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Comparative Genomics Reveals Unique Genetic Determinants of Biofilm Formation in *Campylobacter*

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Abstract

Biofilm is a complex microbial community that provides protection to bacterial cells against various stress conditions, including harsh environments, antimicrobial treatments, and host immune responses. This protective mechanism enhances *Campylobacter* survival during food processing and storage and promotes transmission to humans. Despite its significance, the molecular mechanisms underlying *Campylobacter* biofilm formation and its impact on pathogen persistence remain poorly understood. In this study, we characterized the biofilm-forming ability of 18 *C. jejuni and C. coli* strains isolated from retail meat and performed whole-genome sequencing and comparative genomic analysis to identify strain-specific genes contributing to biofilm formation and maintenance. Phenotypic analysis revealed that *C. jejuni* strains YH001 and YH027 exhibited the strongest biofilm-forming capacity, producing the highest biomass among all isolates. Phylogenetic analysis indicated a close genetic relationship between these two strains, while pangenome analysis identified 19 unique genes/proteins specific to these strains. Functional annotation indicated their critical roles in adhesion, extracellular matrix production, and stress response. These findings demonstrate strain-specific biofilm formation in *Campylobacter* and highlight genetic determinants that may serve as targets for novel therapeutic approaches and intervention strategies to disrupt biofilms, improve food safety, and reduce persistent infections.

Keywords: *Campylobacter*; biofilm; whole-genome sequencing; comparative genomics; pangenome analysis; foodborne pathogen; food safety

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Introduction

Campylobacter is one of the most common foodborne pathogens worldwide and a leading cause of human gastroenteritis, with typical symptoms including diarrhea, abdominal pain, fever, and nausea. According to the Centers for Disease Control and Prevention (CDC), approximately 1.5 million cases of *Campylobacter* infection occur each year in the United States, making it a major public health concern [1]. Among the various species, *Campylobacter jejuni* and *C. coli* are most commonly associated with human illness [2]. These species are prevalent in poultry and other animal-derived food and can also be found in eggs, unpasteurized milk, and untreated water. The primary routes of transmission to humans include consumption of undercooked poultry, cross-contamination in kitchens, and ingestion of contaminated water or raw milk [3,4].

Biofilms are structured microbial communities, in which cells are aggregated and embedded in a matrix of extracellular polymeric substances (EPS). This matrix, produced either by the cells themselves or by surrounding bacteria, consists of polysaccharides, structural proteins (*e.g.*, flagella and pili), nucleic acids (extracellular DNA (eDNA) and RNA (eRNA)), lipids, and other biomolecules [5]. Polysaccharides are essential for biofilm formation contributing to cell adhesion to surfaces and maintenance of structural integrity [6]. Biofilm proteins play important roles in biofilm development and survival of the cells by facilitating access to nutrients and regulating biofilm integrity. They are involved in attaching cells to surfaces, developing three-dimensional biofilm structures, and maintaining biofilm stability through interactions with exopolysaccharide and nucleic acids [7,8]. As a key component of biofilm structure, eDNA contributes to biofilm formation, adhesion, and structural integrity by binding and connecting cells within biofilms [9].

Biofilms formed by pathogenic bacteria present a major challenge in the food processing industry. Common foodborne pathogens, including *Campylobacter*, *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus*, readily develop biofilms on both biotic and abiotic surfaces during food processing and storage. These biofilms significantly enhance bacterial resistance to environmental stresses, disinfectants, and antibiotics, making intervention and eradication efforts difficult [10]. It has been reported that biofilm embedded cells can exhibit remarkably higher (10-1000-fold) resistance to antimicrobials compared with planktonic cells due to the protective properties of the matrix and altered physiology. Consequently, biofilms contribute to persistent contamination, reduced sanitation efficacy, and elevated food safety risks across production environments.

Campylobacter is a Gram-negative, spiral-shaped, microaerophilic bacterium that thrives in low-oxygen environments, typically requiring 3-10% oxygen, 5-10% CO_2 and temperature of 37-42 °C for optimal growth. These physiological constraints favor its persistence in niches such as the gastrointestinal tract of animals, as well as in water systems and plumbing within animal husbandry facilities and food processing plants. In these environments, *Campylobacter* frequently forms biofilms. Studies have shown that *C. jejuni* cells encased in a biofilm matrix exhibit significantly greater stress tolerance compared to planktonic cells. This enhanced resilience makes biofilm-associated *Campylobacter* extremely difficult to eradicate, leading to persistent contamination in food processing facilities and increasing the risk of foodborne infections [11–14].

Campylobacter biofilm formation exhibits significant strain-to-strain variation, influenced by genetic factors and environmental conditions such as low nutrients, extracellular DNA, aerobic/microaerobic conditions, and the presence of co-cultivated bacteria [15,16]. Our current understanding of the molecular mechanisms underlying *Campylobacter* biofilm formation remains limited. Although some genes involved in flagella synthesis, stress response, and quorum sensing have been reported to influence biofilm development, the overall genetic basis and regulatory networks remain poorly characterized [17–19].

Given the implications for public health, identifying the specific genes and proteins involved in biofilm formation is crucial for developing targeted interventions to disrupt biofilm development, reduce antibiotic resistance, and reduce pathogen contamination in food systems.

To address these gaps, we conducted comprehensive genomic and phenotypic analyses of *Campylobacter* biofilms formed by different strains isolated from retail meat products. A total of18 *Campylobacter* isolates including 9 *C. jejuni* and 9 *C. coli* were examined for their biofilm-forming ability. Whole-genome sequencing, phylogenetic analysis, and pan-genomic profiling were employed to identify genes and proteins associated with biofilm formation and to elucidate genetic relationships among the strains. Furthermore, the functions of unique genes identified exclusively in biofilm-forming strains were annotated to clarify their roles in biofilm formation and maintenance. Together, these findings advance our understanding of the genetic determinants of *Campylobacter* biofilms and inform the design of targeted strategies to improve food safety.

Materials and Methods

Sample Preparation

C. jejuni and *C. coli* strains were isolated from retail meat, including chicken meat, chicken livers, and beef livers, collected between 2011 and 2023 using previously described methods [20]. Briefly, 450 g of meat was combined with 250 ml buffered peptone water (BPW) and homogenized using a stomacher. The homogenate was centrifuged and the pellet was enriched in Bolton broth supplemented with horse blood and selective antibiotics (cefoperazone, trimethoprim, vancomycin, and cycloheximide) at 42 °C for 24 h under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2) using a CampyPak (Becton, Dickinson and Company, Franklin Lakes, New Jersey) in an airtight jar. Following enrichment, passive filtration onto Brucella agar was employed for strain isolation based on the highly motile nature of *Campylobacter*. Colonies were re-streaked twice for strain purification, and genus and species identification was performed using a multiplex qPCR assay previously developed for differentiating *C. jejuni* and *C. coli* [21].

Table 1. Sources and genome information of *C. jejuni* and *C. coli* isolates.

Strain and species	Source	Genome size (bp)	%GC	Accession No.	Reference
C. jejuni YH001	Veal livers	1,712,361	30.5	CP010058	[22]
C. jejuni YH008	Drumsticks	1,792,424	30.5	CP172380	This work
C. jejuni (S27Cj) YH009	Chicken thighs	1,663,226	30.5	CP131444	[23]
C. jejuni (S33Cj) YH010	Chicken thighs	1,748,761	30.5	CP131442	[23]
C. jejuni YH012	Chicken livers	1,698,963	30.5	CP172815	This work
C. jejuni YH013	Chicken livers	1,691,848	30.5	CP172379	This work
C. jejuni YH014	Chicken livers	1,802,039	30.5	CP172376	This work
C. jejuni YH027	Calf livers	1,710,959	30.5	CP172352	This work
C. jejuni YH028	Beef livers	1,667,698	30.5	CP172351	This work
C. coli YH501	Drumsticks	1,668,523	31.5	CP015528	[24]
C. coli YH502	Drumsticks	1,718,974	31.0	CP018900	[25]
C. coli YH504	Drumsticks	1,722,143	31.0	CP091644	[24]
C. coli YH507	Chicken livers	1,756,096	31.0	CP172392	This work
C. coli YH508	Chicken thighs	1,703,740	31.5	CP172391	This work
C. coli YH509	Chicken livers	1,697,113	31.5	CP172390	This work
C. coli YH510	Chicken livers	1,812,356	31.0	CP172387	This work
C. coli YH511	Chicken livers	1,674,288	31.5	CP172385	This work
C. coli YH512	Chicken livers	1,754,135	31.5	CP172384	This work

Biofilm Formation

Campylobacter isolates were streaked from -80 °C frozen stocks onto Mueller-Hinton (MH) agar plates and incubated overnight under microaerobic conditions (5% O_2, 10% CO_2, 85% N_2) at 42 °C. Fresh colonies were scraped from the agar plates and resuspended in 2 mL of MH broth, followed by overnight incubation under the same conditions. Subsequently, 50 mL of the overnight culture was inoculated into 5 mL of MH broth and homogenized. Two aliquots of 2 mL of the diluted culture were transferred into 10 cm^2 /10ml polystyrene tissue culture tubes with a flat surface and vent cap (Techno Plastic Products AG, Canton Schaffhausen, Switzerland) and incubated horizontally for 5 days to allow biofilm development. All incubations were performed under microaerobic conditions at 42 °C.

Biofilm Quantification

Biofilm cultures were filtered onto a 40 mm cell strainer fitted on a 50 mL conical tube. The strainer containing aggregated cells was rinsed with 2 mL of fresh MH broth, transferred to a 6-well plate and then submerged in 6 ml 0.1% crystal violet solution. After a 30-min incubation at room temperature, the strainers and aggregates were rinsed three times with 5 mL of sterile water and photographed on a light box. The strainer was then placed in a fresh 6-well plate and submerged in 6 mL of 100% ethanol. After 30 min of incubation at room temperature with gentle shaking, a pipette was used to dislodge aggregates from the strainer surface and mix the ethanol. The absorbance of the ethanol solution (200 mL in each well, in triplicate) was measured at 590 nm using a Cytation 5 plate reader (BioTek/ Agilent Life Sciences, Winooski, Vermont).

Genome Sequencing, Assembly, and Annotation

Genomic DNA was extracted using the Qiagen genomic tip 100/G kit (Valencia, CA) and quantified with a Qubit 3.0 fluorometer (Thermo Fisher Scientific, Waltham, MA) following the manufacturers' instructions. Whole-genome sequencing was performed using Illumina MiSeq and Pacific Biosciences (PacBio) RSII and/or Sequel platforms. Genome assemblies were generated from PacBio long reads using Canu v2.2 [26]. Overhangs of assembled contigs were trimmed and reoriented using Circlator v1.5.5 [27]to produce complete circularized genomes.

In a few cases where reorientation and trimming failed, Illumina MiSeq reads were used to correct sequencing errors in assembled contigs: MiSeq reads were mapped to Canu assemblies using BWA v0.7.17-r1188 [28]; errors were corrected using Pilon v1.22 [29]; pilon correction was repeated iteratively until no errors were detected. Finally, the assemblies were trimmed and reoriented using Circlator v1.5.5 to generate complete circular genomes.

Table 1 summarizes the source and assembled genome information for *C. jejuni* and *C. coli* isolates. For each strain, the complete genome of approximately 1.6–1.8 Mbp, consistent with the size of previously reported *Campylobacter* chromosomes, was annotated using the RAST server [30,31]. Chromosomal integrity was verified by confirming the presence of the start gene *dnaA*, three copies of rRNA operons (23S, 16S, and 5S rRNA), and minimal repeat sequences. Smaller contigs were assessed for potential plasmid sequences.

Pangenome & Phylogenetic Analysis

The *Campylobacter* pangenome was constructed using the KBase web server [32]. First, genome assemblies were annotated with RASTtk v1.073, then the pangenome was constructed using OrthoMCL v0.0.8 [33]. To investigate the relatedness of different genome clusters, we constructed a phylogenetic parsimony tree from all SNPs using kSNP4 v4.1 [34] with a k-mer size of 19. Bootstrap support values were calculated with IQ-Tree v 2.1.2 [35] on the CIPRES Scientific Gateway [36]. Briefly, a maximum likelihood consensus tree was built using a General Time Reversible (GTR) model with a correction for Ascertainment bias. The kSNP4 parsimony tree was served as the starting tree, and a non-parametric bootstrap analysis was performed with 1000 replicates to assess branch support. Whole-genome sequences (accession numbers) used for this analysis are listed in Table 1. The resulting phylogenetic tree was visualized using Iroki [37].

To determine which genes were unique or shared among the *Campylobacter* genomes, we constructed a pangenome using OrthoMCL, based on RASTtk annotations generated through the KBase Server. Heatmaps depicting gene presence/absence and the number of shared genes among the genomes were generated in R v4.4.0 (R Core Team 2024) using the ggplot2 and viridis packages [38].

Results and Discussion

1. Determination of Biofilm-Forming Ability of C. jejuni and C. coli Food Isolates

Biofilm formation was examined in 18 food-derived *Campylobacter* isolates (9 *C. jejuni* and 9 *C. coli*). After incubating duplicate samples in polystyrene tubes under microaerobic conditions at 42°C for five days, adherent cells were washed and stained with crystal violet solution. The results are shown in Figure 1. Biofilm-forming ability varied significantly among the strains: *C. jejuni* YH001 and YH027 produced the largest amount of biofilm biomass, as indicated by crystal violet staining, whereas *C. coli* YH504 and YH507 formed moderate biofilms. The remaining strains showed negligible biofilm under the same conditions. The stronger adhesion and aggregation observed in *C. jejuni* YH001 and YH027 compared to other isolates indicate that biofilm formation in *Campylobacter* is strain-dependent, a finding consistent with prior studies showing variability across strains and species [39].

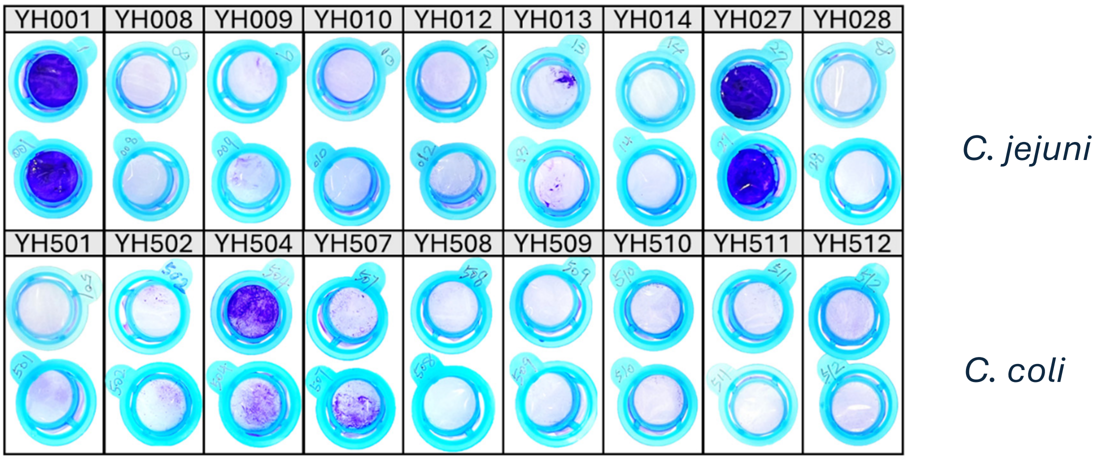

Figure 1. Biofilms of *C. jejuni* (upper panel) and *C. coli* (low panel) strains stained with crystal violet. Biofilms were developed in duplicate samples under microaerobic conditions at 42 °C for five days and visualized after staining.

2. Comparison Between Genetic Relatedness and Biofilm Formation of Campylobacter Isolates

After crystal violet staining, the biofilm biomass was dislodged and quantified by measuring absorbance at 590 nm. The results confirmed that *C. jejuni* YH001 and YH027 were the strongest biofilm-forming strains, whereas *C. coli* YH504 and YH507 formed moderate biofilms among 18 *Campylobacter* food isolates (Figure 2, right panel).

Biofilm formation in bacteria can be influenced by genetic background, environmental adaptation, and regulatory mechanisms. To compare the genotypic traits associated with *Campylobacter* biofilm formation, a phylogenetic tree was constructed based on SNPs derived from whole-genome sequences of *C. jejuni* and *C. coli* isolates (Figure 2, left panel). Interestingly, *C. jejuni* YH001 and YH027 (two strongest biofilm producers) were clustered together in the tree, indicating close genetic relatedness and shared genomic elements. Similarly, *C. coli* YH504 and YH507 (two moderate biofilm producers) were also clustered closely in the tree. These findings suggest that biofilm-forming ability may correlate with genetic similarity, supporting the observed strain-dependent variability in *Campylobacter* biofilm formation.

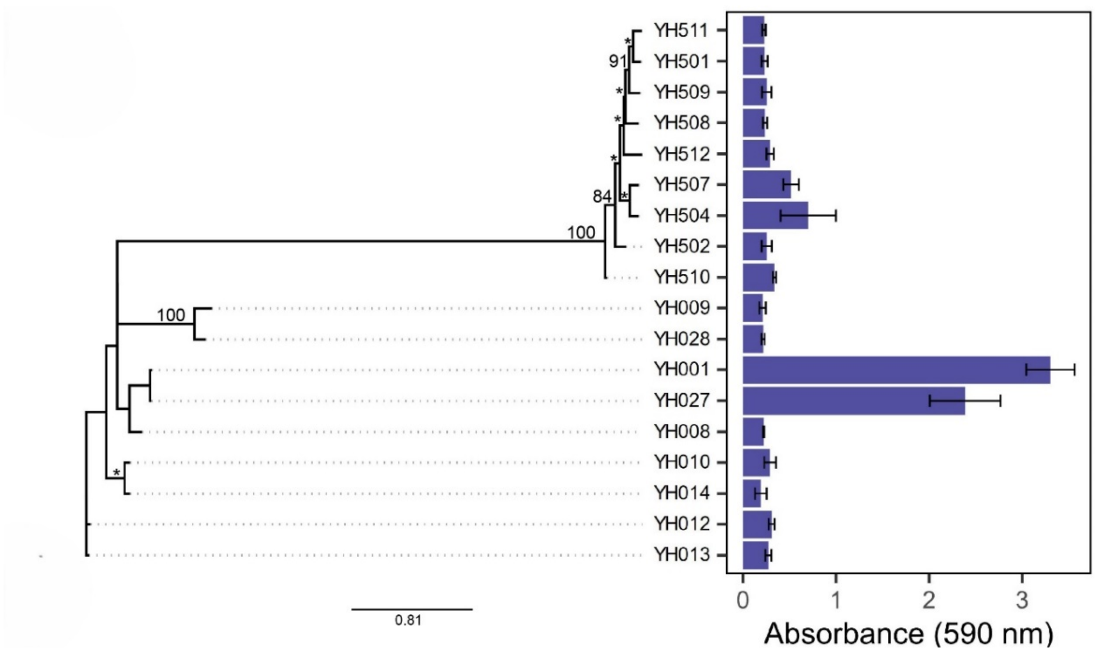

Figure 2. SNP-based phylogenetic tree and biofilm quantification of *C. jejuni* and *C. coli* isolates. Left panel: SNP-based phylogenetic tree constructed from whole-genome sequences of *C. jejuni* (YH001–YH028) and *C. coli* (YH501–YH512) isolates. Bootstrap values on branches correspond with maximum likelihood consensus tree. * indicates a bootstrap value of 100. Right panel: Biofilm formation quantified by crystal violet staining after 5 days of growth in Mueller–Hinton broth under microaerobic conditions at 42 °C without shaking. Absorbance at 590 nm was averaged from two biofilm samples with triplicate measurements each. Error bars represent the standard deviation of the mean.

3. Genetic Traits Associated with Biofilm Formation and Stability

To identify core genes shared by all 18 *Campylobacter* isolates and variable genes unique to specific strains, a pangenome analysis was performed. The complete results are provided in Supplemental Table 1. The heatmap in Figure 3 illustrates the presence and absence of genes across *C. jejuni* and *C. coli* isolates, revealing highly diverse genetic profiles with no two strains exhibiting identical genome patterns.

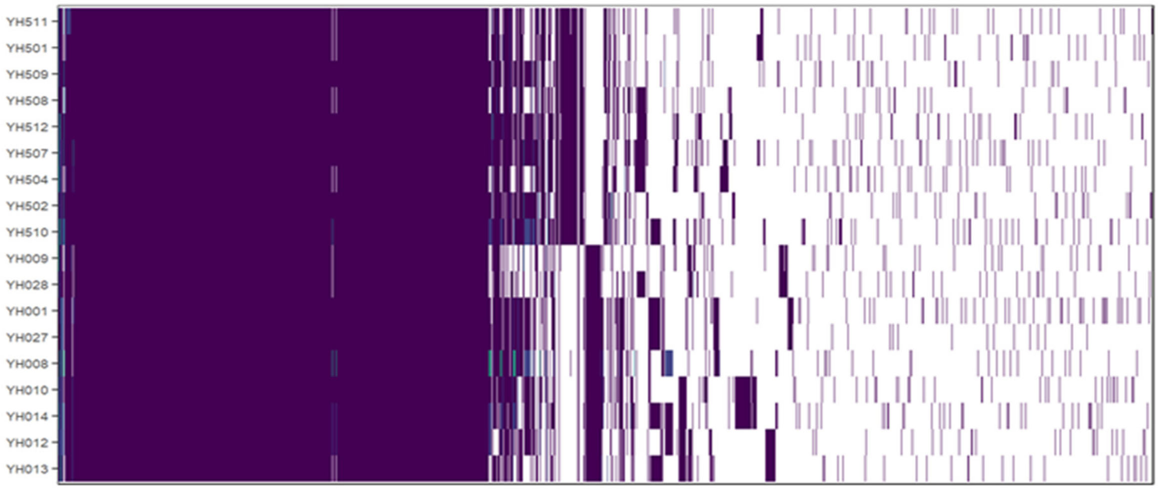

Figure 3. Pangenome analysis of *C. jejuni* and *C. coli* isolates. The heatmap shows the presence (purple) and absence (white) of genes or gene clusters across *C. jejuni* and *C. coli* genomes. The x-axis represents gene clusters, but their position does not correspond to chromosomal location. This visualization highlights the genetic diversity among isolates.

Identification of Strain-Specific Genes Associated with Biofilm Formation

To identify genes potentially involved in *Campylobacter* biofilm formation, we searched for strain-specific genes and annotated proteins using the complete pangenome dataset (Supplemental Table 1). This analysis revealed 19 unique genes/proteins, including 5 hypothetical proteins, present in *C. jejuni* YH001 and YH027, two isolates characterized as strong biofilm producers (Table 2).

Table 2. Annotated proteins uniquely present in *C. jejuni* strains YH001 and YH027.

Annotated proteins	YH001	YH027	The rest strains
Putative Dihydrolipoamide dehydrogenase (EC 1.8.1.4); Mercuric ion reductase (EC 1.16.1.1); PF00070 family, FAD-dependent NAD(P)-disulphide oxidoreductase	1	1	0
D-alanyl-D-alanine carboxypeptidase (EC 3.4.16.4)	1	1	0
FIG00471123: hypothetical protein	1	1	0
DNA-binding protein Roi	1	1	0
FIG00470265: hypothetical protein	1	1	0
FIG00471635: hypothetical protein	1	1	0
FIG00470314: hypothetical protein	1	1	0
hydrogenase, (NiFe)/(NiFeSe) small subunit family	1	1	0
L-Proline/Glycine betaine transporter ProP	1	1	0

Uncharacterized membrane protein, YraQ family	1	1	0
Alpha-ketoglutarate permease	1	1	0
Putative efflux protein	1	1	0
Ammonium transporter	1	1	0
C4-dicarboxylate transporter	1	1	0
Sodium-dependent phosphate transporter	1	1	0
Multi antimicrobial extrusion protein (Na(+)/drug antiporter), MATE family of MDR efflux pumps	1	1	0
Hypothetical protein Cj0566	1	1	0
Potassium-transporting ATPase A chain (EC 3.6.3.12) (TC 3.A.3.7.1)	1	1	0
Cytochrome c family protein	1	1	0

“1” and “0” indicated the presence and absence of the genes in the isolates, respectively.

Functional annotation revealed that these proteins play important roles in biofilm formation and/or maintenance, primarily by mediating cell adhesion to surfaces, extracellular matrix production, and signaling pathways that regulate biofilm stability.

Specifically, Dihydrolipoamide dehydrogenase (DLDH) is a central metabolic enzyme that converts pyruvate to acetyl-CoA for energy production, which is critical for bacterial survival and growth under nutrient-limited conditions. This enzyme is also essential for bacterial adherence, biofilm development, structure integrity, and virulence, and it has been detected in the exopolysaccharide (EPS) of *Pseudomonas aeruginosa* biofilms [40].

FAD-dependent NAD(P)-disulfide oxidoreductases catalyze disulfide bond formation, which stabilizes biofilm matrix proteins and therefore enhances cohesion and resilience within the biofilm. They also participate in polysaccharide biosynthesis, contributing to the extracellular polymeric substance (EPS) that forms the biofilm matrix [41,42].

DD-carboxypeptidase is a key enzyme in peptidoglycan (PG) biosynthesis. PG is a major structural component of bacterial cell walls and is essential for biofilm formation and function by providing cell structural integrity and shape. Proper PG synthesis supports cell-cell interactions and surface attachment [43,44]. Studies have shown that, in *C. jejuni*, mutations in DD-carboxypeptidase lead to defective PG assembly and impaired biofilm formation [45].

DNA-binding protein Roi is important in structuring the biofilm matrix, which is formed largely by extracellular DNA (eDNA) along with exopolysaccharides (EPS) and other components. DNA-binding proteins act as molecular glues, linking eDNA to other matrix components and cells within the biofilm, which is a function critical for biofilm formation and maintaining structural integrity [46].

NiFe hydrogenases have been identified in diverse biofilm communities by metagenomic studies. They are involved in a variety of metabolic pathways within biofilms, including hydrogen metabolism and energy conversion [47].

L-Proline/Glycine betaine transporter (ProP) facilitates the transport of proline and glycine betaine across the cell membrane, enabling cells to maintain osmotic balance and survive under stressful conditions. It plays a crucial role in biofilm formation as it enhances surface attachment and the synthesis of exopolysaccharides necessary for biofilm structure [48].

The YraQ family of membrane proteins facilitates cell adhesion and contributes to the overall structure and stability of the biofilm. Specifically, YraQ family proteins can be involved in initial cell attachment and the production of extracellular polymeric substance (EPS) matrix, which is a key component of biofilms [49].

Alpha-ketoglutarate permease (KgtP) transports α-ketoglutarate across cell membranes. KgtP is involved in carbon and nitrogen utilization, which is crucial for bacterial survival and adaptation. In a biofilm environment, cells often experience nutrient limitations and stress. By enabling the efficient uptake of α-ketoglutarate, KgtP may contribute to the growth and survival of bacteria within a biofilm, potentially influencing biofilm development and structure [50].

Efflux proteins can export EPSs, the primary components of the biofilm matrix, potentially impacting the structure and density of the biofilm. In addition, efflux pumps can contribute to biofilm development by exporting quorum sensing (QS) molecules, regulating transcriptional factors

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involved in biofilm formation, and facilitating bacterial aggregation and adhesion to surfaces by exporting molecules that affect these processes [51,52].

Ammonium transporters mediate the transport of ammonium across bacterial cell membranes, which are essential for biofilm physiology, facilitating nutrient acquisition, waste removal, and potentially influencing biofilm development through their interactions with biofilm components and other regulatory mechanisms. The activity of ammonium transporters can influence biofilm structure and function, impacting the growth and survival of microorganisms within the biofilm [53].

Sodium-dependent phosphate transporters are important for biofilm formation by enabling bacteria to acquire and utilize phosphate, a vital nutrient for cell metabolism and growth. The PstS protein, a subunit of the Pst phosphate transporter, is particularly important in this process, as it not only facilitates phosphate uptake but also influences the structure of bacterial biofilms [54].

Multi-antimicrobial extrusion proteins (MATEs) are essential components of bacterial defense mechanisms, particularly within biofilms. Their ability to extrude various antimicrobial substances contributes to the high levels of resistance and tolerance observed in biofilms [51,55].

Potassium-transporting ATPase A chain is involved in bacterial biofilm formation and maintenance by transporting potassium and maintaining proper intracellular potassium levels for essential functions like membrane potential, pH balance, and cell growth. Biofilms are complex communities of bacteria encased in a self-produced extracellular matrix, and disrupting potassium transporters can alter biofilm development and function [56].

Cytochrome c family proteins are essential for electron transfer, redox balancing, and energy production in bacteria, which is important for biofilm formation and cell respiration within the biofilm [57].

Together, these functional attributes underscore the importance of these proteins in promoting *Campylobacter* biofilm matrix production, structural integrity, and stability under diverse conditions. Future research should focus on functional validation of these candidate genes and proteins, elucidating their regulatory networks, and exploring innovative biofilm disruption strategies, including targeted inhibitors or environmental interventions, to mitigate *Campylobacter* persistence in food production systems. Additionally, given the association between biofilm formation and increased antimicrobial tolerance, further studies are needed to assess the role of these mechanisms in antimicrobial resistance and their impact on public health.

Conclusion

This study demonstrates the strain-specific nature of biofilm formation in *Campylobacter* spp., identifying two strong biofilm-forming strains among 18 food isolates. Through whole-genome sequencing and comparative genomic analysis of biofilm-forming and non-forming strains, we uncovered key genes and pathways associated with biofilm development and stability, providing new insights into the genetic determinants underlying this phenotype. Phylogenetic and pangenomic analyses provided valuable insights into the genetic basis of biofilm formation, and highlighted strain-specific ability to utilize this mechanism for survival in challenging conditions, particularly those encountered during food processing and storage. Importantly, the identification of specific proteins linked to both biofilm formation and pathogenesis in *C. jejuni* strains YH001 and YH027 offers promising molecular targets for novel therapeutic approaches and intervention strategies aimed at disrupting biofilms. These findings not only advance our understanding of the molecular basis of *Campylobacter* biofilms but also have significant implications for improving food safety and reducing the risk of persistent contamination and chronic infections.

Author Contributions: Yiping He, Gretchen Dykes, Chin-Yi Chen and Joseph Capobianco: design and perform experiments, analyze data, interpret results, and prepare manuscript. Heather Koppenhofer: conduct experiments, analyze results, and review manuscript.

Data Availability Statement: All the assembled genome sequences of *Campylobacter* isolates were deposited and are available in GenBank, NCBI under the accession numbers listed in Table 1.

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Conflicts of Interest: The authors declare no conflict of interest.

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