

Identification of genetic differences between two *Campylobacter jejuni* strains with different colonization potentials

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The consumption of poultry meat contaminated with *Campylobacter jejuni* is considered to be a risk factor for human campylobacteriosis. The development of targeted strategies to control campylobacters in broilers would benefit from knowledge of those bacterial factors important in colonization of the avian gut. During preliminary studies it was noted that *C. jejuni* NCTC 11168 was a poorer colonizer of chickens than strain 81116. This poor colonization could not be fully restored by *in vivo* passage, suggesting that it was a genetically endowed property of strain 11168. As the genome sequence is available for this strain, the technique of subtractive hybridization was used to identify gene fragments of strain 81116 not present in strain 11168. After two screening cycles, 24 out of 42 clones were identified as having DNA inserts specific for strain 81116. Six of these 24 clones contained gene fragment inserts with similarities to restriction–modification enzymes found in other bacteria. Two inserts had similarity to arsenic-resistance genes, whereas four others had similarities to cytochrome c oxidase III, dTDP-glucose 4,6-dehydratase, γ -glutamyl transpeptidase and an abortive phage-resistance protein. At least some of these genes may be involved with colonization. A further six inserts had weak similarities to hypothetical proteins or to proteins with assigned functions from strain 11168. The remaining six clones had gene-fragment inserts with no database matches. Southern-blot analysis confirmed that strain-dependent variation existed for each of these DNA inserts. These results indicate that subtractive hybridization can successfully identify genes that are absent from the only *C. jejuni* strain for which the genome sequence is currently available.

Keywords: subtractive hybridization

INTRODUCTION

Campylobacter jejuni is a major cause of human acute bacterial enteritis worldwide (Tauxe, 1992). The sources and routes of infection are debatable, but the consumption of contaminated poultry meat is considered to be a significant risk factor. Surveys have shown that broilers frequently carry large numbers of these organisms in their intestinal contents (Newell &

Wagenaar, 2000). This carriage is both chronic and asymptomatic. The spillage of gut contents during processing can contaminate the retail poultry product and the abattoir environment (Newell *et al.*, 2001). Currently, the reduction or elimination of campylobacters in the food chain is largely focussed at the poultry farm level. Unfortunately, enhanced biosecurity has largely failed to prevent campylobacter entering the broiler house from the environment. In part, this failure is a reflection of the low infectious dose, rapid bacterial growth within the avian gut and subsequent rapid transmission to adjacent animals. All of the evidence suggests that *C. jejuni* has evolved to efficiently colonize the avian gut as a commensal. Therefore, a better

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Abbreviations: AP, alkaline phosphatase; R–M, restriction–modification.
The GenBank accession numbers for the sequences reported in this paper can be found in Table 2.