Investigation of the effects of pH and bile on the growth of oral *Campylobacter concisus* strains isolated from patients with inflammatory bowel disease and controls

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*Campylobacter concisus* is an oral bacterium that is associated with inflammatory bowel disease (IBD). This study examined the impact of pH and bile on the growth of oral *C. concisus* strains isolated from patients with IBD and controls. The growth of 58 *C. concisus* strains on horse blood agar (HBA) plates following exposure to media with various pH values for different time points was examined. Furthermore, the growth of *C. concisus* strains on HBA plates containing different concentrations of ox bile was investigated. Following exposure to pH 2 for 30 min, none of the 58 oral *C. concisus* strains grew on HBA plates. Following exposure to pH 3.5 for 30 min, only four of 20 oral strains examined grew on HBA plates, with a log10 c.f.u. reduction of 0.7–2.5 compared to the same strains without low pH exposure. Exposure to pH 5 for 120 min had minimal effects on *C. concisus* growth. Approximately half of the oral strains (55.2%, 32/58) grew on HBA containing 2% bile. Bile inhibited the growth of *C. concisus* in a dose- and strain-dependent manner. These data suggest that both bacterial and intestinal environmental factors may play a role in the determination of *C. concisus* colonization in the different parts of the gastrointestinal tract and that increased gastric pH and reduced intestinal bile may be risk factors for increased gastric and intestinal *C. concisus* colonization.

INTRODUCTION

*Campylobacter concisus* is a Gram-negative bacterium of the human oral cavity (Tanner et al., 1981; Zhang et al., 2010). *C. concisus* has been shown to be associated with inflammatory bowel disease (IBD). IBD is the chronic inflammation of the gastrointestinal tract and it usually manifests as either Crohn’s disease (CD) or ulcerative colitis (UC) (Cosnes et al., 2011). A significantly higher prevalence of *C. concisus* was detected using PCR in both the intestinal biopsies and faecal samples collected from patients with IBD as compared to controls (Mahendran et al., 2011; Man et al., 2010; Mukhopadhya et al., 2011; Zhang et al., 2009). In addition to IBD, *C. concisus* has also been associated with diarrhoeal disease, being frequently isolated from diarrhoeal faecal samples (Engberg et al., 2000; Lastovica, 2009; Nielsen et al., 2013).

*C. concisus* was previously isolated from the saliva samples of nearly all people tested (Zhang et al., 2010). However, in healthy individuals, intestinal colonization by *C. concisus* is rare. Previous studies have reported that the isolation rate of *C. concisus* from faecal samples is low, having been isolated from only 3% (3/107) of healthy individuals (Engberg et al., 2000) and from no (0 of 108) healthy individuals at all (Nielsen et al., 2013). *C. concisus* strains isolated from intestinal biopsies of patients with IBD were genetically identical or closely related to oral *C. concisus* strains isolated from the same or other patients, suggesting that *C. concisus* strains that colonize the human intestinal tract originate from oral *C. concisus* strains (Ismail et al., 2012).

**Abbreviations:** CD, Crohn’s disease; IBD, inflammatory bowel disease; UC, ulcerative colitis.
Healthy people produce between 1 and 1.5 litres of saliva daily (Humphrey & Williamson, 2001), most of which is swallowed. Using saliva as a vector, *C. concisus* is transported down to the lower parts of the gastrointestinal tract. However, the low isolation rates of *C. concisus* from faecal samples of healthy individuals indicate that the swallowed *C. concisus* do not establish colonization in the intestinal tract in the majority of healthy individuals.

The environmental factors in the human gastrointestinal tract that may prevent oral *C. concisus* from colonizing it have not been systematically investigated. Furthermore, it is not clear whether *C. concisus* strains colonizing the oral cavity of patients with IBD and controls differ in their abilities to overcome the bacterial inhibitory factors of the gastrointestinal tract. In this study, we examined the impact of pH and bile on the growth of *C. concisus* strains isolated from patients with IBD and controls.

**METHODS**

*C. concisus* strains and cultivation conditions. A total of 58 oral *C. concisus* strains isolated from saliva samples were used in this study. These strains were isolated in our previous studies; identities were confirmed by 16S rRNA gene sequencing and analysis of whole-cell protein profiles (Mahendran et al., 2011; Zhang et al., 2010, 2009). Of the 58 oral *C. concisus* strains, 19 strains were from patients with CD, 14 strains from patients with UC and 25 strains from healthy controls. The details of the *C. concisus* strains used in this study are shown in Table 1.

*C. concisus* strains were cultured under anaerobic conditions generated with a commercially available gas generating system enriched with 5% hydrogen using sodium borohydride as previously described (Lee et al., 2014). The gas generating system was purchased from Oxoid. We previously reported that *C. concisus* does not grow under microaerobic conditions without the presence of H₂, but is able to grow under anaerobic conditions without H₂ (Lee et al., 2014). This suggests that O₂ gas may stress *C. concisus*. To minimize the confounding factors in assessing the impact of pH and bile on the growth of *C. concisus*, anaerobic conditions enriched with H₂, which are the optimal atmospheric conditions for *C. concisus* growth in laboratory cultivation based on our previous study, were used for cultivation of *C. concisus* in this study (Lee et al., 2014). Given that *C. concisus* requires H₂ for optimal growth and H₂ has extremely low solubility in liquid, a plate culture method was used to assess *C. concisus* growth (Lee et al., 2014).

Examination of the impact of exposure to pH 2.0 for 30 min on the growth of *C. concisus* strains. All 58 *C. concisus* strains were examined. *C. concisus* strains were first cultured on horse blood agar (HBA) plates composed of Blood Agar Base No. 2 (Oxoid) supplemented with 6% (v/v) defibrinated horse blood for 48 h at 37 °C. The bacterial cells of each strain were collected and resuspended into PBS and the bacterial concentration was adjusted to OD₅₉₅ 0.1. A bacterial suspension of each *C. concisus* strain was placed in two 1.5 ml Eppendorf tubes (1 ml in each tube) and the Eppendorf tubes were centrifuged at 7900 g for 2 min. The supernatant was removed and the bacterial pellet in one tube was resuspended in 1 ml heart infusion broth (Oxoid) supplemented with 5% fetal bovine serum (Invitrogen) (HIB) with a pH of 7.0 and the bacterial pellet in the second tube was resuspended in 1 ml HIB with a pH of 2.0. Both of the bacterial suspension tubes were then incubated at 37 °C while rotated at 160 r.p.m. for 30 min under the cultivation conditions described above. Following this, the tubes were centrifuged again, the supernatant removed and the bacterial pellets were resuspended in 1 ml HIB of pH 7.0. From each of these suspensions, 10 μl was inoculated onto HBA plates and the plates were incubated for 48 h as described above. The growth of *C. concisus* strains on HBA plates was determined by the appearance of bacterial colonies examined under a stereomicroscope. The morphology of *C. concisus* was confirmed by the observation of cells under a phase-contrast microscope.

**Table 1. Fifty-eight oral *C. concisus* strains used in this study**

| Strain name | H1O1 | H2O1 | H3O1 | H4O1 | H5O1 | H6O1 | H7O-S1 | H8O-S1, H8O-S2, H8O-S3 | H9O-S1, H9O-S2, H9O-S3 | H10O-S1 | H11O-S1, H11O-S2 | H12O-S1 | H13O-S1 | H14O-S1 | H15O-S1 | H16O-S1 | H17O-S1 | H18O-S1 | H19O-S1 | H20O-S1 |
|-------------|------|------|------|------|------|------|--------|------------------------|------------------------|---------|----------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Strain name | P1CD010, P1CD018 | P2CD01, P2CD02, P2CD03 | P4CD01, P4CD0-S2, P4CD0-S3 | P5CD01 | P6CD01 | P9CD0-S1 | P10CD0-S1, P10CD0-S2 | P11CD0-S1 | P12CD0-S1, P12CD0-S2 | P17CD0-S1 | P18CD0-S1 | P19CD0-S1 | P3UC01 | P7UC01 | P8UC01 | P13UC0-S1, P13UC0-S2, P13UC0-S3 | P14UC0-S1, P14UC0-S2, P14UC0-S3 | P15UC0-S1, P15UC0-S2, P15UC0-S3 | P16UC0-S1, P16UC0-S2 | H1O1 | H2O1 | H3O1 | H4O1 | H5O1 | H6O1 | H7O-S1 | H8O-S1, H8O-S2, H8O-S3 | H9O-S1, H9O-S2, H9O-S3 | H10O-S1 | H11O-S1, H11O-S2 | H12O-S1 | H13O-S1 | H14O-S1 | H15O-S1 | H16O-S1 | H17O-S1 | H18O-S1 | H19O-S1 | H20O-S1 |

Strains named with the letter P or H were isolated from patients with IBD and healthy controls respectively. The number following denotes each unique individual. The following CD or UC indicates the patient disease state being CD or UC. Multiple strains were isolated from some individuals; for example, P2CDO1, P2CDO2 and P2CDO3 are three strains isolated from the same CD patient.
**Determination of c.f.u. of *C. concisus* strains following exposure to pH 3.5 for 30 min.** Twenty *C. concisus* strains, which were randomly selected from the above 58 strains, were further examined for their quantitative growth following exposure to pH 3.5 for 30 min. These strains are indicated in Table 1. These strains were first cultured on HBA for 48 h at 37 °C and the bacterial cells were then incubated in HIB of pH 3.5 and pH 7 at 37 °C for 30 min as described above. Serial dilutions were then prepared from 10\(^{-1}\) to 10\(^{-7}\) and each dilution of the bacterial suspensions (5 µl of individual *C. concisus* strains was then dropped onto HBA plates in quadruplicate and the plates were incubated further for 48 h at 37 °C to determine the c.f.u.

**Determination of c.f.u. of *C. concisus* strains following exposure to different pH for various periods.** The growth of three oral *C. concisus* strains following exposure to different pH for various time points was examined. *C. concisus* strains P2CDO-S2, P3UCO-S1 and H10-S1 were used in this experiment. These three strains were randomly chosen from strains isolated from individuals with different health conditions (CD, UC and healthy controls) that are listed in Table 1.

The *C. concisus* strains were first cultured on HBA plates for 48 h at 37 °C. The bacterial cells were then collected and suspended into separate tubes containing 500 µl PBS and 100 µl was removed to determine the original c.f.u., which was considered as the 0 min count. From the suspensions, 100 µl was pipetted into 9.9 ml HIB with pH values of 5, 3.5 and 2.5, respectively. The HIB solutions with pH values of 5, 3.5 and 2.5 containing *C. concisus* were then incubated as described above and at 15, 30 and 120 min, 1 ml solution was removed from each tube. The gas packs and sodium borohydride were replaced each time the bacterial suspension was removed. The removed *C. concisus* suspension was centrifuged for 2 min at 7900 g and the supernatant discarded. The bacterial cell pellet was resuspended into 1 ml pH 7 HIB and serial dilutions were then prepared from 10\(^{-2}\) to 10\(^{-7}\) and used for the determination of c.f.u. as described above.

**Examination of the growth of different *C. concisus* strains on culture plates containing 2 % ox bile.** Examination of the growth of bacterial species on media containing 2 % ox bile is a standard method to assess bacterial bile resistance (Vandamme et al., 2005). All 58 *C. concisus* strains were examined. *C. concisus* strains were firstly grown on HBA plates. Following a 48 h incubation period at 37 °C, cells of each *C. concisus* strain were collected and resuspended into individual tubes containing 1 ml PBS and the bacterial suspensions were adjusted to OD\(_{595}\) 0.1. From the bacterial suspension of each *C. concisus* strain, 10 µl was inoculated onto an HBA plate and two HBA plates with 2 % (w/v) ox bile (Sigma Aldrich) (HBA\(_{2\text{ bile}}\)).

After incubating for a further 48 h at 37 °C, the growth of each *C. concisus* strain on both HBA and HBA\(_{2\text{ bile}}\) plates was determined by the appearance of bacterial colonies under a stereomicroscope. The morphology of *C. concisus* was confirmed by the observation of cells under phase-contrast microscopy. For *C. concisus* strains that did not show growth on HBA\(_{2\text{ bile}}\) plates, bacterial cells were removed from the primary inoculation site and reincubated onto HBA plates. HBA plates were further cultured for 48 h and *C. concisus* growth was examined again as described above.

**Determination of c.f.u. of *C. concisus* strains grown on HBA and HBA\(_{2\text{ bile}}\) plates.** To quantify the inhibitory effects of bile on *C. concisus* growth, the c.f.u. of 20 strains grown on HBA and HBA\(_{2\text{ bile}}\) plates were determined. These 20 *C. concisus* strains were randomly chosen from strains that were able to grow on HBA\(_{2\text{ bile}}\) plates in the above experiment.

*C. concisus* strains were first cultured at 37 °C for 48 h. Bacterial cells of each *C. concisus* strain were collected and suspended into PBS. The suspensions were then adjusted to an OD\(_{595}\) of 0.1. The suspension of each *C. concisus* strain (10 µl) was inoculated onto two HBA plates and two HBA\(_{2\text{ bile}}\) plates.

Following incubation at 37 °C for 48 h, *C. concisus* cells from each plate were collected into 1 ml PBS, from which nine serial dilutions were prepared (10\(^{-1}\) to 10\(^{-7}\)). Each of the nine dilutions of the bacterial suspension (5 µl of individual *C. concisus* strain was then dropped onto HBA plates in quadruplicate and the plates were incubated further for 48 h at 37 °C and the c.f.u. determined.

**Effects of different concentrations of bile on *C. concisus* growth.** *C. concisus* strain P1CDO-S2, a strain isolated from a patient with CD, was used in this experiment. This strain was randomly chosen from strains that were able to grow in the presence of 2 % ox bile.

The strain was first cultured at 37 °C for 48 h. Bacterial cells were collected to prepare a suspension with an OD\(_{595}\) of 0.1 and 10 µl of the suspension was inoculated onto six HBA plates containing different concentrations of ox bile including 0, 0.01, 0.1, 0.25, 1.5 and 2 % ox bile. Following incubation at 37 °C for 48 h, cells were collected from each plate and resuspended into 1 ml PBS. Six serial dilutions (10\(^{-2}\) to 10\(^{-7}\)) were prepared from the *C. concisus* suspension collected from each HBA plate containing different concentrations of bile and used for determination of c.f.u. as described above.

**Statistical analysis.** Fisher’s exact test (two-tailed) was performed to compare the prevalence of *C. concisus* strains that were resistant to 2 % bile in strains isolated from patients with IBD and healthy controls. Student’s t-tests (unpaired, two-tailed) were used to compare the c.f.u. of *C. concisus* strains. All statistical analysis was performed using Graphpad Prism software, version 6. A P value of less than 0.05 was considered significant.

**RESULTS**

**The growth of *C. concisus* strains after exposure to pH 2.0 for 30 min**

Following the exposure to HIB solution at pH 2.0 for 30 min, none of the 58 *C. concisus* strains examined was able to grow on HBA plates. The same strains exposed to the control HIB solution at pH 7.0 for the same time period all grew on HBA plates.

**The quantitative growth of *C. concisus* strains following exposure to pH 3.5 for 30 min**

Of the 20 strains examined, four strains (4/20, 20 %) grew on HBA plates following exposure to HIB solution at pH 3.5 for 30 min; these four strains were P2CDO2, P10CDO-S2, H100-S1 and H120-S1. Exposure to pH 3.5 for 30 min reduced the growth of the four strains to a significantly lower c.f.u. compared to the same strains exposed to pH 7 for 30 min (P<0.01) (Table 2).

**The quantitative growth of *C. concisus* strains following exposure to different pH for varied periods**

Three *C. concisus* strains were examined. None of the three *C. concisus* strains grew on HBA plates following exposure to HIB medium at pH 2.5 for 15, 30 and 120 min. All three strains were able to grow after 15 min of exposure to HIB.
medium at pH 3.5; however, a reduced number of c.f.u. were obtained. Only the P2CDO2 strain grew following exposure to HIB at pH 3.5 for 30 min; the c.f.u. count was similar to that after exposure for 15 min. After exposure to pH 3.5 for 120 min, none of the three strains grew. Exposure to pH 5 did not appear to affect the growth of the three *C. concisus* strains (Table 3).

### Growth of *C. concisus* on HBA^2% bile plates

Of the 58 *C. concisus* strains examined, 32 strains (55.2%, 32/58) grew on HBA^2% bile. Of the strains isolated from patients with CD and UC, 57.9% (11/19) and 50% (7/14) of strains grew on HBA^2% bile plates, values that were not significantly different to those from healthy controls (56%, 14/25) (*P* > 0.05). The 26 oral strains that did not grow on HBA^2% bile were reinoculated from HBA^2% bile onto HBA plates and cultured for a further 48 h. Following this, 61.5% of the strains (16/26) grew on HBA plates.

### Quantitative measurement of the inhibitory effects of 2% bile to the growth of *C. concisus* strains

The c.f.u. of 20 *C. concisus* strains grown on HBA^2% bile and HBA plates were determined. The presence of 2% bile inhibited the growth of *C. concisus* and the c.f.u. of all strains grown on HBA^2% bile plates were significantly decreased compared to the c.f.u. of the same strains grown on HBA plates (*P* < 0.05, Table 4). The reduction in c.f.u. induced by 2% bile varied among strains. Of the seven *C. concisus* strains isolated from patients with CD, two strains had a log10 c.f.u. reduction of 1.1 and 1.5 (P2CDO3 and P1CDO18) and the remaining strains had a reduction of between 2.2 and 4.0. Of the eight oral *C. concisus* strains isolated from UC patients, the log10 c.f.u. reductions were from 2.1 to 5.7. The five strains isolated from healthy controls had a c.f.u. reduction between 2.4 to 5.9 log10 units (Table 4).

### The impact of different concentrations of bile on *C. concisus* growth

*C. concisus* strain P1CDO2 was used to examine the impact of varying bile concentrations on growth. As the concentration of bile increased, a significant decrease in c.f.u. was observed (*P* < 0.05). The exception was between HBA plates containing 0.5% and 1% bile; the c.f.u. was not significantly different (Fig. 1).

### DISCUSSION

This study examined the effects of pH and ox bile on the growth of oral *C. concisus* strains isolated from patients...
with IBD and controls. The differential growth of C. concisus strains on HBA plates with various pH values and on HBA plates with or without ox bile reported in this study may aid our understanding of the bacterial and environmental factors that may contribute to the colonization of different parts of the gastrointestinal tract by C. concisus. It should be noted that the experimental conditions used in this study do not strictly reflect the

Table 3. Quantitative assessment of the growth of three oral C. concisus strains on HBA plates following exposure to media with pH 2.5, 3.5 and 5 for different times

<table>
<thead>
<tr>
<th>pH</th>
<th>Time (min)</th>
<th>P2CDO2</th>
<th>P3UCO1</th>
<th>H1O1</th>
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<tr>
<td></td>
<td></td>
<td>log_{10} c.f.u. (mean ± SD)</td>
<td>log_{10} c.f.u. (mean ± SD)</td>
<td>log_{10} c.f.u. (mean ± SD)</td>
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<td>0</td>
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<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
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<td>9.5±7.8</td>
<td>9.1±7.9</td>
<td>9.3±8.1</td>
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P2CDO2 was isolated from a patient with CD, P3UCO1 was isolated from a patient with UC and H1O1 was isolated from a healthy individual. Time 0 refers to the log_{10} c.f.u. before exposure to pH change. The mean log_{10} c.f.u. was calculated from the mean of the quadruplicate c.f.u. counts at each condition.

Table 4. Quantitative assessment of the growth of C. concisus strains on HBA and HBA^{2% bile} plates

<table>
<thead>
<tr>
<th>Strain name</th>
<th>log_{10} c.f.u. on HBA (mean ± sd)</th>
<th>log_{10} c.f.u. on HBA^{2% bile} (mean ± sd)</th>
<th>log_{10} c.f.u. reduction</th>
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<td>P1CDO18</td>
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<td>H16O-S1</td>
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</table>

The log_{10} c.f.u. was calculated from quadruplicate counts. The log_{10} c.f.u. reduction refers to the difference between the log_{10} mean c.f.u. on HBA and HBA^{2% bile}. The c.f.u. of all strains on HBA^{2% bile} were significantly lower than those of the same strains on HBA plates (P<0.05). P1CDO18–P12CDO-S3 strains are oral strains from patients with CD. P3UCO-S1–P16UCO-S2 strains are oral strains from patients with UC. H7O-S1–H16O-S1 strains are oral strains from healthy controls.
physiological conditions of the human gastrointestinal tract and the data generated from this study should not be interpreted as mirroring the actual growth of \textit{C. concisus} in the human gastrointestinal tract.

In this study, we found that following exposure to media at pH 2 for 30 min, none of the 58 oral strains of \textit{C. concisus} was able to grow on HBA plates; suggesting that pH 2 either was bactericidal to \textit{C. concisus} or made \textit{C. concisus} enter into the viable but nonculturable state (Xu et al., 1982). \textit{C. concisus} survived better in a higher pH environment. All 58 strains grew on control HBA plates with pH 7. Exposure to pH 5 for 120 min did not appear to have effects on the growth of \textit{C. concisus} (Table 3). Some oral \textit{C. concisus} strains were found to be more resistant to low pH values compared to others. For example, after exposure to pH 3.5 for 30 min, only four of the 20 oral strains examined were able to grow on HBA plates. Of these four strains, P2CDO2 had lowest c.f.u. reduction following exposure to pH 3.5 for 30 min (Table 3). The primary colonization site of \textit{C. concisus} is the human oral cavity, which is between pH 5.3 and 7.8 (Humphrey & Williamson, 2001). The human stomach has an acidic environment, with a range of pH 0.95–2.6 (Press et al., 1998). The finding from this study that \textit{C. concisus} is highly sensitive to low pH suggests that gastric acid may play an important role in preventing swallowed \textit{C. concisus} from colonizing the stomach and the intestinal tract. Both the levels of pH and the exposure time have impact on the growth of \textit{C. concisus}; \textit{C. concisus} strains had different c.f.u. following exposure to different pH for various time points (Table 3). These data suggest that individuals with increased gastric pH, such as those receiving H\textsubscript{2} receptor antagonists or treatment with proton pump inhibitors, may have a higher risk for gastric or intestinal \textit{C. concisus} colonization. A study by Press et al. (1998) showed that in the fasted state, the gastric pH ranged between pH 1.5 and 4.1 (median pH 2.4) in patients with CD and the values ranged from pH 1.5 to 4.4 (median pH 1.95) in patients with UC and pH 0.95 and 2.6 (median pH 1.55) in controls. This suggests that increased gastric pH may be a factor that has contributed to the increased intestinal colonization of \textit{C. concisus} in patients with IBD.

Despite the high sensitivity to low pH, some viable \textit{C. concisus} bacteria may enter the intestinal tract together with food, which may increase the gastric pH, or with liquid, which may permit survival due to a shorter half gastric emptying time. Whether these viable \textit{C. concisus} are able to establish colonization in the intestinal tract may be determined by both the characteristics of a given \textit{C. concisus} strain such as its ability to resist the antimicrobial activity of the bile and an individual’s intestinal environment. The bile enters into the human intestinal tract at the duodenum and the majority of bile acids (95 %) are reabsorbed from the terminal ileum and transported back to the liver. Thus the bile acid concentration starts to fall in the terminal ileum (Chiang, 2009).

The bile acids are known to inhibit bacterial growth, contributing to the low bacterial content of the small intestine (Guarner & Malagelada, 2003). Bacterial species that inhabit the human intestinal tract have intrinsic abilities to resist the antimicrobial activity of bile (Imhoff, 2005). Pathogenic bacterial species that cause human enteric diseases are also bile resistant. For example, \textit{Salmonella enterica} subsp. \textit{enterica} serovar Typhi, which causes typhoid fever, is capable of surviving in the gall bladder (Gonzalez-Escobedo et al., 2011). The majority of the strains of \textit{Campylobacter jejuni} (60–93 %), which can cause gastroenteritis, were reported to be able to grow in the presence of 2 % ox bile (Garrity et al., 2005). A study by Fox et al. (2007) showed that when grown in culture media containing 2.5 % ox bile, the c.f.u. of \textit{C. jejuni} strain NCTC 11168 decreased only 0.625 log\textsubscript{10} units compared to that cultured in media without bile.

Of the 58 oral strains examined in this study, 32 \textit{C. concisus} strains (55.2 %) were able to grow on HBA plates.
containing 2% ox bile. Of the 26 strains that did not grow on HBA\textsuperscript{2\% bile} plates, 16 strains were still able to grow on HBA plates when removed from HBA\textsuperscript{2\% bile} plates following 48 h of incubation, suggesting that the 2% bile had inhibitory effects toward the growth of these \textit{C. concisus} strains, but was not bactericidal.

Despite the finding that approximately 50% of oral \textit{C. concisus} strains were able to grow in the presence of 2% ox bile, the bile appeared to be a potent inhibitor of the growth of these \textit{C. concisus} strains. The c.f.u. reduction induced by 2% ox bile varied considerably among strains and the majority of \textit{C. concisus} strains had more than 2 log c.f.u. reduction in the presence of 2% ox bile (Table 4). The substantial inhibitory effects of bile on \textit{C. concisus} growth suggest that the intestinal tract is not an optimal habitat for \textit{C. concisus}, which may be one of the reasons why \textit{C. concisus} has chosen the oral cavity as its primary colonization site (Zhang \textit{et al.}, 2010).

A small number of oral \textit{C. concisus} strains appeared to have a higher ability to resist the inhibitory effects of bile. For example, while all other strains in Table 4 had more than 2 log c.f.u. reductions in the presence of 2% ox bile, strains P1CDO18 and P2CDO3 had c.f.u. reductions of 1.5 and 1.1. Interestingly, the strain that had the lowest c.f.u. reduction, P2CDO3, was from a relapsed patient with CD who had ileal resection surgery (Mahendran \textit{et al.}, 2013). None of the other patients whose \textit{C. concisus} strains were included in this study had bowel resection. It is possible that CD patients who are colonized with higher bile-resistant \textit{C. concisus} strains in their oral cavity are more likely to develop severe small intestine lesions, increasing the likelihood of requiring surgery. This hypothesis remains to be investigated.

A further finding from this study was that the inhibition of bile to \textit{C. concisus} growth was concentration dependent; a higher concentration of ox bile had a greater inhibition of \textit{C. concisus} growth (Fig. 1). These data suggest that some clinical conditions that have altered bile or bile acid recirculation such as primary sclerosing cholangitis or ileal resection may have effects on \textit{C. concisus} intestinal colonization, which remains to be investigated (Stiehl \textit{et al.}, 1988).

In summary, this study has systematically examined the effects of pH and bile on the growth of \textit{C. concisus}. The study showed that \textit{C. concisus} was very sensitive to low pH. About half of the oral \textit{C. concisus} strains were resistant to 2% bile, but their growth was greatly inhibited by 2% bile. The bile inhibited the growth of \textit{C. concisus} in a dose-dependent manner. Furthermore, \textit{C. concisus} strains were found to have differential abilities to resist low pH and bile. Taken together, these data suggest that both the intrinsic characteristics of \textit{C. concisus} strains and an individual’s gastrointestinal environment may play a role in the determination of \textit{C. concisus} colonization in different parts of the gastrointestinal tract and that increased gastric pH and reduced intestinal bile may be risk factors for increased gastric and intestinal \textit{C. concisus} colonization.

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