water for prolonged periods leading to a waterborne transmission route being proposed but not conclusively identified. This paper describes studies using mature heterotrophic mixed-species biofilms grown under oligotrophic conditions using a continuous-culture chemostat system. The biofilms were challenged with *Helicobacter pylori* (NCTC 11637). Results indicate the presence of *H pylori* associated with the biofilm for up to 192h post-challenge, suggesting that biofilms in water distribution systems could be a possible and as yet unrecognised reservoir of *H pylori*. © 1998 Published by Elsevier Science Ltd on behalf of the IAWQ. All rights reserved

KEYWORDS

Helicobacter pylori; biofilms; drinking water.

INTRODUCTION

Microbial and clinical significance H pylori is a unipolar, flagellated, spiral or S-shaped organism when viewed *in vivo* (Goodwin and Warsley, 1993). The organism does not form spores but does undergo morphological change from spiral to coccoid morphology accompanied by a distinct loss of culturability (Owen, 1995). The coccoid form of the organism, however, is believed to retain the capacity to return to the culturable state given the required stimuli (Gribbon and Barer, 1995). Infection of the stomach by *H pylori* is arguably the most prevalent bacterial infection world-wide and plays a major role in several gastrointestinal diseases including type B gastritis (McGuigan, 1996), gastric ulcer, peptic ulcer, MALT and gastric adenocarcinoma (Moran, 1997). Transmission routes and reservoirs of *H pylori* are poorly understood. Several non-human reservoirs, such as dogs (Eaton *et al.*, 1996), cats (Fox, 1995) and monkeys (Drazek, 1994), have been suggested. Person-to-person transmission with faecal-oral (Megraud, 1995) and oral-oral (Madinier, 1995) routes have been proposed with socio-economic status and density of living as associated factors (Van Zanten, 1995). The existence of an environmental reservoir is at present unproven. *H*

pylori is microaerophilic and thus cannot survive in air; however, the coccoid form of the organism possesses a polysaccharide coat which may protect it from adverse environmental conditions (Vincent, 1995) and survival in water over a wide range of environmental conditions has been demonstrated (West *et al.*, 1992). Two separate studies found that infection was linked to the quality of potable water available to the population (Klein *et al.*, 1991; Hulten *et al.*, 1996).

Biofilms in water distribution systems - microorganisms rapidly adhere to exposed surfaces in aquatic environments. Firmly attached cells grow overwhelmingly as microcolonies which often synthesise extracellular polymeric compounds that enclose the cells in a gelatinous-like matrix (Ladd and Costerton, 1990) and this complex of cells and their products have become known as a biofilm (Donlan, 1994). In nutrient deprived or otherwise hostile environments, concentration and growth of microbial cells on solid surfaces confers considerable advantages such as improved growth prospects through concentration of scarce nutrients and resistance to antimicrobial agents (Gilbert *et al.*, 1993). Biofilms are ubiquitous in water distribution systems (Kalmbach *et al.*, 1997) and their re-growth poses a serious risk with respect to the microbial quality of potable water supplies (Donlan *et al.*, 1994). Current models of biofilm structure suggest a complex 3-dimensional assemblage of 'mushrooms' or 'strings' (Costerton, 1995) emerging from a basal layer (Figure 1). These biofilms will lose cells during two processes: (i) erosion of material from the tips of the streamers or (ii) through sloughing - the catastrophic loss of large amounts of biofilm material. The complex structure found in a mixed species heterotrophic biofilm will include areas of reduced O_2 tension allowing microaerophilic organisms to survive and thrive. Further, the two mechanisms for the removal of biofilm material mean that (i) a very low level of cells is released (probably below detection limits) or (ii) catastrophic releases occur which are chaotic in nature and thus are unlikely to be represented in a grab sample survey. This would account for the failure of previous workers to have identified drinking water as an environmental reservoir of *H pylori*.

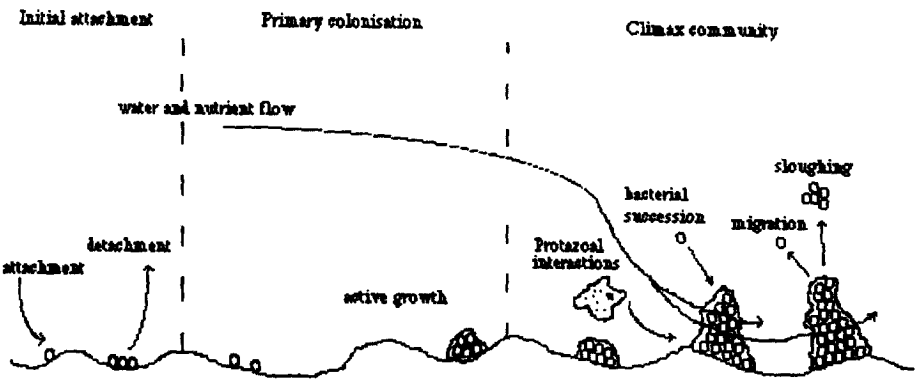

Figure 1. Development of a mixed species biofilm (Rogers and Keevil, 1995).

MATERIALS AND METHODS

Cultivation of Helicobacter pylori - H pylori NCTC 11637 was cultivated routinely on Columbia agar base (BBL Microbiology) supplemented with 10% defibrinated horse blood (TCS Microbiology) under microaerophilic conditions (5% O_2; 10% CO2; 85% N_2) in a variable atmosphere incubator (Don Whitely Scientific). Liquid culturing was achieved in Ho's broth (Ho and Vijayakumari, 1993). Culture identity was confirmed by Gram stain and rapid urease test (Cowan and Steel, 1995).

Development of PCR protocol - PCR was carried out using primers Hp 1 and Hp 2 which amplified a specific 500bp fragment of the 16S rRNA gene of *H pylori* (Engstrand, 1992). The sensitivity of the Hp1, Hp 2 primer set was established as $1x10^2$ cfu/reaction on lysed cells (Thoreson *et al.*, 1995).

Detection of Helicobacter pylori in a Single-Stage Continuous Culture-coupled modified Robbins device (SSCC-MRD) system by PCR - 10L of water collected from Mannofield treatment works, Aberdeen prior to chlorination was passed through a 0.2μm nylon membrane (Millipore) and used to run a Single-Stage Continuous Culture-coupled modified Robbins device (Mackerness *et al.*, 1993; Lappin-Scott *et al.*, 1993; Figures 2 and 3). The SSCC-MRD was challenged with 4.5mL of an overnight culture of *H pylori* NCTC 11637 harvested and re-suspended in PBS buffer. The presence of *H pylori* on the stainless steel coupons was determined by PCR analysis. Coupons were removed from the Robbins device and placed in 1mL of sterile TE buffer. The coupons were then sonicated and the buffer stored at -20°C prior to PCR analysis.

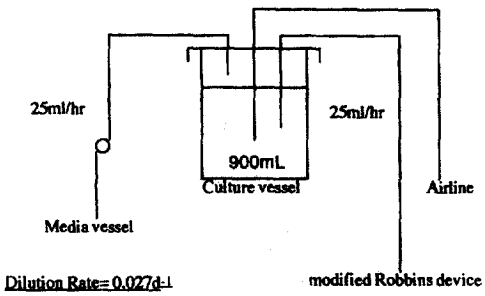

Figure 2. Schematic representation of the Single-Stage Continuous Culture system (SSCC).

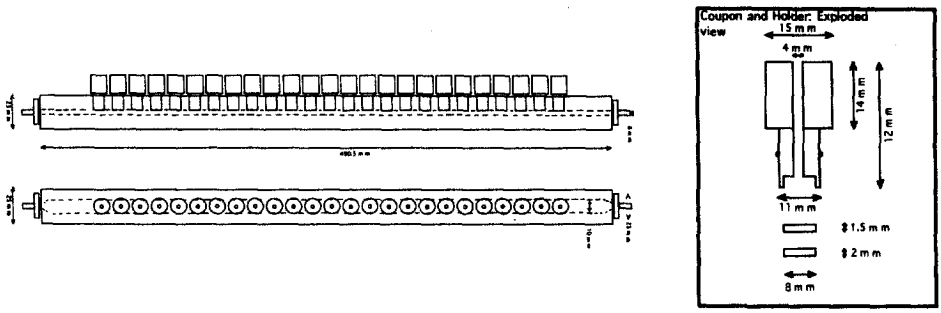

Figure 3. Schematic representation of the modified Robbins device.

RESULTS

Inoculation of the SSCC-modified Robbins device with H pylori NCTC 11637 H pylori was not detected in biofilm material by PCR prior to its addition into the SSCC. For a period of 192h post-challenge, *H pylori* was detected in the mixed species heterotrophic biofilm present on the stainless steel coupons by PCR (Figures 4 and 5).

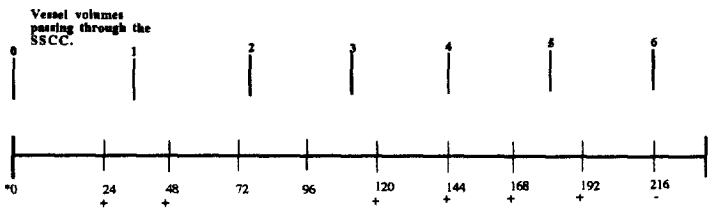

Figure 4. Detection of *H pylori* NCTC 11637 in biofilms by PCR (*time h elapsed; -/+ *H pylori* not detected/detected by PCR).

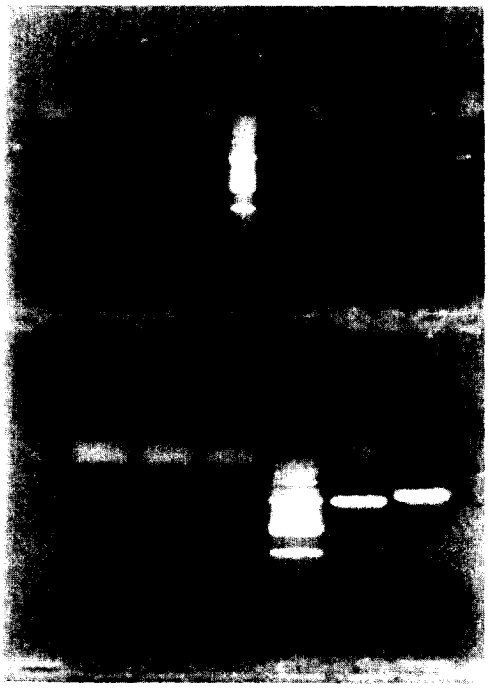

Figure 5. Electrophoresis of the PCR products obtained from biofilm samples removed from the stainless steel coupons. [Lanes 1 and 2 = positive control (purified *H pylori* DNA); Lanes 3 and 11 = molecular weight markers; Lanes 4 and 5 = pre-challenge; Lanes 6 and 7 = 48h; Lane 8 = 72h; Lane 9 = 144h; Lane 10 = 168h; Lane 11 = 192h; Lane 13 = 216h; Lane 14 = 240h; Lane 15 = 312h].

CONCLUSIONS

H pylori was detected in the biofilm material removed from the stainless steel coupons for a period of 192h post-challenge after a total of five vessel-volumes had passed through the SSCC-MRD. These results give a strong indication that viable *H pylori* may survive in water distribution systems by becoming associated with the biofilm community present in such systems. Work is being undertaken to investigate these results further.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support of the Wellcome Trust who funded this work (Award 047548/Z/96/Z).

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