School of Biological Sciences

Glucose utilisation by *Campylobacter jejuni* subsp. *jejuni* strains isolated from farm associated Norway rats

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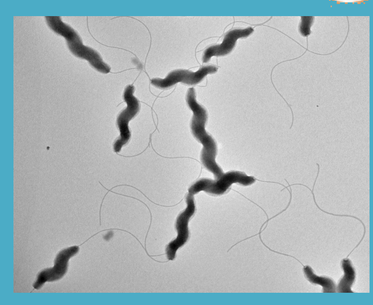

TEM, Dg147, ST 6562, RG-1 group, Reading University

Introduction

Campylobacter has been classically defined as non-glycolytic, microaerophilic, spiral bacteria. Recently, it has been demonstrated that several strains of *C. coli* and *C. jejuni* subsp. *doylei* possess a functional Entner-Doudoroff (ED) pathway permitting catabolism of glucose (1,2). A Glc transporter and enzymes of the ED pathway are encoded by a mobile *glc* locus inserted within an *rrn* operon (1). Analysis of draft genome sequences also identified presence of the *glc* locus in a small percent of sequenced *C. jejuni* strains almost exclusively from wild birds and Norway rats (2). Within the rat isolate collection of 137 strains, 32.7% of *C. jejuni* and 18.7% of *C. coli* were positive for the *glc* locus. Most of the *C. jejuni* belonged to a broad novel group (RG-2) most closely related to *C. jejuni* clonal complex CC-45 and clearly distinct from a second unique clade of rat isolates RG-1 (ED negative, 25.8% of *C. jejuni*) (Figure 1). Strains of RG-2, and also RG-1, appeared widespread within farm-associated rats in the S.E of England. The divergence of these groups from common clonal complexes of *C. jejuni* may be a consequence of adaptation to a specific host or environment.

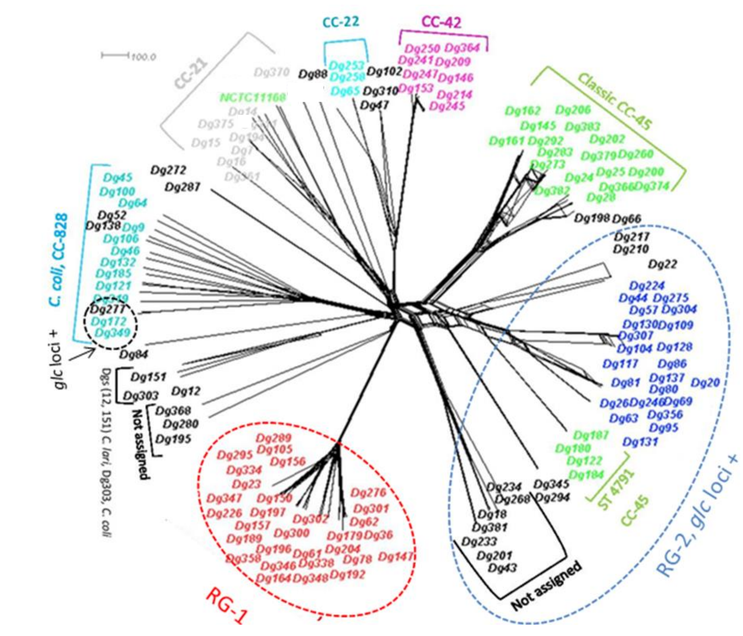

Figure 1: WGS phylogenetic network of *Campylobacter* strains from Norway rats. Comparison of whole genome sequences of *C. jejuni/ coli* strains isolated from rat faecal pellets from 8 farms in S.E. England over 2 years. Data available via PubMLST.org/ Campylobacter.
RG-1, red dotted circle (Rat group1, unique in MLST database); RG-2, blue dotted circle (Rat group 2), positive for *glc* loci and ED genes, blue highlighted strains complete *glc* locus; CC- clonal complex and ST-sequence type.

Aims

In this study, we investigate functionality of the ED pathway in two of the RG-2 rat isolates of *C. jejuni* subsp. *jejuni* (Figure 1) and assess the ability of both RG-1 and RG-2 strains to colonise chickens.

Results

Localisation of the *glc* locus within an *rrn* locus in *C. jejuni* Dg275

- 16 of 34 contigs of the draft genome sequence of the RG-2 strain, Dg275, mapped to the reference strain NCTC11168 using CONTIGuator . *ansB* identified at the end of contig 2, gap16 (Figure 2 A) using Artemis Comparison Tool (ACT) program
- Primers were designed to amplify and sequence DNA corresponding to gap16. A complete *glc* locus (8.67 kbp) was identified between *ansB* (contig 2) and *ggt* (contig 19) (Figure 2 B).
- Comparison with the same *rrn* locus in different strains, highlighted conservation of *ansB* and *recJ* upstream of 16SrRNA, but heterogeneity downstream of 5SrRNA, in addition to insertion of the *glc* locus (shown for Dg275 Dg 349 (*C coli)*

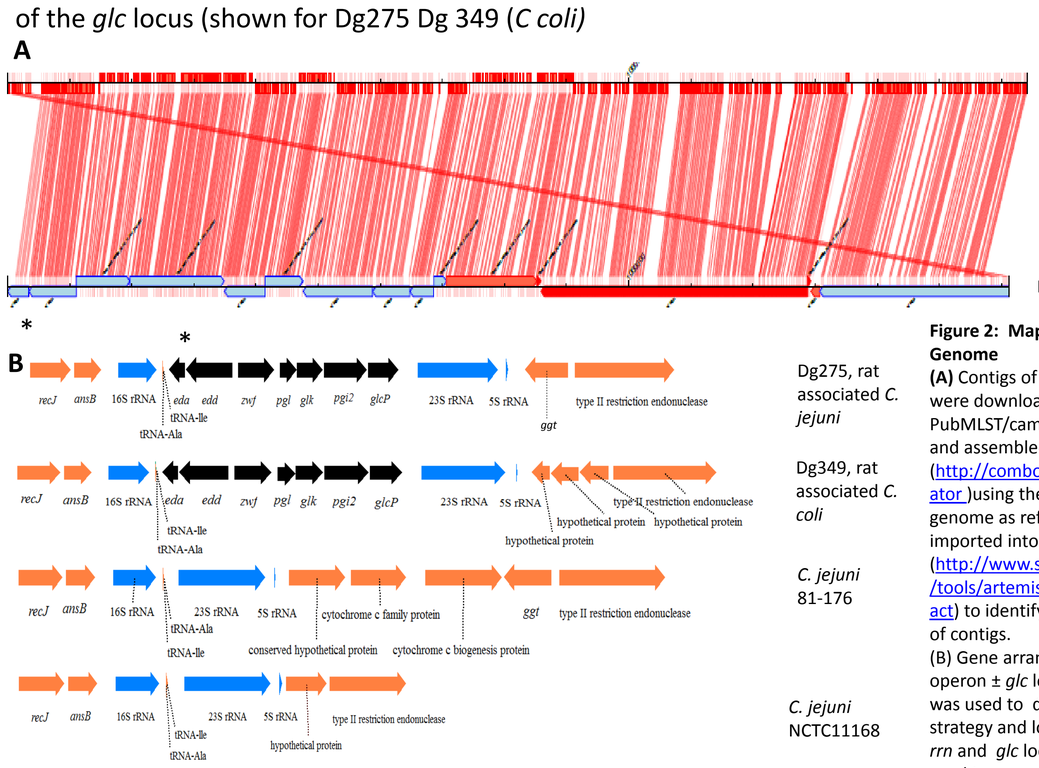

Figure 2: Mapping Dg275 draft Genome
(A) Contigs of WGS of Dg275 were downloaded from PubMLST/campylobacter database and assembled with CONTIGuator (http://combo.dbe.unifi.it/contiguator)using the NCTC11168 genome as reference. Files were imported into ACT software (http://www.sanger.ac.uk/science/tools/artemis-comparison-tool-act) to identify sequences at ends of contigs.
(B) Gene arrangements of rRNA operon ± *glc* loci. DNA Dynamo was used to design a sequencing strategy and locate and assemble *rrn* and *glc* loci. Vector NTI was used to gene assignment.

Diauxic growth – Serine and Aspartate preferred over glucose

- Functionality of ED pathway demonstrated for 24 RG-2 strains of *C. jejuni* (*glc*+) by enhanced growth in a microtitre plate minimal media assay (not shown).
- Glucose utilisation confirmed for strain Dg275, during growth in rich (MHB, Figure 3) and minimal media (not shown), supplemented with 20 mM glucose
- Serine and aspartate rapidly depleted – by early stationary phase and only then was glucose metabolised (after 24 h, stationary phase onwards). As recognised for other *C. jejuni* (3), proline was the least preferred of the 4 amino acids utilised.
- Ability to utilise glucose promoted survival to late stationary phase (72h) in contrast to growth without glucose and to ED negative strain, Dg200 (not shown)

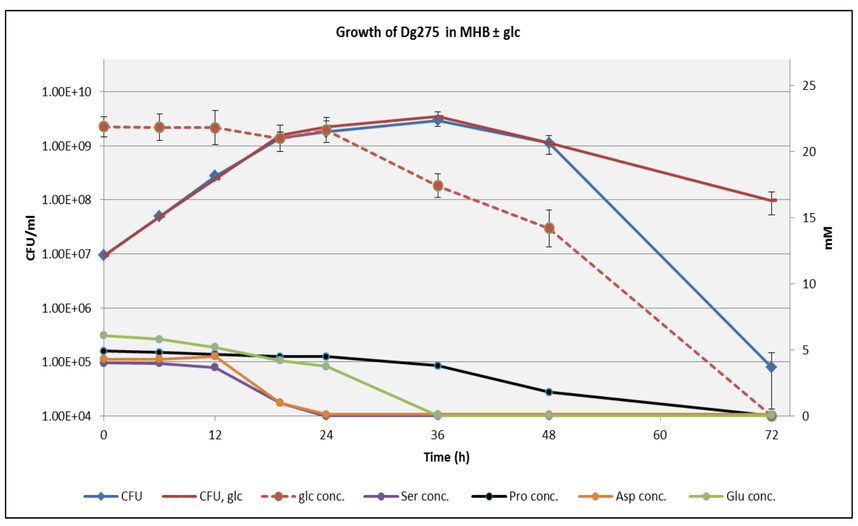

Figure 3: Growth of *C.jejuni* in rich media with and without glucose. *C. jejuni* Dg275 was incubated in 10 ml MHB, 10% FBS ± 20mM glc, with antibiotic for 72h at 37°C , at 150 rpm . Aliquots taken during growth were monitored for viable cells (CFU), glucose (glc) concentration using a Merk Glucose Assay Kit and all amino acids using a EZ-Faast-kit followed by GC-MS. Only amino acids showing a decrease in concentration are shown.

RG-2 strains colonise chickens less efficiently; RG-1 strain not at all

Colonisation assay:

- Ability of RG-1 and RG-2 isolates to colonise chickens was compared to colonisation by predicted 'generalist' rat isolates of clonal complexes, CC-21 and CC-45
- Groups of 14, 19 day old Ross 308 broiler chickens were infected through oral gavage with approximately ~7 $\log_{10}$ CFU of the test strain and housed individually
- 3 and 7 days post-infection, 7 birds/ group sacrificed and *Campylobacters* in caecal, ileum and jejunum contents quantitated by Miles and Misra on mCCDA (Figures 4 and 5).
- Identity of recovered strains, confirmed by selected ST alleles and *glc* utilisation

Figure 4: **Don Whitley DWS M35 cabinet**. Viable count of recovered colonies in a Don Whitley M35 cabinet, at 42°C for 48h, in a microaerophilic atmosphere of 5% O_2, 5% CO_2, 2% H_2 and 88% N_2

- ED positive strains, Dg95 and Dg275 colonised chicken caecum, although significantly less efficiently than the CC-45, CC42, ST 21 CC-21 (*Average for 7 chickens: Dg95 and 275, 5.2 and 4.7 $\log_{10}$ CFU/g respectively cf CC-45, CC42, ST 21 CC-21, 7.9, 7.16 and 7.57 $\log_{10}$ CFU/g respectively , P<0.000*)
- Interestingly, Dg147 (RG-1) did not colonize chickens and the ST 50 CC-21 strain colonised very poorly. One consistent difference with the RG-1 group is that none of them possess cytotoxin genes *cdtA* and *cdtC*

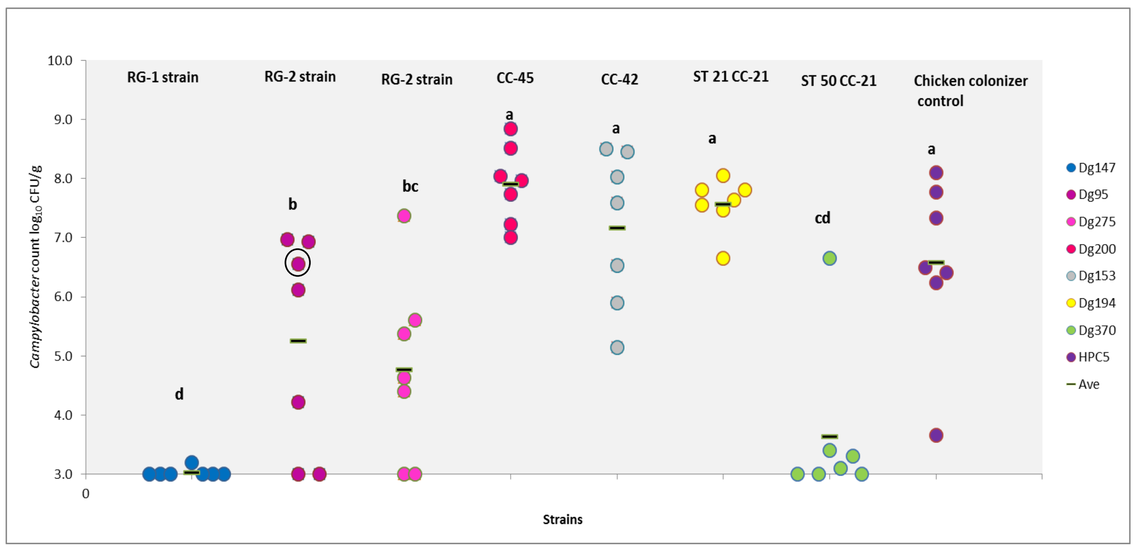

Figure 5: 7-day colonization of chicken caecum with novel *C. jejuni* (RG-1, RG-2) and strains belonging to generalist clonal complexes. 7 chickens / strain administered with ~7 $\log_{10}$ CFU as indicated. Each point, average of 6 counts from triplicate serial dilutions of caecal content from one chicken. Circled point, ED negative recovered strain. Black bar, average, statistical comparison SPSS 21; a, b, c, d- P < 0.000

Conclusions

This is the first demonstration of glucose utilisation by *C. jejuni* subsp. *jejuni.* For these strains glucose provides an additional substrate following amino acid depletion, permitting much longer survival. The absence of benefit of glucose catabolism to chicken colonisation by these strains is supported by the observed lower efficiency of chicken colonisation, recovery of a non-glucose utilising mutant (Figure 5) and the absence of the *glc* locus in draft genome data of strains recovered from chickens (2). A high percent of the rat associated *C. jejuni* were shown to possess a functional ED pathway. This raises the possibility that utilisation of glucose somehow benefits colonisation of the rat host or prolonged survival in the environment. Interestingly, Dg147 of the novel RG-1 group was unable to colonize chicken caecum, consistent with the hypothesis these may be rat adapted strains and a potential future model.

References

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