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RESEARCH ARTICLE

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

Dr. Khairallah A.S. Mohammed[1] and Dr. Mahmoud A. Halablab[2].
1. Southern Technical University, Health & Medical Technology College, Basrah/IRAQ.
2. College of Science & Information Systems, Hariri Canadian University, LEBANON.

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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. coil* have marked differences in the oxidation rates of pyruvate. Whereas *C. coil* 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. coil* 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* [1], *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 [2, 3]. 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 [4]. *C. jejuni* is particularly common in birds, including poultry [5]. In contrast, *C. coli* is the predominant species found in pigs [6]. As person-to-person spread is uncommon [7], cases are sporadic, with outbreaks being rare and since the bacteria do not replicate

Corresponding Author:- Khairallah A.S. Mohammed.
Address:- Southern Technical University, Health & Medical Technology College, Basrah/Iraq.

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 orthophospahate 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.

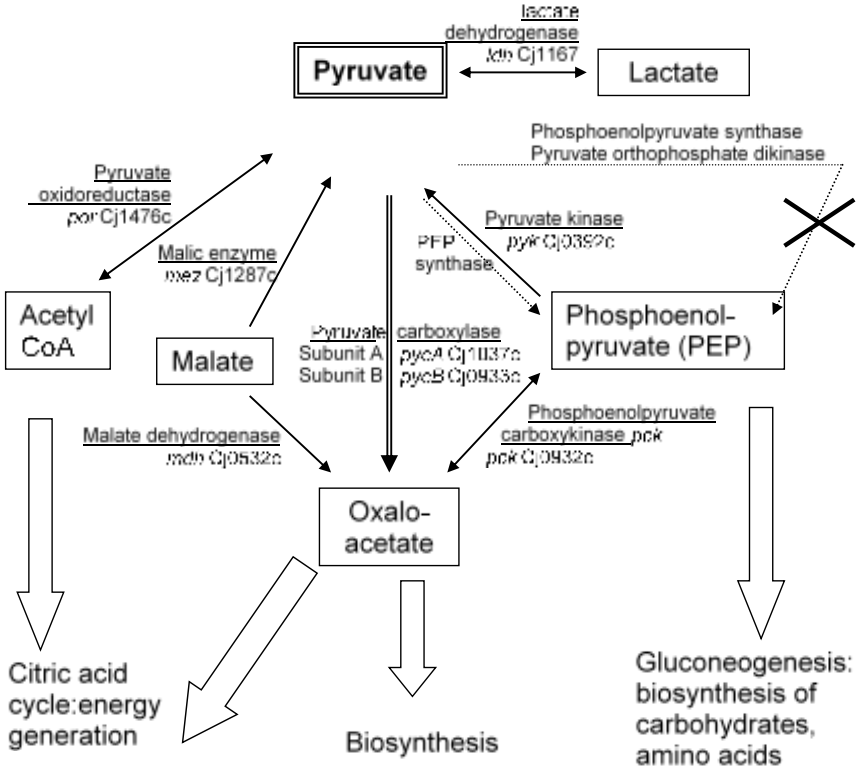

Fig. 1:- The central role of pyruvate in cellular metabolism and key enzymatic steps discussed in the text. Block arrows depict major pathways of catabolism and anabolism. Thin black arrows depict direct enzymatic conversions. Enzyme names (underlined) and gene annotation (with gene name in italics) 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 [13]. 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 [14].

Table 1:- *Campylobacter* strains (100 isolates) used in this study

Campylobacter strain	Source	*Campylobacter* strain	Source
[a]*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
[b]E3529	Human stool	NSP2106 –110	Human stool
3817	Human stool	NSP2113 -18	Human stool
3861	Human stool	[c]*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		

[a] NCTC: National Collection of Type Culture; [b] All isolates used were obtained from U.K. Human isolates were isolated in 1998-2001and 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 [15]. 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) [16]. 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 [17]. 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/Vmax and $-1/K_m$ respectively [17]. 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 Km 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,

$$K_m = \frac{b.(\text{initial substrate concentration})}{a}$$

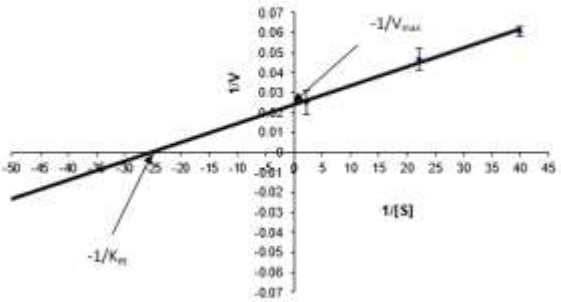

Fig. 2:-. Estimation of K_m and V_{max} values from double reciprocal plots of the rate of substrate oxidation against substrate concentration. Representative curve showing the low K_m value and the oxidation of pyruvate by *C. jejuni* isolate (NSP02067). The pyruvate concentrations used

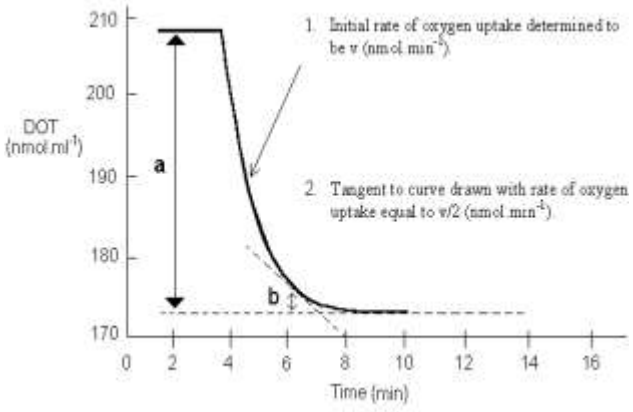

Fig.3:- Estimation of K_m values for substrate utilisation from curves of dissolved oxygen tension versus time representing the complete oxidation of substrate. Representative curve showing oxidation of Pyruvate (0.045 mM) by *C. coli* strain (C346).

were 0.025, 0.045, 0.45 mM.

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

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 $\leq$ 3 μM (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 ($\leq$ 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 (< 300μM) 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 1000μM (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 puruvate 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 [18]. Generally, oxidation of pyruvate can be achieved through three different metabolic pathways [19]. 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 [12, 10]. However, only the gene corresponding to POR enzyme has been found in the genome sequencing data of *C. jejuni* 11168 [9].

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

	Oxidation Rate at various concentrations pyruvate a			Kmb value (μM)	
Strain	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. jejumi 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*.

	Oxidation Rate at various concentrations pyruvate a	
C. coli strain	Control (0.045mM, 4.5 mM pyruvate)	[b]FP (0.25 g l^{-1})
NCTC 11366	85	ND
EX69	40	ND
NSP2010	18.5	ND

NSP2069	37		ND			
NSP003	44		ND			
1999/0248	76		ND			
1999/0246	90		ND			
	0.045 mM pyruvate		0.45 mM pyruvate		4.5 mM pyruvate	
C. jejuni strain	Control	[b]FP	control	[b]FP	control	[b]FP
NCTC 11168	7.5	2.9	18	6.4	126	40
NSP2033	26	4.4	50	8.8	90	34
NSP203	16	4.4	40	6.6	104	30
NSP2018	5.8	3	13	5.8	138	64
NSP202	20	8.8	48	14	96	20
NSP2019	8	4	23	6.6	116	26
NSP2015	15	6.2	34	13.8	124	29
NSP2067	20	8	32	12.5	90	36

ND: not detected; aoxidation rate given as nmol O2. min^{-1}. mg^{-1} cell protein. Oxidation rates by *C. coli* and increasing the concentration of pyruvate did not affect the inhibitory effect of β-fluoropyruvate (FP); **b:**β-fluoropyruvate was added at a final concentration of 0.25 g l^{-1}.

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.

$$\text{pyruvate} + \text{NAD} \xrightarrow{\text{POR}} \text{acetyl Co A} + \text{NADH}$$

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.

$$\text{Pyruvate} + \text{ATP} + HCO_3^- \longrightarrow \text{Oxaloacetate} + \text{ADP} + \text{P}$$

$$\text{Pyruvate} + CO_2 + \text{NADPH} + H^+ \longrightarrow \text{L-malate} + \text{NADP}$$

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.

$$\text{Phosphoenolpyruvate} + CO_2 + \text{GDP} \longrightarrow \text{oxaloacetate} + \text{GTP}$$

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 μM) by *C. coli* (1. mol oxygen per mol substrate) was less than that needed (2.5 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 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 mol oxygen per mol substrate during oxidation of pyruvate (0.045 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 mol oxygen per mol substrate) and more than that required for the oxidation of pyruvate to acetate (2.5 mol oxygen per

mol substrate). These results suggest that puryvate 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.

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

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.

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