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Phenotypic MicroArray (PM) profiles (carbon sources and sensitivity to osmolytes and pH) of *Campylobacter jejuni* ATCC 33560 in response to temperature

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Abstract: The present study aimed to provide an insight of *C. jejuni* ATCC 33560 phenotype profiles (carbon sources and sensitivity to osmolytes and pH) using Phenotypic MicroArray (PM) system in response to optimal and suboptimal temperature. *C. jejuni* ATCC 33560 showed utilization carbon sources from amino acids and carboxylates but not from sugars. *C. jejuni* ATCC 33560 is sensitive to NaCl at 2% and above but showed survival in a wide range of food preservatives (sodium lactate, sodium phosphate, sodium benzoate, ammonium sulphate and sodium nitrate). When incubated at suboptimal temperature, no phenotype loss was observed in carbon source plates. Phenotype loss of *C. jejuni* ATCC 33560 was observed in sodium chloride (1%), sodium sulphate (2-3%), sodium formate (1%), sodium lactate (7-12%), sodium phosphate pH7 (100mM and 200mM), ammonium sulphate pH8 (50mM), sodium nitrate (60mM, 80mM and 100mM), sodium nitrite (10mM), and growth in pH5. The phenotypic profile from present study will provide a better insight related to survival of *C. jejuni* ATCC 33560.

Keywords: phenotypic MicroArray profile, *C. jejuni* ATCC 33560, temperature and food preservatives

Introduction

Campylobacter jejuni has been implicated to be the most common species in the genus *Campylobacter* to cause illness in man. *C. jejuni* is known to be very sensitive organisms to environmental stresses (Park, 2002). *Campylobacter* is easily loss its culturability due to environmental stresses and thus making it difficult to be detected. *Campylobacter* had previously been under-reported due to its fastidious and require specific condition to grow. Despite of being sensitive and fastidious organisms, *C. jejuni* turn out to be an important food-borne pathogen (Park, 2002). In order to be successful foodborne pathogens, *C. jejuni* has to overcome the environmental stresses outside the host to get into the risk factors that contribute to human infections. *C. jejuni* is thought to survive in environment rather than grow in the environment as the minimal growth temperatures varies from 32°C to 36°C (Hazeleger et al., 1998).

Food has long been considered as the main factor in causing *Campylobacter* infections in human. Besides that, man can also be infected by *Campylobacter* through environmental factors, such as recreational waters and pet animals. *C. jejuni* is recognised to be prevalent in animal products (Denis et al., 2001; Jacobs-Reitsma et al., 2008; Tang et al., 2010). However, recent investigation has also shown that *C. jejuni* is also prevalent in fresh produce (Chai et al., 2007). It is very surprising and interesting that how such sensitive and fragile organism survive through environment and food preparation chain to be one of the most important foodborne pathogens in recent years. Despite *C. jejuni* being an important

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food-borne pathogen, its physiology has not been studied in as much detail as other enteric pathogens (Kelly, 2005). *C. jejuni* is a difficult organism to culture, has complex nutritional requirements and is microaerophilic. Kelly (2005) highlighted combinations of such conditions has hindered progress in elucidating many aspects of *C. jejuni* physiology and metabolism.

Technology breakthrough in global phenotyping of bacteria had provided better insight on bacteria physiology and metabolism (Bochner et al., 2001; Bochner, 2009). Recently, Phenotypic MicroArray (PM) profiling and analysis on bacteria have gained popularity (Eiff et al., 2006; Funchain et al., 2000; Tracy et al., 2002; Zhou et al., 2003). This technology is an integrated system of cellular assays, instrumentation, and bioinformatic software for high throughput screening of cellular phenotypes (Bochner et al., 2001; Bochner, 2009).

This study describes the phenotypic profiles of *C. jejuni* ATCC 33560 at optimal growth temperatures and at temperatures below its minimal growth temperatures. The significance of the present study is that it provides the insight regarding *Campylobacter jejuni* ATCC 33560 respiration in substrates, osmolytes and pH range in response to temperature.

Materials and Methods

Bacteria strain

C. jejuni ATCC 33560 in glycerol stock was revived in Bolton broth under microaerophilic condition for 48 h at 42°C. The cells were subcultured on Brain Heart Infusion agar (Merck, Germany) with 5% lysed horse blood added and incubated for 48 h at 42°C.

Preparation of PM1 and PM2A

C. jejuni cells were inoculated into 8 mL of 1.2x IF-0a to get 16%T (transmittance) using turbidimeter (Biolog, Hayward, USA). IF-0a is buffer salt solution which maintains the viability of bacteria cells but does not contain any carbon source. The cell suspension was added to 16 mL of PM1 and PM2A inoculating fluid and the final cell density was 52%T. Each PM1 and PM2A inoculating fluid consist of 10 mL IF-0a GN/GP (1.2x), 0.12 mL dye mix D (100x), 1.0 mL PM additive (12x) and 0.88 mL sterile distilled water. PM additives (12x) for PM1 and PM2A consist of 0.6% Bovine Serum Albumin (Sigma) and 15 mM $NaHCO_3$ (Sigma). The prepared cell suspension (52%T) was used to inoculate PM1 and PM2A with 100 µL/well.

Preparation of PM9 and PM10

C. jejuni cells were inoculated into 8 mL of 1.2x IF-10a to get 16%T (transmittance) using turbidimeter (Biolog, Hayward, USA). IF-10a is a solution which contains nutrients that supports growth. The cell suspension was added to 16 mL of PM9 and PM10 inoculating fluid and the final cell density was 72%T. Each PM9 and PM10 inoculating fluid consist of 10 mL IF-10a GN/GP (1.2x), 0.12 mL dye mix D (100x), 1.0 mL PM additive (12x) and 0.88 mL sterile distilled water. PM additives (12x) for PM9 and PM10 consist of 0.6% Bovine Serum Albumin (Sigma), 15 mM $NaHCO_3$ (Sigma) and 12 mM α-ketoglutarate (Sigma). The prepared cell suspension (72%T) was used to inoculate PM9 and PM10 with 100 µL/well.

Incubation and data recording

The PM panels were placed without lid into gas impermeable bag (Biolog, Hayward, USA) with microaerophilic environment generated by CO_2 Gen Compact (Oxoid, Hampshire, England) and sealed using impulse sealer. The PM panels were incubated in the OmniLog instrument at 42°C (optimal temperature) and 30°C (suboptimal temperature). The recordings of phenotypic data were performed by OmniLog instrument which captured a digital image of the microarray and stored the quantitative color changes values in computer file. The computer files could be displayed in the form of kinetic graphs. For each incubation temperature, 382 phenotypes were recorded four times each hour by the OmniLog. Of these, 370 phenotypes were of interest in the present study.

Results and Discussions

Summary on PM profile of *C. jejuni* ATCC 33560 in various substrate, osmolytes and pH at optimal growth temperature (42°C) is shown in Table 1, 2, 3, and 4. Out of 190 carbon source tested (Table 1 and 2), *C. jejuni* ATCC 33560 was found to grow in L-aspartic acid (PM1,A7), L-proline (PM1,A8), L-lactic acid (PM1,B9), L-glutamic acid (PM1,B12), L-asparagine (PM1,D1), L-serine (PM1,G3), succinic acid (PM1,A5), D, L-malic acid (PM1,C3), α-hydroxy-butyric-acid (PM1,E7), fumaric acid (PM1,F5), bromo-succinic acid (PM1,F6), mono methyl succinate (PM1,G9), methyl pyruvate (PM1,G10), D-malic acid (PM1,G11) and L-malic acid (PM1,G12).

For sensitivity to osmolytes and pH (Table 3 and 4), *C. jejuni* ATCC 33560 found to be very sensitive to sodium chloride (NaCl) with detectable growth

Table 1. Phenotype microarray profile for *C. jejuni* ATCC 33560 on PM1 plates incubated at 42°C and 30°C.
(a) PM profile from well A1 to D12, (b) PM profile from well E1 to H12.

(a)

°C		
42	30	
-	-	(A1) Negative Control
-	-	(A2) L-Arabinose
-	-	(A3) N-Acetyl-D-Glucosamine
-	-	(A4) D-Sacharic Acid
+	+	(A5) Succinic Acid
-	-	(A6) D-Galactose
+	+	(A7) L-Aspartic Acid
+	+	(A8) L-Proline
-	-	(A9) D-Alanine
-	-	(A10) D-Trehalose
-	-	(A11) D-Mannose
-	-	(A12) Dulcitol
-	-	(B1) D-Serine
-	-	(B2) D-Sorbitol
-	-	(B3) Glycerol
-	-	(B4) L-Fucose
-	-	(B5) D-Glucuronic Acid
-	-	(B6) D-Gluconic Acid
-	-	(B7) D,L-a-Glycerol- Phosphate
-	-	(B8) D-Xylose
+	+	(B9) L-Lactic Acid
-	-	(B10) Formic Acid
-	-	(B11) D-Mannitol
+	+	(B12) L-Glutamic Acid
-	-	(C1) D-Glucose-6-Phosphate
-	-	(C2) D-Galactonic Acid-g-Lactone
+	+	(C3) D,L-Malic Acid
-	-	(C4) D-Ribose
-	-	(C5) Tween 20
-	-	(C6) L-Rhamnose
-	-	(C7) D-Fructose
-	-	(C8) Acetic Acid
-	-	(C9) a-D-Glucose
-	-	(C10) Maltose
-	-	(C11) D-Melibiose
-	-	(C12) Thymidine
+	+	(D1) L-Asparagine
-	-	(D2) D-Aspartic Acid
-	-	(D3) D-Glucosaminic Acid
-	-	(D4) 1,2-Propanediol
-	-	(D5) Tween 40
-	-	(D6) a-Keto-Gluatic Acid
-	-	(D7) a-Keto-Butyric Acid
-	-	(D8) a-Methyl-D-Galatoside
-	-	(D9) a-D-Lactose
-	-	(D10) Lactulose
-	-	(D11) Sucrose
-	-	(D12) Uridine

(b)

°C		
42	30	
-	-	(E1) L-Glutamine
-	-	(E2) m-Tartaric Acid
-	-	(E3) D-Glucose-1-Phosphate
-	-	(E4) D-Fructose-6-Phosphate
-	-	(E5) Tween 80
-	-	(E6) a-Hydroxy Glutaric Acid-g-Lactone
+	+	(E7) a-Hydroxy-Butyric Acid
-	-	(E8) β-Methyl-D-Glucoside
-	-	(E9) Adonitol
-	-	(E10) Maltotriose
-	-	(E11) 2'-Deoxy-Adenosine
-	-	(E12) Adenosine
-	-	(F1) Glycyl-L-Aspartic Acid
-	-	(F2) Citric Acid
-	-	(F3) m-Inositol
-	-	(F4) D-Threonine
+	+	(F5) Fumaric Acid
+	+	(F6) Bromo-Succinic Acid
-	-	(F7) Propionic Acid
-	-	(F8) Mucic Acid
-	-	(F9) Glycolic Acid
-	-	(F10) Glyoxylic Acid
-	-	(F11) D-Cellobiose
-	-	(F12) Inosine
-	-	(G1) Glycyl-L-Glutamic Acid
-	-	(G2) Tricarballylic Acid
+	+	(G3) L-Serine
-	-	(G4) L-Threonine
-	-	(G5) L-Alanine
-	-	(G6) L-Alanyl-Glycine
-	-	(G7) Acetoacetic Acid
-	-	(G8) N-Acetyl-b-D-Mannosamine
+	+	(G9) Mono Methyl Succinate
+	+	(G10) Methyl Pyruvate
+	+	(G11) D-Malic Acid
+	+	(G12) L-Malic Acid
-	-	(H1) Glycyl-L-Proline
-	-	(H2) p-Hydroxy-Phenylacetic Acid
-	-	(H3) m-Hydroxy-Phenylacetic Acid
-	-	(H4) Tyramine
-	-	(H5) D-Psicose
-	-	(H6) L-Lyxose
-	-	(H7) Glucuronamide
-	-	(H8) Pyruvic Acid
-	-	(H9) L-Galactonic Acid-g-Lactone
-	-	(H10) D-Galacturonic Acid
-	-	(H11) β-Phenylethylamine
-	-	(H12) Ethanolamine

Notes: (A1), (row,column); "+", positive respiration; "-", no respiration.

Table 2. Phenotype microarray profile for *C. jejuni* ATCC 33560 on PM2A plates incubated at 42°C and 30°C.
(a) PM profile from well A1 to D12, (b) PM profile from well E1 to H12.

(a)

42	30	°C
+	+	(A1) Negative Control
+	+	(A2) Chondroitin Sulfate C
+	+	(A3) α-Cyclodextrin
+	+	(A4) β-Cyclodextrin
+	+	(A5) γ-Cyclodextrin
+	+	(A6) Dextrin
+	+	(A7) (A1) Gelatin
+	+	(A8) Glycogen
+	+	(A9) Inulin
+	+	(A10) Laminarin
+	+	(A11) Mannan
+	+	(A12) Pectin
+	+	(B1) N-Acetyl-D-Galactosamine
+	+	(B2) N-Acetyl-Neuraminic Acid
+	+	(B3) β-D-Allose
+	+	(B4) Amygdalin
+	+	(B5) D-Arabinose
+	+	(B6) D-Arabitol
+	+	(B7) L-Arabitol
+	+	(B8) Arbutin
+	+	(B9) 2-Deoxy-D-Ribose
+	+	(B10) i-Erythritol
+	+	(B11) D-Fucose
+	+	(B12) 3-0-b-D-Galactopyranosyl-D-Arabinose
+	+	(C1) Gentiobiose
+	+	(C2) L-Glucose
+	+	(C3) D-Lactitol
+	+	(C4) D-Melezitose
+	+	(C5) Maltitol
+	+	(C6) α-Methyl-D-Glucoside
+	+	(C7) β-Methyl-D-Galactoside
+	+	(C8) 3-O-Methyl-Glucose
+	+	(C9) β-Methyl-D-Glucuronic Acid
+	+	(C10) α-Methyl-D-Mannoside
+	+	(C11) β-Methyl-D-Xyloside
+	+	(C12) Palatinose
+	+	(D1) D-Raffinose
+	+	(D2) Salicin
+	+	(D3) Sedoheptulosan
+	+	(D4) L-Sorbose
+	+	(D5) Stachyose
+	+	(D6) D-Tagatose
+	+	(D7) Turanose
+	+	(D8) Xylitol
+	+	(D9) N-Acetyl-D-Glucosaminitol
+	+	(D10) γ-Amino-Butyric Acid
+	+	(D11) δ-Amino-Valeric Acid
+	+	(D12) Butyric Acid

(b)

42	30	°C
+	+	(E1) Capric Acid
+	+	(E2) Caproic Acid
+	+	(E3) Citraconic Acid
+	+	(E4) D,L-Citramalic Acid
+	+	(E5) D-Glucosamine
+	+	(E6) 2-Hydroxy-Benzoic Acid
+	+	(E7) 4-Hydroxy-Benzoic Acid
+	+	(E8) β-Hydroxy-Butyric Acid
+	+	(E9) γ-Hydroxy-Butyric Acid
+	+	(E10) α-Keto-Valeric Acid
+	+	(E11) Itaconic Acid
+	+	(E12) 5-Keto-D-Gluconic Acid
+	+	(F1) D-Lactic Acid Methyl Ester
+	+	(F2) Malonic Acid
+	+	(F3) Melibionic Acid
+	+	(F4) Oxalic Acid
+	+	(F5) Oxalomalic Acid
+	+	(F6) Quinic Acid
+	+	(F7) D-Ribono-1,4-Lactone
+	+	(F8) Sebacic Acid
+	+	(F9) Sorbic Acid
+	+	(F10) Succinamic Acid
+	+	(F11) D-Tartaric Acid
+	+	(F12) L-Tartaric Acid
+	+	(G1) Acetamide
+	+	(G2) L-Alaninamide
+	+	(G3) N-Acetyl-L-Glutamic Acid
+	+	(G4) L-Arginine
+	+	(G5) Glycine
+	+	(G6) L-Histidine
+	+	(G7) L-Homoserine
+	+	(G8) 4-Hydroxy-L-Proline (trans)
+	+	(G9) L-Isoleucine
+	+	(G10) L-Leucine
+	+	(G11) L-Lysine
+	+	(G12) L-Methionine
+	+	(H1) L-Ornithine
+	+	(H2) L-Phenylalanine
+	+	(H3) L-Pyroglutamic Acid
+	+	(H4) L-Valine
+	+	(H5) D,L-Carnitine
+	+	(H6) Butylamine (sec)
+	+	(H7) D,L-Octopamine
+	+	(H8) Putrescine
+	+	(H9) Dihydroxy-Acetone
+	+	(H10) 2,3-Butanediol
+	+	(H11) 2,3-Butanone
+	+	(H12) 3-Hydroxy-2-Butanone

Notes: (A1), (row,column); "+", positive respiration; "-", no respiration.

Table 3. Phenotype microarray profile for *C. jejuni* ATCC 33560 on PM9 plates incubated at 42°C and 30°C.
(a) PM profile from well A1 to D12, (b) PM profile from well E1 to H12.

(a)

°C	
42	+
30	-

(A1) 1% NaCl	-
(A2) 2% NaCl	-
(A3) 3% NaCl	-
(A4) 4% NaCl	-
(A5) 5% NaCl	-
(A6) 5.5% NaCl	-
(A7) 6% NaCl	-
(A8) 6.5% NaCl	-
(A9) 7% NaCl	-
(A10) 8% NaCl	-
(A11) 9% NaCl	-
(A12) 10% NaCl	-
(B1) 6% NaCl	-
(B2) 6% NaCl + Betaine	-
(B3) 6% NaCl + N-N Dmethyl Glycine	-
(B4) 6% NaCl + Sarcosine	-
(B5) 6% NaCl + Dimethyl Sulphonyl Propionate	-
(B6) 6% NaCl + MOPS	-
(B7) 6% NaCl + Ectoine	-
(B8) 6% NaCl + Choline	-
(B9) 6% NaCl + Phosphorylcholine	-
(B10) 6% NaCl + Creatine	-
(B11) 6% NaCl + Creatinine	-
(B12) 6% NaCl + L-Carnitine	-
(C1) 6% NaCl + KCl	-
(C2) 6% NaCl + L-Proline	-
(C3) 6% NaCl + N-Acetyl-L-Glutamine	-
(C4) 6% NaCl + ?-Glutamic Acid	-
(C5) 6% NaCl + ?–Amino-N-Butyric Acid	-
(C6) 6% NaCl + Glutathione	-
(C7) 6% NaCl + Glycerol	-
(C8) 6% NaCl + Trehalose	-
(C9) 6% NaCl + Trimethylamine-N-Oxide	-
(C10) 6% NaCl + Trimethylamine	-
(C11) 6% NaCl + Octopine	-
(C12) 6% NaCl + Trigonelline	-
(D1) 3% Potassium Chloride	-
(D2) 4% Potassium Chloride	-
(D3) 5% Potassium Chloride	-
(D4) 6% Potassium Chloride	-
(D5) 2% Sodium Sulfate	+
(D6) 3% Sodium Sulfate	+
(D7) 4% Sodium Sulfate	-
(D8) 5% Sodium Sulfate	-
(D9) 5% Ethylene Glycol	+
(D10) 10% Ethylene Glycol	+
(D11) 15% Ethylene Glycol	+
(D12) 20% Ethylene Glycol	+

(b)

°C	
42	+
30	-

(E1) 1% Sodium Formate	-
(E2) 2% Sodium Formate	-
(E3) 3% Sodium Formate	-
(E4) 4% Sodium Formate	-
(E5) 5% Sodium Formate	-
(E6) 6% Sodium Formate	-
(E7) 2% Urea	+
(E8) 3% Urea	-
(E9) 4% Urea	-
(E10) 5% Urea	-
(E11) 6% Urea	-
(E12) 7% Urea	-
(F1) 1% Sodium Lactate	+
(F2) 2% Sodium Lactate	+
(F3) 3% Sodium Lactate	+
(F4) 4% Sodium Lactate	+
(F5) 5% Sodium Lactate	+
(F6) 6% Sodium Lactate	+
(F7) 7% Sodium Lactate	-
(F8) 8% Sodium Lactate	-
(F9) 9% Sodium Lactate	-
(F10) 10% Sodium Lactate	-
(F11) 11% Sodium Lactate	-
(F12) 12% Sodium Lactate	-
(G1) 20mM Sodium Phosphate pH7	+
(G2) 50mM Sodium Phosphate pH7	+
(G3) 100mM Sodium Phosphate pH7	-
(G4) 200mM Sodium Phosphate pH7	-
(G5) 20mM Sodium Benzoate pH5.2	-
(G6) 50mM Sodium Benzoate pH5.2	-
(G7) 100mM Sodium Benzoate pH5.2	-
(G8) 200mM Sodium Benzoate pH5.2	-
(G9) 10mM Ammonium Sulfate pH8	+
(G10) 20mM Ammonium Sulfate pH8	+
(G11) 50mM Ammonium Sulfate pH8	-
(G12) 100mM Ammonium Sulfate pH8	-
(H1) 10mM Sodium Nitrate	+
(H2) 20mM Sodium Nitrate	+
(H3) 40mM Sodium Nitrate	+
(H4) 60mM Sodium Nitrate	-
(H5) 80mM Sodium Nitrate	-
(H6) 100mM Sodium Nitrate	-
(H7) 10mM Sodium Nitrite	-
(H8) 20mM Sodium Nitrite	-
(H9) 40mM Sodium Nitrite	-
(H10) 60mM Sodium Nitrite	-
(H11) 80mM Sodium Nitrite	-
(H12) 100mM Sodium Nitrite	-

Notes: (A1), (row,column); "+", positive respiration; "-", no respiration.

Table 4. Phenotype microarray profile for *C. jejuni* ATCC 33560 on PM10 plates incubated at 42°C and 30°C.
(a) PM profile from well A1 to D12, (b) PM profile from well E1 to G12.

(a)

42 °C	30 °C	
-	-	(A1) pH 3.5
-	-	(A2) pH 4
-	-	(A3) pH 4.5
-	+	(A4) pH 5
+	+	(A5) pH 5.5
+	+	(A6) pH 6
+	+	(A7) pH 7
+	+	(A8) pH 8
-	-	(A9) pH 8.5
-	-	(A10) pH 9
-	-	(A11) pH 9.5
-	-	(A12) pH 10
-	-	(B1) pH 4.5
-	-	(B2) pH 4.5 + L-Alanine
-	-	(B3) pH 4.5 + L-Arginine
-	-	(B4) pH 4.5 + L-Asparagine
-	-	(B5) pH 4.5 + L-Aspartic Acid
-	-	(B6) pH 4.5 + L-Glutamic Acid
-	-	(B7) pH 4.5 + L-Glutamine
-	-	(B8) pH 4.5 + Glycine
-	-	(B9) pH 4.5 + L-Histidine
-	-	(B10) pH 4.5 + L-Isoleucine
-	-	(B11) pH 4.5 + L-Leucine
-	-	(B12) pH 4.5 + L-Lysine
-	-	(C1) pH 4.5 + L-Methionine
-	-	(C2) pH 4.5 + L-Phenylalanine
-	-	(C3) pH 4.5 + L-Proline
-	-	(C4) pH 4.5 + L-Serine
-	-	(C5) pH 4.5 + L-Threonine
-	-	(C6) pH 4.5 + L-Tryptophan
-	-	(C7) pH 4.5 + L-Tyrosine
-	-	(C8) pH 4.5 + L-Valine
-	-	(C9) pH 4.5 + Hydroxy-L-Proline
-	-	(C10) pH 4.5 + L-Ornithine
-	-	(C11) pH 4.5 + L-Homoarginine
-	-	(C12) pH 4.5 + L-Homoserine
-	-	(D1) pH 4.5 + Anthranilic Acid
-	-	(D2) pH 4.5 + L-Norleucine
-	-	(D3) pH 4.5 + L-Norvaline
-	-	(D4) pH 4.5 + a- Amino-N-Butyric Acid
-	-	(D5) pH 4.5 + a-Amino Malonate
-	-	(D6) pH 4.5 + b-Hydroxy Glutamate
-	-	(D7) pH 4.5 + g-Hydroxy Glutamic Acid
-	-	(D8) pH 4.5 + 5-Hydroxy-L-Lysine
-	-	(D9) pH 4.5 + 5-Hydroxy-L-Tryptophan
-	-	(D10) pH 4.5 + D,L Diamino-Pimelic Acid
-	-	(D11) pH 4.5 + Trimethylamine-N-Oxide
-	-	(D12) pH 4.5 + Urea

(b)

42 °C	30 °C	
-	-	(E1) pH 9.5
-	-	(E2) pH 9.5 + L-Alanine
-	-	(E3) pH 9.5 + L-Arginine
-	-	(E4) pH 9.5 + L-Asparagine
-	-	(E5) pH 9.5 + L-Aspartic Acid
-	-	(E6) pH 9.5 + L-Glutamic Acid
-	-	(E7) pH 9.5 + L-Glutamine
-	-	(E8) pH 9.5 + Glycine
-	-	(E9) pH 9.5 + L-Histidine
-	-	(E10) pH 9.5 + L-Isoleucine
-	-	(E11) pH 9.5 + L-Leucine
-	-	(E12) pH 9.5 + L-Lysine
-	-	(F1) pH 9.5 + L-Methionine
-	-	(F2) pH 9.5 + L-Phenylalanine
-	-	(F3) pH 9.5 + L-Proline
-	-	(F4) pH 9.5 + L-Serine
-	-	(F5) pH 9.5 + L-Threonine
-	-	(F6) pH 9.5 + L-Tryptophan
-	-	(F7) pH 9.5 + L-Tyrosine
-	-	(F8) pH 9.5 + L-Valine
-	-	(F9) pH 9.5 + Hydroxy-L-Proline
-	-	(F10) pH 9.5 + L-Ornithine
-	-	(F11) pH 9.5 + L-Homoarginine
-	-	(F12) pH 9.5 + L-Homoserine
-	-	(G1) pH 9.5 + Anthranilic Acid
-	-	(G2) pH 9.5 + L-Norleucine
-	-	(G3) pH 9.5 + L-Norvaline
-	-	(G4) pH 9.5 + Agmatine
-	-	(G5) pH 9.5 + Cadaverine
-	-	(G6) pH 9.5 + Putrescine
-	-	(G7) pH 9.5 + Histamine
-	-	(G8) pH 9.5 + Phenylethylamine
-	-	(G9) pH 9.5 + Tyramine
-	-	(G10) pH 9.5 + Tryptamine
-	-	(G11) pH 9.5 + Trimethylamine-N-Oxide
-	-	(G12) pH 9.5 + Urea

Notes: (A1), (row,column); "+", positive respiration; "-", no respiration.

in 1% NaCl (PM9,A1). However, *C. jejuni* ATCC 33560 found to be able to grow in other osmolytes and ions such as sodium sulphate (PM9,D5-6), ethylene glycol (PM9,D9-12), sodium formate (PM9,E1), urea (PM9,E7), sodium lactate (PM9,F1-12), sodium phosphate (PM9,G1-4), ammonium sulphate (PM9,G9-12), sodium nitrate (PM9,H1-6) and sodium nitrite (PM9,H7). *C. jejuni* ATCC 33560 grow in pH range from pH5 (PM10,A4) to pH8 (PM10,A8).

C. jejuni ATCC 33560 profile when incubated at below minimal growth temperature (30°C), phenotype loss was found in 1% sodium chloride (PM9,A1), 2-3% sodium sulphate (PM9,D5-6), 1% sodium formate (PM9,E1), 7-12% sodium lactate (PM9,F7-12), 100mM and 200mM sodium phosphate pH7 (PM9,G3-4), 50mM and 100mM ammonium sulphate pH8 (PM9,G11-12), 60mM, 80mM and 100mM sodium nitrate (PM9,H4-6), 10mM sodium nitrite (PM9,H7), and growth in pH5 (PM10,A4).

Previous reports (Bochner et al., 2001; Bochner, 2009; Eiff et al., 2006; Tracy et al., 2002) proved Phenotype MicroArray (PM) plates have several advantages: 1) color changes is easy to monitor and quantitate; 2) color changes is very sensitive and highly reproducible; 3) measuring cell respiration which can occur independent of cell growth. However, Bochner (2009) highlighted limitation of PM plates in which abiotic reduction of tetrazolium dye is possible to occur at alkaline pH and with the pentose reducing sugars. In order to overcome this problem, a negative control with identical protocol and incubations for each plate without *C. jejuni* culture were ran concurrently in this study. Thus, the true positive growth can be determined.

C. jejuni ATCC 33560 was found to have active respiration in a number of single carbon substrate in limited nutrient condition. Although *C. jejuni* is known to be fastidious and require specific condition to grow, single carbon source from amino acids and carboxylates proved to support its metabolism. *C. jejuni* utilize carbon source mainly from amino acids and also intermediates in Kreb cycles. There is no positive utilization of carbon source from sugars. Kelly (2005) reported *C. jejuni* is unable to metabolise sugars as carbon sources and this is confirmed in the present study using PM assays. This is due to absence of the key glycolytic enzyme 6-phosphofructokinase and predicted the major carbon source used by *C. jejuni* are amino acids as it possesses several enzymes for the amino acid deamination (Kelly, 2005). In addition to that, several types carboxylate are capable to act as electron donors (Kelly, 2005). Chicken excreta was reported to be high in amino acids, namely aspartate, glutamate, proline and serine (Parson et al., 1982), these might serve as the supporting ground for *C. jejuni* to survive in chicken samples due to cross contamination. Chai et al. (2007) have discussed that chicken manure used as fertilizer in vegetables farms might be the source of *Campylobacter* contamination in fresh produce. Among the amino acids that support *C. jejuni* growth, L-lactic acid was found to be most favoured substrate. L-lactic acid is found in fermented milk products or leftover milk. This might suggest *C. jejuni* able to grow well in leftover milk or fermented milk products. There are reports on campylobacter outbreak due to consumption of milk in either raw or due to pasteurization failure (Jacobs-Reitsma et al., 2008; Fahey et al., 1995).

Little is known about *C. jejuni* response to osmotic stress but Svensson et al. (2008) discussed *C. jejuni* is more sensitive to salt than other foodborne bacterial pathogens and typically unable to grow at above 2% NaCl. In the current observation had agreed well with the statement in which *C. jejuni* showed positive respiration at 1% NaCl but not 2% NaCl. However, interesting findings in the current study is that though *C. jejuni* is sensitive to salt but it survive well in a broad range of other ions or osmolytes, namely sodium lactate, sodium phosphate, sodium benzoate, ammonium sulphate and sodium nitrate, which are used as food additives or preservatives. This suggest *C. jejuni* survive and might grow in the additives or preservatives used in food industry.

The present study revealed that *C. jejuni* incubated at temperature below minimal growth temperature (30°C) have similar phenotypic profile as incubated at optimum temperature (42°C) in carbon source plates and some phenotypic loss in terms of sensitivity to osmolytes, ions and pH. This indicates *C. jejuni* is still metabolically active in an unfavourable growth temperature.

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