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Growth and Survival of *Campylobacter* Pathogens in the Presence of Different Metabolic Inhibitors

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The effect of metabolic inhibitors on the growth of *Campylobacter* (100 human, animal and environmental isolates) was investigated. These inhibitors included β -fluoropyruvate (FP), iodoacetate (IAA, an inhibitor of glycolysis) and α -methyl-D-glucoside (MG, a glucose analogue). In the presence of FP (0.8-1 g L⁻¹), the growth *C. jejuni* was inhibited for 24 h then growth occurred efficiently. However, the growth of *C. coli* was reduced by more than 50% even after 72 h. The growth of *C. doylei* was totally inhibited by the FP concentrations employed. In the presence of IAA (0.0048 g L⁻¹), non of the tested species was able to grow; when half of the IAA concentration was used, *C. coli* grew after 24 h whereas *C. jejuni* grew after 48 h. On the other hand, *C. doylei* was unable to grow even after 72 h. All the strains tested were relatively resistant to high concentration of MG; the growth of *C. jejuni* and *C. doylei* was completely inhibited in the presence of 50 and 40 g L⁻¹ of MG whereas *C. coli* was resistant to MG concentration (70 g L⁻¹) when grown in brain heart infusion medium. This investigation attempts to not only understand better the survival of the organism in the environment but also should assist in finding ways to control *Campylobacter* in the environment and the food chain and hence reduce the risk of infection to human beings.

Key words: *Campylobacter*, metabolic inhibitors, β -fluoropyruvate, iodoacetate, α -methyl-D-glucoside

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INTRODUCTION

Campylobacter species are Gram negative, microaerophilic and/or anaerobic, mainly spiral shaped, bacteria that have a wide range of diversity regarding natural habitats, potential for disease production and procedures for isolation and culturing (Penner and Mills, 1997). *Campylobacter* species can be found as commensal microflora or pathogen in the intestinal tracts of birds, animals or human, human oral cavity and in the genital tract of animals (Tenover and Fennell, 1992; Harvey *et al.*, 2003). Also, *Campylobacter* has been isolated from fresh water and seawater (Terzieva and McFeters, 1991; Moore *et al.*, 2002; Cools *et al.*, 2003) and from shellfish (Sails *et al.*, 2003).

Campylobacters vary in producing disease such as diarrhoea, bacteraemia, septic abortion, infertility and possibly disease of human teeth (MacLaren and Agumbah, 1988; Abderhalden *et al.*, 1995; Akiba *et al.*, 2002; Belongia *et al.*, 2003; Patel *et al.*, 2003). Since 1981, *Campylobacter* have been the most commonly reported cause of human gastro-intestinal illness in England and Wales (Frost *et al.*, 2002). In addition, in a small minority of patients who are infected with a particular serotype of *C. jejuni* may develop Guillain-Barre' syndrome (Ogawara *et al.*, 2003), Miller-Fisher syndrome (Ang *et al.*, 2002) or reactive arthritis (Soderlin *et al.*, 2003). The handling or consumption of raw or undercooked meat products and occasionally contaminated water or raw milk, can be considered as main vehicles for campylobacteriosis transmission (Sopwith *et al.*, 2003). A number of species may be involved in human disease, but the majority of cases are caused by *C. jejuni* (Frost *et al.*, 2002). In addition, campylobacters are varied in their requirements for growth, composition of the medium, temperature and atmospheric conditions. All *Campylobacter* species utilise organic amino acids or citric acid cycle intermediates to obtain energy and non-of them ferment or oxidise carbohydrates (Smibert, 1978).

Campylobacters have been classified and reclassified as the progress of research into the organism has advanced. Currently, the genus *Campylobacter* contains 16 species and 6 subspecies. Recently, Penner and Mills (1997) reviewed and divided *Campylobacter* species into three groups on the basis of the optimum temperature for growth, the habitat and the diseases associated with the organism. These groups included thermophilic enteropathogenic species, animal pathogens and commensals that may infect human and species associated with periodontal disease.

Until 1973, it was unknown that this group is the most important agent linked to diarrhoeal disease in humans. However, the true significance of

Campylobacter to human health was established in 1977 when a selective medium was designed for their isolation (Skirrow, 1977). Since that time, it was found that all the members of this group are pathogenic and rarely recovered from healthy humans (On, 2001; Belongia *et al.*, 2003). Thermophilic *Campylobacter* species, particularly, *C. jejuni* and *C. coli* are now recognised as the most common causative agents of bacterial gastroenteritis in humans (Coker *et al.*, 2002; Frost *et al.*, 2002; Belongia *et al.*, 2003; Sandberg *et al.*, 2006; Hansson *et al.*, 2007). In developing countries, the highest incidence is in infants and young children (Ketley, 1997; Coker *et al.*, 2002; Fullerton *et al.*, 2007) whereas in developed countries young adults are mostly the targets, aged 18-29 years old (Rosenquist *et al.*, 2003).

Campylobacter spp. continue to be the greatest cause of bacterial gastrointestinal infections in humans worldwide. Some foodborne pathogens tend to be widely distributed in the environment and have different means of surviving and being transmitted to different hosts. Therefore, multi intervention strategies may be required to successfully reduce the risk of food pathogens in poultry, livestock and humans. Because of its impact on human and animal health, this preliminary study is aimed at trying to reduce the incidence of *Campylobacter* in the environment and possibly through the food chain.

MATERIALS AND METHODS

Bacterial strains: The strains tested were type strains (*C. jejuni* subsp. *jejuni* NCTC 11168, *C. jejuni* subsp. *doylei* NCTC 11951 and *C. coli* NCTC 11366) obtained from the National Collection of Type Culture, Colindale, UK, human clinical isolates (80 isolates) and animal, poultry and environmental isolates (10 *C. jejuni* and 7 *C. coli*). Upon receipt, all the strains were grown on Preston medium (Oxoid), for 48-72 h at 37°C under microaerobic conditions (5% O₂, 10% CO₂, 85% N₂, CampyGen™ Oxoid) (Bolton *et al.*, 1997). Typical *Campylobacter* colonies were subcultured in brain heart infusion (BHI) broth medium (Oxoid) and incubated under similar conditions. The resulting cultures were dispensed into cryotubes containing 14-20% glycerol and kept at -70°C for further use.

Campylobacter strains were identified by Gram stain, morphology and conventional biochemical tests, API Campy or hippurate test, nitrate reduction, catalase and oxidase tests.

Culture media

Brain heart infusion broth medium: Brain Heart Infusion (BHI) (Oxoid, CM225) is a nutritionally complex rich medium, originally devised for the growth of fastidious

bacteria. The cells grown in BHI were used to study the effect of biochemical inhibitors on the growth and metabolism of *Campylobacter*. The medium was prepared as described by the manufacturer and was autoclaved at 15 lbs per square inch (psi), equivalent to 121°C for 15 min.

Basal medium: Basal medium was used to study the effect of different concentrations of glucose, glucose analogue on the growth of *Campylobacter* strains. The composition of this medium was as follows: Trypticase 20 g L⁻¹, Yeast extract 2 g L⁻¹, Sodium bisulphite 0.1 g L⁻¹, Sodium chloride 5 g L⁻¹, pH 7.4. The medium was sterilised by autoclaving as above.

Assessment of growth optical density measurements:

Optical Density (OD) was used to measure the growth of organisms in broth medium (basal and BHI medium). The OD was measured using Gallenkamp Visispectrophotometer using 600_{nm} filter. At different time intervals, a sample was taken from growing organisms in the medium with or without the biochemical inhibitor. The OD_{600_{nm}} of each sample was measured and the growth pattern was established.

RESULTS AND DISCUSSION

Effect of β-fluoropyruvate (FP) on the growth of *Campylobacter* species in broth media:

The effect of FP on the growth of *C. jejuni* and *C. coli*, in broth media, was varied (Fig. 1-2). The inhibitory effect was observed at 0.8-1 g L⁻¹. FP inhibited the growth of *C. jejuni*, but growth subsequently occurred, efficiently, within 24 h (Fig. 1). On the other hand, a marked inhibitory effect of FP on growth of *C. coli* (more than 50% reduction) was constant after 72 h incubation at 37°C (Fig. 2). Whereas the growth of *C. subsp. doylei* was completely inhibited for even more than 96 h (Fig. 3). These results indicated variability in the resistance between *C. jejuni* subsp. *jejuni* and *C. coli* to FP. *C. jejuni* adapted to grow well in the presence of 0.8 to 1 g L⁻¹ FP after 24 h incubation, whereas the reduction in the growth of *C. coli* was steady for 72 h in the presence of the same concentrations of the inhibitor (Fig. 1-2). In contrast, *C. subsp. doylei* was extremely sensitive and the growth was inhibited even for more than 96 h (Fig. 3).

The inhibitory effects of β-fluoropyruvate on the growth of *Campylobacter* strains may be due to the binding of the FP to the TPP component of pyruvate oxidoreductase and consequently inactivate this enzyme.

The results showed that both of *C. jejuni* and *C. coli* are relatively resistant to FP. However, the initial effects of

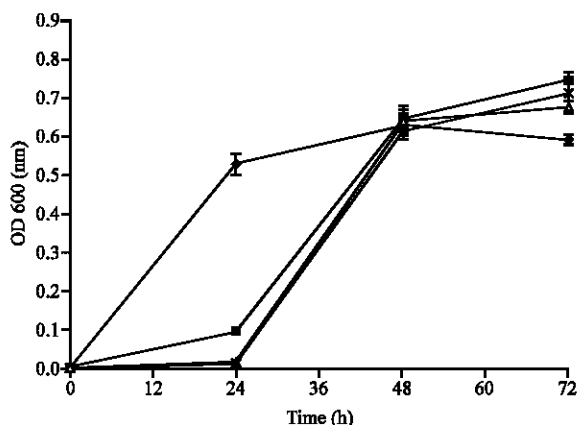


Fig. 1: Effect of various concentrations of β-fluoropyruvate (FP) on the growth of *C. jejuni* NCTC type strain (11168). Growth was measured by optical density (OD), (◆): growth with out FP 0; (■): growth with FP 0.2 g L⁻¹; (▲): growth with FP 0.4 g L⁻¹; (×): growth with FP 0.8 g L⁻¹

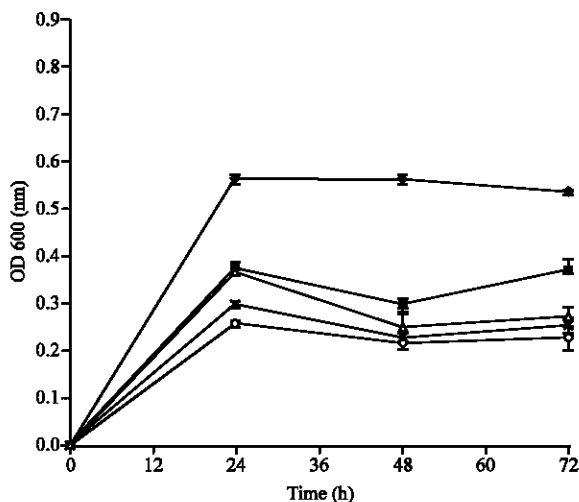


Fig. 2: Effect of various concentrations of β-fluoropyruvate (FP) on the growth of *C. coli* NCTC type strain (11366). Growth was measured by optical density (OD), (◆): growth without FP; (■): growth with FP 0.2 g L⁻¹; (▲): growth with FP 0.4 g L⁻¹; (×): growth with FP 0.8 g L⁻¹; (○): growth with FP 1 g L⁻¹

FP (0.8-1 g L⁻¹) on strains tested suggested that FP may have inhibitory effects on the metabolic pathways of these organisms. In addition, the differences in the period of the initial inhibition effects on the growth of *Campylobacter* species (24 h for *C. jejuni* and 72 h for *C. coli* and more than 96 h for *C. jejuni* subsp. *doylei*) suggested that these species may have different

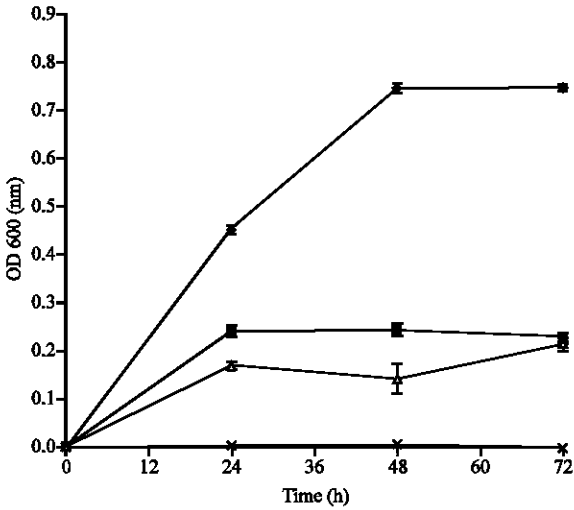


Fig. 3: Effect of various concentrations of β -fluoropyruvate (FP) on the growth of *C. doylei* NCTC type strain (11951). Growth was measured by optical density (OD), (◆): growth without FP; (■): growth with FP 0.2 g L⁻¹; (▲): growth with FP 0.4 g L⁻¹; (×): growth with FP 0.8 g L⁻¹

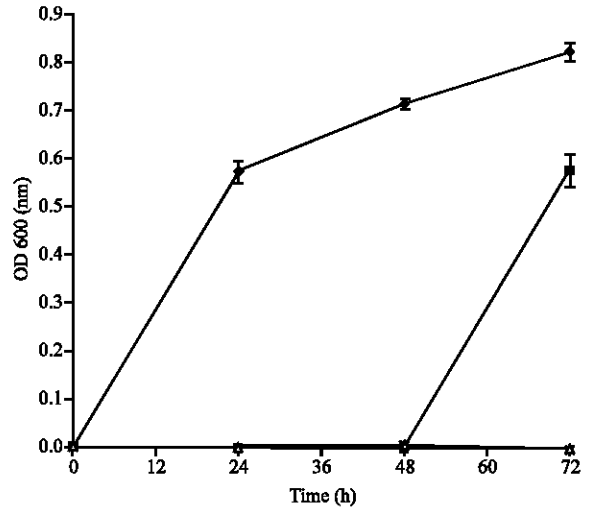


Fig. 4: Effect of various concentrations of iodoacetic acid (IAA) on the growth of *C. jejuni* NCTC type strain (11168). Growth was measured by optical density (OD), (◆): growth without IAA; (■): growth with IAA 0.0024 g L⁻¹; (▲): growth with IAA 0.0048 g L⁻¹; (×): growth with IAA 0.0096 g L⁻¹

metabolic pathways for pyruvate. On the other hand, the significant sensitivity of *C. jejuni* subsp. *doylei* to FP can distinguish it from *C. jejuni* subsp. *jejuni* and *C. coli*. The data presented are representative of a total of 100 strains tested.

Effect of iodoacetate (IAA) on the growth of *Campylobacter* species in broth medium: The effect of IAA on the growth of *Campylobacter* species was studied in broth medium. None of the tested species was able to grow in the presence of IAA at concentration of 0.0048 g L⁻¹ (Fig. 4-6). However, varied effect of IAA at concentration of 0.0024 g L⁻¹ on the growth of *Campylobacter* species was noted (Fig. 4-6). *C. coli* was able to grow, in the presence 0.0024 g L⁻¹ of IAA, after 24 h of incubation, whereas *C. jejuni* was able to grow after 48 h in the presence of the same concentration of IAA. On the other hand, *C. doylei* was unable to grow even after 72 h (Fig. 6).

Since the glycolytic enzyme, glyceraldehydes 3-phosphate dehydrogenase, is the presumed target for IAA, it was envisaged that non fermentative organisms or those which do not oxidise glucose would not be susceptible to the inhibitory effects of IAA. However, this was not the case in this study. All *Campylobacter* species tested were relatively sensitive to IAA. This suggests that iodoacetate may inhibit glucose synthesis pathway (gluconeogenesis) in these organisms. All the enzymes of glucose synthesis pathway

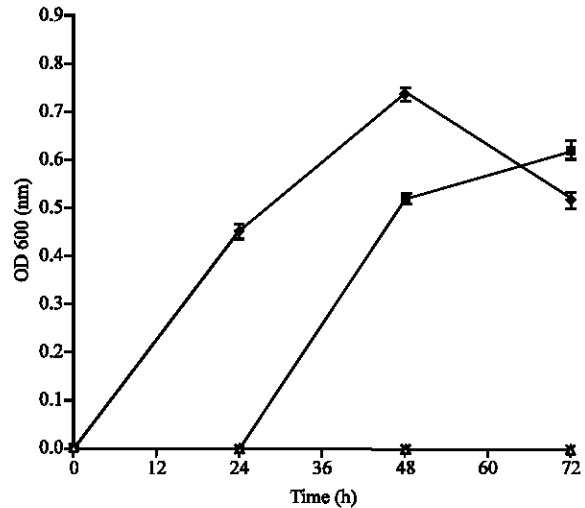


Fig. 5: Effect of various concentrations of iodoacetic acid (IAA) on the growth of *C. coli* NCTC type strain (11366). Growth was measured by optical density (OD), (◆): growth without IAA; (■): growth with IAA 0.0024 g L⁻¹; (▲): growth with IAA 0.0048 g L⁻¹; (×): growth with IAA 0.0096 g L⁻¹

have been demonstrated in *Campylobacter* including glyceraldehydes 3-phosphate dehydrogenase (Wang, 1975; Parkhill *et al.*, 2000). The variation in the inhibitory effects on the growth of *Campylobacter* species, in the lag phase, at low concentration of IAA (Fig. 4-6) might

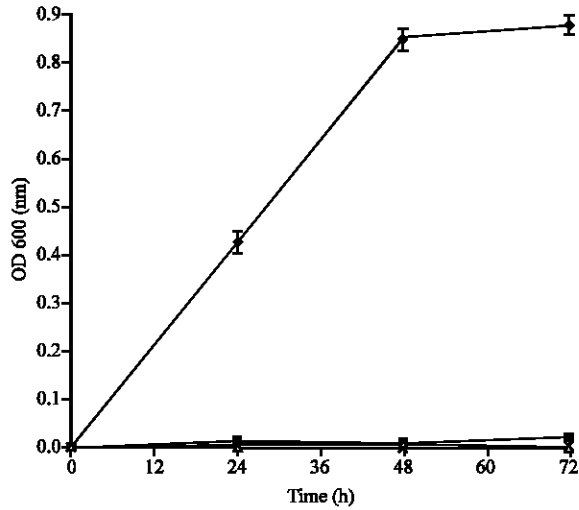


Fig. 6: Effect of various concentrations of iodoacetic acid (IAA) on the growth of *C. doylei* NCTC type strain (11951). Growth was measured by optical density (OD), (◆): growth without IAA; (■): growth with IAA 0.0024 g L⁻¹; (▲): growth with IAA 0.0048 g L⁻¹; (×): growth with IAA 0.0096 g L⁻¹

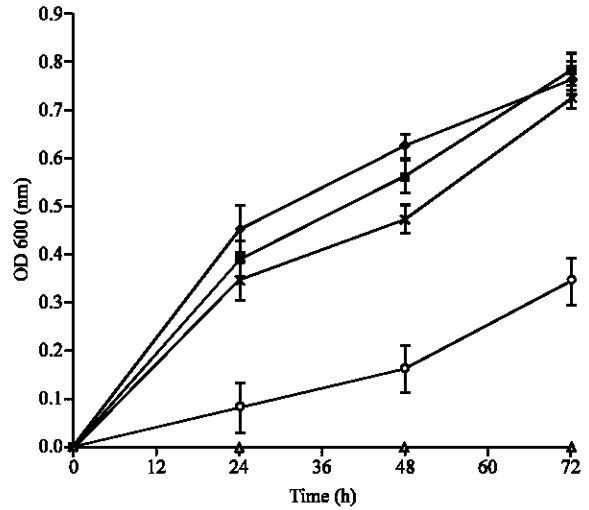


Fig. 7: Effect of various concentrations of α -methyl-D-glucoside (MG) on the growth of *C. jejuni* NCTC type strain (11168) in brain heart infusion liquid medium. Growth was measured by optical density (OD), (◆): growth without MG; (■): growth with MG 10 g L⁻¹; (×): growth with MG 30 g L⁻¹; (○): growth with MG 40 g L⁻¹; (▲): growth with MG 50 g L⁻¹

be useful in the development of diagnostic tests or to improve selective media. In addition, these results suggest that *Campylobacter* species (*C. coli* and *C. jejuni*) may have inducible enzymes, which may become active and enable these organisms to adapt and grow under stressed conditions. The results also showed that such supposed enzymes were varied in their response to the presence of IAA.

Effect of α -methyl-D-glucoside (MG) on the growth of *Campylobacter* species in broth medium: All the strains tested were relatively resistant to high concentration of MG. However, the resistance to MG was varied amongst *Campylobacter* species (Fig. 7-9). Whereas *C. coli* was resistant to MG concentration of 70 g L⁻¹, the growth of *C. doylei* and *C. jejuni* was completely inhibited in the presence of 40 and 50 g L⁻¹ of MG, respectively (the growth was in BHI medium).

The susceptibility of *Campylobacter* to MG was investigated in three types of media containing different amounts of glucose (BHI containing glucose, glucose free BHI and glucose free basal medium).

The susceptibility of strains tested would be increased in medium containing lowest level of glucose. To the contrary, decreased glucose concentration resulted in a marked increase in resistance to MG.

In glucose-free BHI medium, the resistance of all strains tested was markedly increased (Fig. 8). These

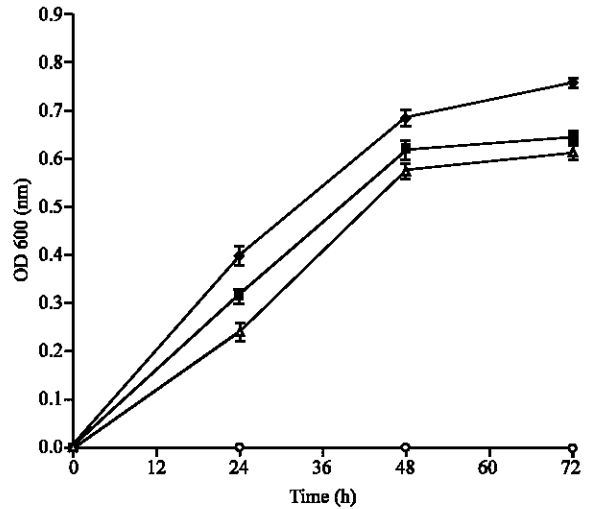


Fig. 8: Effect of various concentrations of α -methyl-D-glucoside (MG) on the growth of *C. doylei* NCTC type strain (11951) in brain heart infusion medium. Growth was measured by optical density (OD), (◆): growth without MG; (■): growth with MG 10 g L⁻¹; (×): growth with MG 30 g L⁻¹; (○): growth with MG 40 g L⁻¹

results suggest that the inhibitory effect of high concentration of MG may be due to an increased medium osmotic pressure rather than blocking glucose transport.

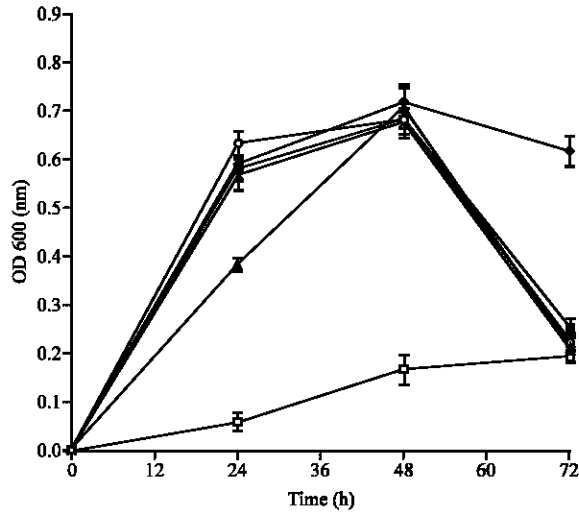


Fig. 9: Effect of various concentrations of α -methyl-D-glucoside (MG) on the growth of *C. coli* NCTC type strain (11366) in brain heart infusion medium. Growth was measured by optical density (OD), (◆): growth without MG; (■): growth with MG 10 g L⁻¹; (×): growth with MG 30 g L⁻¹; (○): growth with MG 40 g L⁻¹; (▲): growth with MG 50 g L⁻¹; (□): OD growth with MG 70 g L⁻¹

The effect of increased glucose and glucose analogue concentrations on growth of *C. jejuni* in basal medium was also investigated (data not shown). The results indicated that the growth was delayed when glucose concentrations was increased (50 g L⁻¹). This inhibition was the same as that caused by equivalent concentration of MG indicating that the inhibitory effect may have been caused by the increase in medium osmotic pressure rather than direct inhibition by the biochemical (Fig. 9). However, the relative resistance of *Campylobacter* species to high concentration of MG and the variation in their resistance did differentiate them and might be useful in the development of diagnostic tests or selective media.

CONCLUSION

The employment of the metabolic inhibitors, used in this study, should throw some light at better understanding how these organisms can survive in the environment. Bearing in mind that, in the laboratory, the organism grows only in microaerophilic conditions and yet in the environment such conditions are not easily available. Because different species behaved differently in the presence of different metabolic inhibitors, it is becoming possible to appreciate the metabolic diversity

within campylobacters. Some species may have adapted different pathways for the metabolism of certain substrates depending on the conditions they are exposed to. Ultimately, the control of campylobacters, which are widely distributed in the environment and have been responsible for several cases of gastroenteritis in humans, is a necessity.

REFERENCES

- Abderhalden, R., I. Hosli, A. Dallas and R. Frei, 1995. Septic abortion in *Campylobacter jejuni* infection. *Geburtshilfe Frauenheilkd*, 55 (10): 99-601.
- Akiba, T., K. Akiba, N. Suto, K. Kumagai, M. Sakamoto and N. Yazak, 2002. *Campylobacter coli* bacteraemia in an 11-year-old boy. *Paediat. Int.*, 4 (5): 543-544.
- Ang, C.W., P.G. Noordzij, M.A. de Klerk, H.P. Endtz, P.A. van Doorn and J.D. Laman, 2002. Ganglioside mimicry of *Campylobacter jejuni* lipopolysaccharides determines antiganglioside specificity in rabbits. *Infect. Immun.*, 9: 5081-5085.
- Belongia, E.A., P.H. Chyou, R.T. Greenlee, G. Perez-Perez, W.F. Bibb and E.O. DeVries, 2003. Diarrhoea incidence and farm-related risk factors for *Escherichia coli* O₁₅₇:H₇ and *Campylobacter jejuni* anti bodies among rural children. *J. Infect. Dis.*, 187 (9): 1460-1468.
- Bolton, F.J., D.R.A. Wareing and A.D. Sails, 1997. Comparison of a novel microaerobic system with three other gas-generating systems for the recovery of *Campylobacter* species from human faecal sample. *Eur. J. Clin. Microbiol. Infect. Dis.*, 16 (11): 839-842.
- Coker, A.O., R.D. Isokpehi, B.N. Thomas, K.O. Amisu and C.L. Obi, 2002. Human campylobacteriosis in developing countries. *Emerging. Infect. Dis.*, 8 (3): 237-244.
- Cools, I.M. Uyttendaele, C. Caro, E. D'Haese, H.J. Nelis and J. Debevere, 2003. Survival of *Campylobacter jejuni* strains of different origin in drinking water. *J. Applied Microbiol.*, 94 (5): 886-892.
- Frost, J.A., I.A. Gillespie and S.J. O'Brien, 2002. Public health implications of *Campylobacter* outbreaks in England and Wales, 1995-9: Epidemiological and microbiological investigations. *Epidemiol. Infect.*, 128 (2): 111-118.
- Fullerton, K.E., L.A. Ingram, T.F. Jones, B.J. Anderson, P.V. McCarthy, S. Hurd, B. Shiferaw, D. Vugia, N. Haubert, T. Hayes, S. Wedel, E. Scallan, O. Henao and F.J. Angulo, 2007. Sporadic *Campylobacter* infection in infants: A population-based surveillance case-control study. *Pediat. Infect. Dis. J.*, 26 (1): 19-24.

- Hansson, I., I. Vagsholm, L. Svensson and E. Olsson-Engvall, 2007. Correlations between *Campylobacter* spp. prevalence in the environment and broiler flocks. J. Applied Microbiol., 103 (3): 640-649.
- Harvey, S.A., P.J. Winch, E. Leontsini, C. Torres-Gayoso, S. Lopez-Romero, R.H. Gilman and R.A. Oberhelma, 2003. Domestic poultry-raising practices in a Peruvian shantytown: Implications for control of *Campylobacter jejuni*-associated diarrhoea. ACTA Tropica, 86 (1): 41-54.
- Ketley, J.M., 1997. Pathogenesis of enteric infection by *Campylobacter*. Microbiology, 143 (Pt 1): 5-21.
- MacLaren, A.P. and G.J. Agumbah, 1988. Infertility in cattle in South-West Scotland caused by an intermediate strain of *Campylobacter fetus* subspecies *fetus* (formerly *Campylobacter fetus intestinalis*). Br. Vet. J., 144 (1): 29-44.
- Moore, J.E., D. Gilpin, E. Crothers, A. Canney, A. Kaneko and M. Matsuda, 2002. Occurrence of *Campylobacter* spp. and *Cryptosporidium* spp. in seagulls (*Larus* spp.). Vect. Borne Zoonotic Dis., 2 (2): 111-114.
- Ogawara, K., S. Kuwabara, M. Koga, M. Mori, N. Yuki and T. Hattori, 2003. Anti-GM1b IgG antibody is associated with acute motor axonal neuropathy and *Campylobacter jejuni* infection. J. Neur. Sci., 210 (1-2): 41-45.
- On, S.L., 2001. Taxonomy of *Campylobacter*, *Arcobacter*, *Helicobacter* and related bacteria: Current status, future prospects and immediate concerns. J. Applied Microbiol., 90: 1S-15S.
- Parkhill, J.B., W. Wren, K. Mungall, J.M. Ketley, C. Churcher, D. Basham, T. Chillingworth, R.M. Davies, T. Feltwell, S. Holroyd, K. Jagels, A.V. Karlyshev, S. Moule, M.J. Pallen, C.W. Pen, M.A. Quail, M.A. Rajandream, K.M. Rutherford, A.H. van Vliet, S. Whitehead and B.G. Barrell, 2000. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. Nature, 403 (6770): 665-668.
- Patel, M., M. Coogan and J.S. Galpin, 2003. Periodontal pathogens in subgingival plaque of HIV-positive subjects with chronic periodontitis. Oral Microbiol. Immunol., 18 (3): 199-201.
- Penner, J.L. and S.D. Mills, 1997. *Campylobacter* and *Arcobacter*. In: Principles and Practice of Clinical Bacteriology, Emmerson, A.M., P.M. Hawkey and S.H. Gillespie (Eds.). John Wiley and Sons Ltd., pp: 555-580.
- Rosenquist, H., N.L. Nielsen, H.M. Sommer, B. Norrung and B.B. Christensen, 2003. Quantitative risk assessment of human campylobacteriosis associated with thermophilic *Campylobacter* species in chickens. Int. J. Food Microbiol., 83 (1): 87-103.
- Sails, A.D., A.J. Fox, F.J. Bolton, D.R. Wareing and D.L. Greenway, 2003. A real-time PCR assay for the detection of *Campylobacter jejuni* in foods after enrichment culture. Applied Environ. Microbiol., 69 (3): 1383-1390.
- Sandberg, M., K. Nygard, H. Meldal, P.S. Valle, H. Kruse and E. Skjerve, 2006. Incidence trend and risk factors for campylobacter infections in humans in Norway. BMC. Public Health, 6 (Jul 7): 179.
- Skirrow, M.B., 1977. *Campylobacter* enteritis: A new disease. Br. Med. J., 2 (6078): 9-11.
- Smibert, R.M., 1978. The genus *Campylobacter*. Ann. Rev. Microbiol., 32: 673-709.
- Soderlin, M.K., H. Kautiainen, M. Puolakkainen, K. Hedman, M. Soderlund-Venermo, T. Skogh and M. Leirisalo-Repo, 2003. Infections preceding early arthritis in southern Sweden: A prospective population-based study. J. Rheum., 30 (3): 459-464.
- Sopwith, W., M. Ashton, J.A. Frost, K. Tocque, S. O'Brien, M. Regan and Q. Syed, 2003. Enhanced surveillance of *Campylobacter* infection in the North West of England 1997-1999. J. Infect., 46 (1): 35-45.
- Tenoverand, F.C. and C.L. Fennell, 1992. The Genera *Campylobacter* and *Helicobacter*. In: A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Application, Balows, A., H.G. Truper and M. Dworkin *et al.* (Eds.). Spring-Verlag, New York, pp: 3488-3511.
- Terzieva, S.I. and G.A. McFeters, 1991. Survival and injury of *E. coli*, *Campylobacter jejuni* and *Yersinia enterocolitica* in stream water. Can. J. Microbiol., 37 (10): 785-790.
- Wang, P.T., 1975. Metabolic pathways of *Campylobacter fetus*. Ph.D Thesis, Virginia Polytechnic Institute and State University.