Abstract

The spiral Gram-negative bacterium *Campylobacter jejuni* is a microaerophile that lives in the intestinal tract of many farmed animals in a commensal-like association, but on infection of humans is pathogenic. It is the leading cause of bacterial gastroenteritis worldwide. Multi-locus sequence typing (MLST) has identified subgroups belonging to ST21CC21 andST45CC45 clonal complexes (CC) as generalists colonising different animals and causing human disease,while others show a narrower host-specificity. *C. jejuni* depends on amino acids, short chainfatty acids and intermediates of the TCA cycle as primary energy and carbon sources and cannot utilise glucose as a growth substrate due to an incomplete Embden-Meyerhof Parnas (EMP)pathway. Recently, a glc locus encoding enzymes of the Entner-Doudoroff (ED) pathway plus *glcP*, encoding a sugar permease, and *glk*, encoding glucose kinase, were identified in the genome sequence of a small number of *C. jejuni* and *C. coli* strains and ability of selected strains to metabolise glucose demonstrated.

This study investigated the association of the *glc* locus with *C. jejuni* strains of farm-associated Norway rat origin. The typed ED pathway of most isolates fell within two broad groups, belonging to either EDMLST types 2, 3, 9 and 11 (including strains Dg275 and Dg95) or to EDMLST types 21 and 23 (including strain Dg43) and this division correlated with distinct phylogenetic branches based on cgMLST. Growth studies combined with DNA amplification and Sanger sequencing confirmed possession of a complete glc locus and ability to utilise glucose for all of these strains and absence of all glc genes from a couple of other distantly related strains belonging to clonal complexes 21 and 45 for which partial glc genes were listed in the annotated genomes. The genetic organisation and insertion of the glc locus within each of the *3 rrn* loci of Dg43 and within *rrnA* and *rrnB* of Dg95 was demonstrated by long range PCR and Sanger sequencing, followed by hybrid Illumina and Nanopore sequencing of the genome.
In a previous study that assessed colonisation of chickens by strains Dg95 and Dg275, all five recovered isolates from one chicken no longer utilised glucose (Mohammed, 2018). Here, inability to use glucose was explained by identification of a mutation in glk in both copies of the *glc* locus for two isolates. Interestingly, in one isolate, Ch95-95-1 the mutation(Gly167STOP) created a pseudogene, while in a second isolate the mutation (GluGlyTrpHis) was within the predicted sugar binding site (consensus GluXGlyHis).
As ED positive strains of *C. jejuni* are notably absent from chicken isolates in the PUBMLST/campylobacter database, these results might reflect some incompatibility of glucose metabolism and competitive colonisation of the chicken caecum by *C. jejuni*.

During this study, glucose was consistently shown to enhance survival of ED+ strains during the decline phase of growth whether in rich or in a minimal medium, under standard conditions (5% O_2,10% CO_2, 2% H_2). Under conditions of aerobic stress (15.9% O2, 4.5% CO2, 0% H2) strains carrying the glc locus also showed a shorter lag phase, indicating that these strains are already primed to deal with aerobic stress. The mutant Ch95-95glk showed no glucose dependent benefit to survival in 5% or 15.9% oxygen. At suboptimal concentrations of oxygen (2% O2, 10% CO2, 2% H2), addition of glucose had no apparent benefit, consistent with less benefit in the caecum. Neither maltose nor lactose could be metabolised, although in vivo access to glucose would likely occur via degradation of these and other sugars by members of the intestinal microbiota.

The metabolism of glucose was also shown to protect cells from the harmful effect of H_2O_2 in the decline phase from 48-72h. Dg275 and Dg95 cells grown in glucose exhibited ~4-8 fold
higher cfu on exposure to H2O2 compared to cells grown without glucose. This correlated with the consistent observation that glucose metabolism by these strains delayed coccoid formation and extended survival of cells in the decline phase of growth. This study provides evidence that glucose metabolism by ED positive strains of *C. jejuni* extends survival under adverse conditions. It would be interesting to further explore the impact of glucose metabolism on survival in different environments, including in the chicken and Norway rat caecum and in environmental locations outside the host.