DIFFERENCES IN PYRUVATE METABOLISM IN C. JEJUNI AND C. COLI.

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Abstract

The ability of Campylobacter jejuni (83 strains) and C. coli (17 strains) to metabolise pyruvate was assessed in vitro using oxygen electrode system. All the strains tested were able to oxidise pyruvate. However, C. jejuni and C. coli have marked differences in the oxidation rates of pyruvate. Whereas C. coli strains oxidize pyruvate at a constant, high rate with high affinity, C. jejuni displayed a low rate at lower concentrations, but at higher concentrations the rate and affinity increased. In the presence of an inhibitor (of β-flouropyruvate), pyruvate oxidation was abolished completely in C. coli but only partly in C. jejuni.

Preliminary molecular studies indicated a significant variation in nucleotide composition of the genes involved in pyruvate metabolism between C. jejuni and C. coli. It is, therefore, possible that C. jejuni strains may have more elaborate pyruvate metabolism pathways than that in C. coli. This has probable consequences on the inability of C. coli to survive under stressful conditions and thus may explain the differences in the occurrence of this organism and C. jejuni.

Introduction:

Pyruvate plays a central role in cellular metabolism, linking carbohydrate and amino acid utilization with energy generation and biosynthetic pathways. It is a component of the Kreb’s cycle, a precursor for alanine, serine, (iso)leucine, valine, succinate and lactate and for the energy carriers acetyl-CoA. All cells require active pyruvate metabolism to maintain homeostasis, as well as the anabolic activity essential for growth. Thus the presence or absence of enzymes involved in pyruvate catabolism are good indicators for the physiology of bacteria, their fitness to a variety of environments and consequently their ability to survive environmental stress.

Of the 17 species within the genus Campylobacter, Campylobacter jejuni and Campylobacter coli are the most important from a food safety point of view. C. jejuni, and less commonly the latter, are common causes of human enteritis. These bacteria are thermophilic and obligate micro-aerophils, and only naturally replicate in a host, where they generally colonize the gastrointestinal tract. Both species asymptomatically colonize a wide variety of warm-blooded animals including livestock and, consequently contaminates meat products. C. jejuni is particularly common in birds, including poultry. In contrast, C. coli is the predominant species found in pigs. As person-to-person spread is uncommon, cases are sporadic, with outbreaks being rare and since the bacteria do not repa...
outside a host, their potential survival *ex vivo* is likely to be of crucial importance to their transmission and in particular *via* food-borne route.

Current molecular tools in addition to genome sequencing has allowed for a greater understanding of the metabolic capacity and epidemiology of *Campylobacter jejuni*[^8]. The importance of the organism in human illness has resulted in at least 18 isolates from 8 different *Campylobacter* species having been or being sequenced[^8]. The genome sequence of *C. jejuni* strain 11168 has been published[^9]. The genes involved in pyruvate metabolism identified are depicted in Figure 1. The annotation, based on gene similarity of bacterial genes with known function, suggests that this *C. jejuni* strain has at least three different pathways for the conversion of pyruvate to oxaloacetate: direct carboxylation, conversion *via* malate, and conversion *via* phosphoenolpyruvate (PEP). It is presently unknown whether this is representative of all strains of the species, or other *Campylobacter* species. The activity of pyruvate oxidoreductase (Por) has been demonstrated in both *C. jejuni* and *C. coli*[^10]. The importance of PEP carboxykinase in *C. jejuni*, encoded by *pck*, has been demonstrated by the inability to produce a knock-out mutant indicating that this gene is essential[^11]. Since no PEP synthase and pyruvate orthophosphate dikinase activities are demonstratable, it seems likely that *pck* encodes the only enzyme to produce PEP. Although, prior evidence suggested that *C. jejuni* can produce pyruvate from PEP, *via* PEP synthase[^12], this gene was not identified in the genome sequence.

The aim of this study was to investigate the patterns and kinetics of pyruvate metabolism by intact cells (and lysates) of *C. jejuni* and *C. coli* strains. In addition, the study was extended to evaluate the molecular basis of pyruvate metabolism in *C. coli* and *C. jejuni*. The results indicated significant differences in pyruvate metabolism between *C. jejuni* and *C. coli*, and, to a lesser extent, within strains of *C. jejuni*. The implications of these observations to survival fitness and potential virulence of the strains investigated are discussed.

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**Fig. 1:** The central role of pyruvate in cellular metabolism and key enzymatic steps discussed in the text. Block arrows depict major pathways of catabolism and anabolism. Thin black arrows depict direct enzymatic conversions. Enzyme names (underlined) and gene annotation (with gene name in italics) are from the annotation of the *C. jejuni* 11168 genome sequence [6]. Dotted, crossed arrows depict pathways not found in *C. jejuni*, with enzyme names not underlined (Phosphoenolpyruvate synthase). The activity of PEP synthase was demonstrated *in vitro* [9] but the gene has not been identified.
Materials and Methods:

Strains and Culture Conditions:
The strains used in this study are shown in a Table 1. The type strains C. jejuni subsp. jejuni NCTC 11168, C. jejuni subsp. doylei NCTC 11951 and C. coli NCTC 11366 were obtained from the National Collection of Type Culture, Colindale, UK. Human clinical isolates (71 C. jejuni and 9 C. coli) were kindly provided by Mr Nigel Pittock, Ashford Borough Council, UK. Animal, poultry and environmental isolates (10 C. jejuni and 7 C. coli) were kindly provided by Professor Diane Newell (Veterinary Laboratories Agency, Weybridge, UK).

All the strains were grown as previously described. Briefly, the strains provided were grown on Preston selective agar medium (Oxoid, Basingstoke, UK) under microaerophilic conditions (CampyGenTM, Oxoid) at 37°C for 48 h. Ten ml of brain heart infusion (BHI) medium (Oxoid) were inoculated with isolated colonies from the agar plates. Microaerophilically grown cultures were dispensed into cryotubes containing 15% glycerol and kept at -70°C until further use. From these stock cultures, 0.1 ml was transferred to sterile universal tubes (capacity 20 ml) containing 10 ml of BHI. After 24 h static incubation at 37°C in air, 0.1 ml aliquots were transferred to 10 ml fresh BHI medium and incubated on a shaker (150 rpm, Gallenkamp, UK) at 37°C in air. Early stationary phase cells were harvested for pyruvate (Sigma) utilization experiments.

The identification and speciation of all strains were carried out using Gram stain, morphology and API Campy (bioMerieux, France) or biochemical tests, such as hippurate, nitrate reduction, catalase and oxidase tests. Speciation was confirmed using species-specific PCR reactions as described in.

| Campylobacter strain | Source | Campylobacter strain | Source |
|-----------------------|--------|-----------------------|--------|
| *C. jejuni NCTC 11168* | Unknown | NSP2075 – 76 | Human stool |
| *C. doylei NCTC 11951* | Unknown | NSP2079 – 82 | Human stool |
| *C. coli NCTC 11366* | Unknown | NSP2085 – 87 | Human stool |
| E3529 | Human stool | NSP2106 – 110 | Human stool |
| 3817 | Human stool | NSP2113 -18 | Human stool |
| 3861 | Human stool | *C. coli* EX 69 | Puddle |
| 1D /1999/ 0252 | Human stool | *C. coli* EX 75 | Puddle |
| 1D /1999/ 0246 | Human stool | *C. coli* PS 41 | Pig |
| 1D /1999/ 0248 | Human stool | *C. coli* PS 70 | Pig |
| ID /1999/ 0249 | Human stool | *C. coli* PS 80 | Pig |
| NSP003 – 4 | Human stool | *C. jejuni* EX 61 | Puddle |
| ID /1999/ 0244 | Human stool | *C. jejuni* EX 63 | Puddle |
| NSP0011 | Human stool | *C. jejuni* EX 33 | Wild bird faeces |
| NSP0013 | Human stool | *C. jejuni* EX 360 | Wild bird faeces |
| NSP202 – 8 | Human stool | *C. jejuni* EX 133 | Poultry |
| NSP2010 – 25 | Human stool | *C. jejuni* EX 726 | Poultry |
| 16703 | Human stool | *C. jejuni* Ch 65 | Poultry |
| 11672 | Human stool | *C. jejuni* OF 20 | Poultry |
| NSP2028 – 39 | Human stool | *C. jejuni* BF 8951 | Poultry |
| NSP2041-54 | Human stool | *C. jejuni* C284/6 | Poultry |
| NSP2056 – 57 | Human stool | *C. coli* C346/2 | Poultry |
| NSP2061 | Human stool | *C. jejuni* Caecum 4 | Poultry |
| NSP2067-69 | Human stool | *C. coli* PS 515 | Pig |
| NSP2072 | Human stool | *C. jejuni* | |

*a* NCTC: National Collection of Type Culture; *b* All isolates used were obtained from U.K. Human isolates were isolated in 1998-2001 and were identified and speciated in the present study; *c* The identification and speciation of animal and environmental isolates were carried out at Veterinary Laboratory Agency, Weybridge, UK.)
Pyruvate oxidation:

Early stationary phase grown cells were harvested by centrifugation (5000 x g for 8 min), washed twice and resuspended in Ringer’s and HEPES buffer. This buffer contained one quarter strength Ringer’s solution (Oxoid), 18g/l HEPES and 160 U/ml catalase (Sigma). The total protein concentration was determined using Markwell's method. Substrate oxidation was determined from changes in dissolved oxygen tension (DOT) using an oxygen electrode system (Rank Brothers, Bottisham, Cambridge, UK.) linked to a chart recorder calibrated with air-saturated water (DOT approximately 210 nmol ml/1 at 37˚C). The water was then replaced by 1 ml of washed cell suspension containing approximately 0.25 mg cell protein which was magnetically stirred at 37˚C and isolated from air using a cylindrical plug with a fine central pore enabling the addition of test substrates. After the cell suspension had been isolated from air and after a steady DOT recording was obtained.

Calculation of Oxidation rates and K_m values:

Saturation constants (K_m values) and rates of substrate metabolism were estimated from recorder tracings of change in DOT as shown in Fig. 2 and 3. Saturation or affinity constants (K_m values) and maximum velocity of substrate utilisation (V_max) were estimated from recorder tracings of change in DOT. It was assumed that the relationship between the rate of substrate metabolism (V) and substrate concentration follows Michaelis kinetics and the K_m value and V_max could be estimated from the plot of 1/s versus 1/v in which the intercepts on Y and X axis are 1/V_max and −1/K_m respectively. Where K_m values were relatively high (> 50 μM), the rates of substrate utilisation were determined at a number of substrate concentrations. The straight line of best fit was then drawn in plot of 1/(rate of O_2 uptake) versus 1/(s). K_m and V_max values were determined as shown in Fig. 2.

Alternatively, where the affinity for substrate was high, (i.e. the maximum rates of substrate utilisation were obtained at low substrate concentration), K_m and V_max values were determined from analysis of single curve representing a complete utilisation of the substrate (Fig. 3). In this case, it was assumed that the rate of change in dissolved oxygen tension (DOT) at any time (t) was proportional to the concentration of the substrate used. In this regard, substrate concentration (s) at time (t) was: (initial substrate concentration x (a-c)/a i.e. initial substrate concentration x b/a where a was the total reduction in DOT following substrate addition and c the reduction in DOT at time (t).

To determine the K_m value, the point when the rate of substrate metabolism had declined to half (V/2) was estimated from the graph and the concentration of the proportion of remaining substrate (b/a) at this time was estimated (Fig. 3). Since the value of K_m is equal to the concentration of utilised substrate when V_max at half of its value,

\[
K_m = \frac{b \text{(initial substrate concentration)}}{a}
\]

Fig. 2: Estimation of K_m and V_max values from double reciprocal plots of the rate of substrate oxidation against substrate concentration. Representative curve showing the low K_m value and the oxidation of pyruvate by C. jejuni isolate (NSP02067). The pyruvate concentrations used

Fig. 3: Estimation of K_m values for substrate utilisation from curves of dissolved oxygen tension versus time representing the complete oxidation of substrate. Representative curve showing oxidation of Pyruvate (0.045 mM) by C. coli strain (C346).
were 0.025, 0.045, 0.45 mM. 

\[ K_m = \frac{b \times (\text{initial substrate concentration})}{a} = \frac{2.5 \times 45}{40} = 2.8 \, \mu M \]

**Effect of β-flouropyruvate on pyruvate oxidation:**
Bacterial cell suspensions were prepared and added to the oxygen electrode vessel. Different concentrations of β-flouropyruvate (Sigma) were then added to the suspension in one reaction vessel (5 min prior to the addition of pyruvate) and monitored the change in dissolved oxygen tension in comparison with untreated cells in another reaction vessel.

**Results:**
All the strains tested were able to oxidise pyruvate. However, oxidation rates and \( K_m \) values varied markedly amongst *C. jejuni* and *C. coli* species. The *C. coli* strains (Table 2) were distinguished from strains of *C. jejuni* by their ability to oxidise pyruvate at high rate in the presence of low concentration (0.045 mM) and by their single and low \( K_m \) value. All the strains tested of *C. coli* were able to oxidise pyruvate at high rates (60 – 90 nmol. min\(^{-1}\). mg cell protein\(^{-1}\)) with high affinity (\( K_m \) values were mostly ≤ 3 \( \mu M \) (Table 2). The rate of oxygen uptake was constant with time until the substrate was almost exhausted and raising the initial concentration of pyruvate to 0.5 mM did not increase oxygen uptake rates (Table 2). However, only one *C. coli* strain (*C. coli* PS 515) behaved, metabolically, in similar manner to *C. jejuni* strains, this will be discussed further in the molecular section.

All *C. jejuni* strains tested oxidised pyruvate at low rate (4 – 60 nmol. min\(^{-1}\). mg cell protein\(^{-1}\)) when 0.045 mM were added to the cell suspensions and the rates were significantly increased when the substrate concentration was increased to 0.45 and 4.5 mM (Table 2). On the basis of oxidation rate of pyruvate at low concentration, *C. jejuni* strains were divided into two subgroups. The first subgroup was characterised by its relatively high rate (>12 nmol. min\(^{-1}\). mg cell protein\(^{-1}\)) at low concentration of pyruvate (0.045 mM) whereas the second group showed low rate (<10 nmol. min\(^{-1}\). mg cell protein\(^{-1}\)) with the same concentration.

In addition, all *C. jejuni* strains tested showed two different \( K_m \) values for pyruvate utilisation. Low \( K_m \) value (<300\( \mu M \)) was obtained at substrate concentration (0.045 to 0.45 mM), and when the concentration of substrate was increased to 5 mM, \( K_m \) value increased to greater than 1000\( \mu M \) (Table 2).

The effect of FP on substrate oxidation was investigated for *C. jejuni* and *C. coli*, by monitoring changes in dissolved oxygen tension. When FP was added to cell suspensions 5 min before the addition of pyruvate there was a marked inhibition of substrate oxidation in *C. coli* compared to *C. jejuni* cells (Table 3). Significant inhibition (50-100%) was apparent at FP concentration of 0.25 g l\(^{-1}\), i.e. 4-fold lower than that required to inhibit the growth of these organisms (data not shown). The effect of FP on substrate oxidation by *C. jejuni* and *C. coli* was largely correlated to the inhibitory effects of FP on the growth of these organisms. It was varied amongst *C. jejuni* and *C. coli*. The oxidation of pyruvate in *C. coli* showed more sensitivity to FP inhibition than that in *C. jejuni* (Table 3). It was also observed that the inhibitory effect of FP was decreased when substrate concentrations were increased (Table 3). In addition, it was noted that the inhibition was less marked when FP was added to cell suspension metabolising substrate than when added before substrate addition. Thus, it appears that FP might compete with organic acids for binding to active sites of organic acid metabolising enzymes.

Preliminary molecular studies on the genes involved in pyruvate metabolism (Fig. 1) showed significant differences in the genetic makeup of these genes between *C. coli* and *C. jejuni* (data not shown).

**Discussion:**
The oxidation of pyruvate to acetyl-Coenzyme A is the key step in the intermediary metabolism of these organisms, which depends on citric acid cycle to generate the required energy. Generally, oxidation of pyruvate can be achieved through three different metabolic pathways. The first reactions carried out by the action of pyruvate dehydrogenase (PDH), which is involved in mammalian systems and other aerobic microorganisms. In the second pathway, mixed acid fermentation systems, oxidation of pyruvate to acetyl CoA achieved by the action of pyruvate – formate lyase. Whereas in the third pathway (obligate anaerobes), pyruvate acceptor oxidoreductase (POR) carry out the oxidation of pyruvate to acetyl CoA.
The activities of the above three enzymes, PDH, pyruvate – formate lyase and POR, have been demonstrated in *C. jejuni* 11168. However, only the gene corresponding to POR enzyme has been found in the genome sequencing data of *C. jejuni* 11168. 

### Table 2:- Representative data for oxidation rates and *K*ₘ values of pyruvate utilisation by *Campylobacter* isolates.

| Strain          | Oxidation Rate at various concentrations pyruvate a | Kₘb value (µM) |
|-----------------|---------------------------------------------------|----------------|
|                 | 0.045 mM | 0.45 mM | 4.5 mM | Low Km | High Km |
| *C. coli* NSP003| 37 ± 6   | 37 ± 6  | 37 ± 6 | ≤ 3    | ≤ 3     |
| *C. coli* 1999/0246 | 103 ± 18 | 103 ± 18 | 103 ± 18 | ≤ 3    | ≤ 3     |
| *C. coli* NCTC 11366 | 92 ± 23  | 92 ± 23 | 92 ± 23 | ≤ 3    | ≤ 3     |
| *C. coli* NSP2075 | 49 ± 6   | 49 ± 6  | 49 ± 6 | ≤ 3    | ≤ 3     |
| *C. coli* NSP2069 | 43 ± 6   | 43 ± 6  | 43 ± 6 | ≤ 3    | ≤ 3     |
| *C. coli* EX69     | 55 ± 18  | 55 ± 18 | 55 ± 18 | ≤ 3    | ≤ 3     |
| *C. coli* EX75     | 48 ± 12  | 48 ± 12 | 48 ± 12 | ≤ 3    | ≤ 3     |
| *C. coli* C346/2   | 66± 11   | 66± 11  | 66± 11 | ≤ 3    | ≤ 3     |
| *C. coli* EX580PS80| 58± 4    | 58± 4   | 58± 4  | ≤ 3    | ≤ 3     |
| *C. coli* 1999/0248| 79 ± 4   | 58± 4   | 58± 4  | ≤ 3    | ≤ 3     |
| **C. coli** PS 515 | **14.7 ± 3** | **22.7 ± 2.7** | **63.6 ± 14** | **29**  | **1000** |
| **Subgroup I**    |          |          |        |        |         |
| *C. jejuni* NSP2033| 26 ± 6   | 45 ± 5  | 93 ± 4  | 40    | 670     |
| *C. jejuni* NSP203 | 18 ± 2   | 46 ± 5  | 100± 13 | 90    | 500     |
| *C. jejuni* NSP202 | 19 ± 6   | 52 ± 5  | 170± 14 | 125   | 2000    |
| *C. jejuni* NSP2076| 37 ± 6   | 71 ± 2  | 166± 12 | 55    | 500     |
| *C. jejuni* NSP2082| 20 ± 2   | 49 ± 10 | 139± 10 | 83    | 1000    |
| *C. jejuni* NSP2067| 22 ± 3   | 41 ± 8  | 130± 38 | 40    | 1330    |
| *C. jejuni* NSP2072| 66 ± 10  | 104± 18 | 181± 13 | 29    | 360     |
| *C. jejuni* NSP2068| 16 ± 6   | 47 ± 5  | 159± 20 | 111   | 1400    |
| *C. jejuni* NSP2029| 18 ± 2   | 47 ± 3  | 177± 17 | ND    | 500     |
| **Subgroup II**   |          |          |        |        |         |
| *C. jejuni* NSP2018| 4.6 ±1.2 | 9.6 ± 3 | 103± 32 | 58    | 1000    |
| *C. jejuni* NSP2023| 3.4 ±0.8 | 12 ± 0.1 | 150± 30 | 166   | >2000   |
| *C. jejuni* NSP2025| 3.6 ±0.4 | 21 ± 5  | 130± 38 | 333   | >2000   |
| *C. jejuni* NSP2036| 4.4 ±0.2 | 12± 2   | 117± 22 | 100   | >2000   |
| *C. jejuni* NSP2080| 8.3 ±0.6 | 40 ± 3  | 125± 18 | 333   | >2000   |
| *C. jejuni* NSP2017| 7.3 ±0.9 | 15± 0.2 | 67± 17  | 55    | 2000    |
| *C. jejuni* NSP2016| 4.7 ±2.0 | 19± 4   | 77± 21  | 166   | 2000    |
| *C. jejuni* NCTC 11168 | 8.4 ±0.7 | 24± 6   | 107± 18 | 105   | 2000    |
| *C. jejuni* NSP2034| 11.0 ±3.0| 33 ± 7  | 91± 24  | 125   | 1000    |
| *C. jejuni* NSP204  | 3.0 ±0.8 | 13 ± 5  | 69± 18  | 166   | 2000    |
| *C. jejuni* NSP2022| 3.5 ±0.5 | 12± 4   | 80± 22  | 250   | 2000    |
| *C. jejuni* NSP2024| 5.8 ±1.8 | 14± 5   | 63± 23  | 71    | 2000    |
| *C. jejuni* NSP2019| 6.4 ±1.8 | 18± 5   | 96± 17  | 80    | 2000    |
| *C. jejuni* 11672  | 8.0 ±1.8 | 22± 4   | 38± 0.4 | 100   | 500     |

a oxidation rate given as nmol O₂. min⁻¹. mg⁻¹ cell protein, referred in the text as 'units'; b low and high Km values were obtained from pyruvate concentrations of 0.025-0.045–0.45 and 0.45-0.9-4.5 mM, respectively.

### Table 3:- Inhibitory effect of β-fluoropyruvate (FP) on pyruvate oxidation by *C. coli* and *C. jejuni*.

| *C. coli* strain | Oxidation Rate at various concentrations pyruvate a | ³FP (0.25 g l⁻¹) |
|------------------|---------------------------------------------------|-----------------|
| *C. coli* strain | Control (0.045M, 4.5 mM pyruvate)                 | "FP (0.25 g l⁻¹)"|
| **NCTC 11366**   | 85                                                | ND              |
| **EX69**          | 40                                                | ND              |
| **NSP2010**       | 18.5                                              | ND              |
As shown in Figure 1 C. jejuni may metabolise pyruvate by different pathways. Most of these pathways end with products that contribute to citric acid cycle. The main reaction for the oxidation of pyruvate is the oxidative decarboxylation by pyruvate flavodoxin oxidoreductase (POR) enzyme. Acetyl CoA is produced from the reaction and then converts either to acetate or contributes into the citric acid cycle.

\[
\text{POR} \quad \text{pyruvate} + \text{NAD} \rightarrow \text{acetyl CoA} + \text{NADH}
\]

The significance of this pathway appears in the production of NADH as energy source, and the production of acetyl CoA, which is the main contributor to the citric acid cycle.

The second reaction by which pyruvate may enter the citric acid cycle is the carboxylation of pyruvate by pyruvate carboxylase or malic acid enzyme to become oxaloacetate and malate respectively.

\[
\text{Pyruvate} + \text{ATP} + \text{HCO}_3^- \rightarrow \text{Oxaloacetate} + \text{ADP} + \text{P} \\
\text{Pyruvate} + \text{CO}_2 + \text{NADPH} + \text{H}^+ \rightarrow \text{L-malate} + \text{NADP}
\]

The genome sequencing of C. jejuni NCTC 11168 showed the presence of the genes corresponding to the pyruvate carboxylase and malic acid enzymes \(^9\).

The alternative pathway by which pyruvate may enter the citric acid cycle is the conversion of pyruvate to phosphoenolpyruvate and then to oxaloacetate by phosphoenol synthase and phosphoenolpyruvate carboxylase respectively.

\[
\text{Phosphoenolpyruvate} + \text{CO}_2 + \text{GDP} \rightarrow \text{oxaloacetate} + \text{GTP}
\]

However, the genes corresponding for phosphoenolpyruvate carboxylase but not for phosphoenolpyruvate synthase have been found in the genome sequencing of C. jejuni 11168 \(^9\) (Fig. 1). This may indicate the absence of the above pathway in these organisms.

The total amount of oxygen consumed during the oxidation of pyruvate (45 \(\mu\text{M}\)) by C. coli (1. \text{mol oxygen per mol substrate}) was less than that needed (2.5 \text{mol oxygen per mol substrate}) for its complete oxidation to CO\(_2\) and water via the TCA cycle through the conversion of pyruvate to acetyl CoA, and is more than that required (0.5 \text{mol oxygen per mol substrate}) for oxidation of pyruvate to acetate plus CO\(_2\). This finding suggests the incorporation of pyruvate skeleton into the Kreb's cycle through the conversion of pyruvate to acetyl CoA.

C. jejuni consumed 1.5 - 2 \text{mol oxygen per mol substrate} during oxidation of pyruvate (0.045 \text{mM}), which is less than the sum of the oxygen needed for complete oxidation of pyruvate to CO\(_2\) and water via Kreb's cycle (2.5 \text{mol oxygen per mol substrate}) and more than that required for the oxidation of pyruvate to acetate (2.5 \text{mol oxygen per...
mol substrate). These results suggest that pyruvate may be oxidized to acetyl CoA, which is either incorporated into TCA cycle or is converted to acetate. On other hand, in the presence of high concentration of pyruvate, another pathway may be operated pyruvate carboxylase and by which pyruvate can enter the Kreb’s cycle as oxaloacetate or malate.

The presence of two different $K_m$ values for pyruvate oxidation suggests that C. jejuni may possess different pathways for pyruvate metabolism or different transport systems for pyruvate transferring. Several experiments have been done, in this study, to find out whether there are two transport systems or two metabolic pathways for pyruvate in C. jejuni. In these experiments, metabolic inhibitors and substrate oxidation by lysed cells have been investigated. The results obtained from lysed cells (data not shown) were similar to that obtained from intact cells indicating the absence of two transport systems for pyruvate in C. jejuni. In contrast, the results obtained from the substrate oxidation and the results from the investigation of the effect of metabolic inhibitors on intact cells may suggest the presence of different pathways for the metabolism of pyruvate in C. jejuni. These pathways might be operated via pyruvate carboxylase and via POR.

The presumed target for β-fluoropyruvate (FP) was the pyruvate dehydrogenase complex (PDHC). The activity of this enzyme (PDHC) has been demonstrated in C. jejuni but has not been detected in C. coli $^{12}$. However, the genetic information indicated that C. jejuni does not possess the gene corresponding for PDHC and instead it does have pyruvate oxidoreductase (POR), which may be involved in oxidation of pyruvate to CO$_2$ and acetyl-coenzymeA $^9$. The activity of POR has been demonstrated in C. jejuni and C. coli $^{10}$. Other studies indicated that POR in C. jejuni is thiamine pyrophosphate (TPP) dependent $^{20}$. Similarly, the decomposition of fluoropyruvate is strictly TPP dependent $^{21}$. Therefore, the inhibitory effects of β-fluoropyruvate on the oxidation of pyruvate by Campylobacter strains may be due to the binding of the FP to TPP and consequently inactivate POR enzyme.

$$R\text{-TPP} + \text{fluoropyruvate} \rightarrow R\text{-TPP- fluoropyruvate}$$

The results obtained in this study suggests that the enzyme involved in the metabolism of pyruvate in C. coli has different properties from that in C. jejuni or it may suggest the presence of more than one pathway for pyruvate metabolism in C. jejuni and only one pathway may be operated in C. coli strains.

The marked variation in the oxidation rates and $K_m$ values between C. jejuni and C. coli strains could be a useful test to differentiate between these species. In addition, the possible presence of two pathways for pyruvate in C. jejuni and one pathway in C. coli may be considered as a possible explanation for the low contribution of C. coli in human disease and may be useful in epidemiological studies.

Pyruvate plays a central role in the general metabolic processes of a cell. Thus, utilization of this substrate may indicate the cell’s ability to grow under specified environmental conditions. The thermophilic campylobacters, C. jejuni and C. coli, are ubiquitous in the environment and, although growth can only occur within the host gut, survival occurs under a wide range of environmental stresses. Therefore, pyruvate metabolism in these bacteria may be important for the fitness of the cells and for the survival of the organism in hostile environments $^{22}$. The successful survival of C. jejuni in these conditions may, indirectly, explain its frequent offence in human disease. On the other hand, the limited repertoire of pyruvate metabolic processes in C. coli may hamper the organism’s survival in adverse environmental conditions and hence it’s infrequent isolation from human cases, although the organism is commonly isolated from pigs, cattle and sheep meat in the food chain.

Furthermore, the ability to oxidize pyruvate at high rate may be considered as a pathogenicity factor, reducing the O2 concentration leading to limit the ability of cellular immune system such as neutrophils activity to generate reactive oxygen intermediates.

Preliminary molecular experiments (data not shown) compliment the metabolic differences between C. jejuni and C. coli based on the amplification, hybridization and sequencing data of the genes involved in the initial steps of pyruvate metabolism (Fig. 1). More future work is needed to study in more details the molecular basis of the metabolic differences between these species.
Acknowledgement:-
We thank Professor Diane Newell and Ahmed IH for their help in carrying out the molecular experiments in their laboratory at Veterinary Laboratory Agency, Weybridge, UK. Also, we thank Mr Nigel Pittock, Ashford Borough Council for providing Campylobacter isolates.

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