MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DE PELOTAS
FACULDADE DE AGRONOMIA ELISEU MACIEL
DEPARTAMENTO DE CIÊNCIA E TECNOLOGIA AGROINDUSTRIAL
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

TESE

Proteômica global de *Campylobacter jejuni* NCTC11168 sob diferentes condições de pH

Tassiana Ramires

Pelotas, 2022

Tassiana Ramires

Proteômica global de *Campylobacter jejuni* NCTC11168 sob diferentes condições de pH

Tese apresentada ao Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Doutor em Ciência e Tecnologia de Alimentos (área de conhecimento: Microbiologia de Alimentos).

Comitê de orientação:

Prof. Dr. Wladimir Padilha da Silva
Profa. Dra. Ângela Maria Fiorentini

Pelotas, 2022

Universidade Federal de Pelotas / Sistema de Bibliotecas
Catalogação na Publicação

R173p Ramires, Tassiana

Proteômica global de *Campylobacter* jejuni NCTC11168 sob diferentes condições de pH / Tassiana Ramires ; Wladimir Padilha da Silva, orientador. — Pelotas, 2022.

81 f. : il.

Tese (Doutorado) — Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, 2022.

1. *Campylobacter* termofílicos. 2. Estresse ácido. 3. pH intestinal. 4. Campilobacteriose. 5. Cortes cárneos de frango. I. Silva, Wladimir Padilha da, orient. II. Título.

CDD : 664

Elaborada por Gabriela Machado Lopes CRB: 10/1842

Tassiana Ramires

Proteômica global de *Campylobacter jejuni* NCTC11168 sob diferentes condições de pH

Tese aprovada, como requisito parcial, para obtenção do grau de Doutora em Ciência e Tecnologia de Alimentos, Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas.

Data da Defesa: 29 de julho de 2022.

Banca examinadora:

Prof. Dr. Wladimir Padilha da Silva (Orientador), Doutor em Ciência dos Alimentos pela Universidade de São Paulo (USP).

Profa. Dra. Ângela Maria Fiorentini (Coorientadora), Doutora em Ciência dos Alimentos pela Universidade Federal de Santa Catarina (UFSC).

Profa. Dra. Graciela Völz Lopes, Doutora em Ciências Veterinárias pela Universidade Federal do Rio Grande do Sul (UFRGS).

Dra. Isabela Schneid Kroning, Doutora em Ciência e Tecnologia de Alimentos pela Universidade Federal de Pelotas (UFPel).

Prof. Dr. Marcelo Mendonça, Doutor em Biotecnologia pela Universidade Federal de Pelotas (UFPel).

Profa. Dra. Rita de Cássia dos Santos da Conceição, Doutora em Ciências pela Universidade Federal de Pelotas (UFPel).

Dedico

À minha mãe, Marly, que nunca mediu esforços a mim e à minha criação, além de sempre ter me ensinado que o conhecimento é a única coisa que ninguém nunca irá tirar de nós...

AGRADECIMENTOS

À minha mãe Marly, por estar sempre presente nos momentos em que eu mais precisei, sem ela com certeza as minhas realizações não seriam possíveis. É por ela que sigo sempre em busca do meu melhor. Mãe, tu és a minha maior e melhor inspiração de vida!

Ao meu pai Paulo, pelo incentivo em momentos difíceis e por se fazer presente sempre como meu grande amigo.

À minha irmã Tatiane, por ser um dos meus exemplos de pessoa e profissional, que não mede esforços para o bem dequeles que ama.

Ao meu afilhado Bernardo e à minha sobrinha Brenda, por serem fonte de amor em nossa família e estímulo para que eu seja um bom exemplo a eles.

Às minhas avós Clélia e Cely (in memoriam), que sempre me motivaram da maneira mais pura e sincera.

A todos os meus familiares, tios e primos que sempre me valorizam e incentivam na área acadêmica.

Ao meu orientador Wladimir Padilha da Silva, por ter acreditado em mim e ter me recebido de braços abertos no Lab Micro desde o princípio. Wladi, se eu for metade do profissional que tu és eu já estarei plenamente realizada... obrigada também pela amizade ao longo dos anos e por ser um paizão para mim, o qual eu quero sempre honrar e orgulhar.

Aos meus amigos, Amanda Escobar, Rodrigo de Lima, Carol Lunkes, Luiz Guilherme, Thiago Franco, Risada, Flávia Voloski, Mari Iglesias e Rafa Vieira, alguns mais distantes fisicamente ao longo dos últimos anos, outros companheiros de todas as horas, meu muito obrigada por tudo... comemorar as conquistas, se partilhar com vocês, teriam o mesmo sabor...

Aos meus últimos amigos conquistados, mas não menos importantes... Débora, Gabi, Si e Sid, obrigada pela amizade e companheirismo dos últimos meses, vocês estavam presentes em momentos de grandes desafios e poder contar com vocês tornou tudo mais fácil!

Aos meus colegas e companheiros do Laboratório de Microbiologia de Alimentos que fazem com que eu me realize em fazer parte dessa equipe... a vocês Andréia, Diego, Gustavo, Itiane, Laís, Letícia, Liss, Pâmela, à professora

Ângela, à professora Graciela, meu muito obrigada pela parceria, pelas conversas, risadas e compartilhamento de conhecimento, além de todo o apoio sempre quando necessário.

Às minhas amigas irmãs, Camila, Isa, Kau e Nata... que sempre tornaram mais agradável qualquer situação, estar com vocês é sempre prazeroso e tenho muita sorte de ter encontrado vocês ao longo do caminho da pós-graduação!

Aos membros da banca examinadora por aceitarem contribuir com este trabalho, Isabela, Graciela, Marcelo e Rita, tenham a certeza de que todos vocês são especiais de alguma forma para mim.

À Capes pela concessão de bolsa de estudos.

À *University of Tasmania* e ao PhD John Bowman, por me receberam durante a realização do meu doutorado Sanduíche.

À Universidade Federal de Pelotas e ao Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, pela oportunidade de executar este trabalho.

A todos que de uma forma ou de outra me auxiliaram e torceram pela realização desse sonho, meu muito obrigada!

RESUMO

Ramires, Tassiana. **Proteômica global de *Campylobacter jejuni* NCTC11168 sob diferentes condições de pH.** 2022. 81f. Tese (Doutorado) - Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos. Universidade Federal de Pelotas, Pelotas.

Campylobacter termofílico é um subgrupo dentro do gênero *Campylobacter*, formado pelas espécies *C. jejuni*, *C. coli*, *C. lari* e *C. upsaliensis,* sendo assim denominado devido a sua temperatura ótima de multiplicação oscilar entre 42 °C. *Campylobacter* termofílicos são, atualmente, as principais bactérias causadoras de doenças transmitidas por alimentos em todo o mundo, além de estarem entre as quatro principais causas globais de doenças diarreicas. Dentre as espécies de *Campylobacter* termofílicos, a mais relacionada à campilobacteriose é *C. jejuni*, seguida por *C. coli*. O principal reservatório de *C. jejuni* são as aves, principalmente os frangos, possivelmente pela temperatura corporal desses animais ser similar à temperatura ótima para a sua multiplicação. Apesar da importância desse patógeno para a saúde pública, pouco ainda se sabe sobre os mecanismos de sobrevivência e de infecção de *C. jejuni.* Com isso, o objetivo desse estudo foi avaliar a proteômica global de *C. jejuni* NCTC11168 quando submetido a diferentes condições de pH, simulando barreiras encontradas no organismo humano e em cortes cárneos de frango. Para isso, a cepa padrão *C. jejuni* NCTC 11168 foi submetida a diferentes valores de pH, a fim de tornar o ambiente *in vitro* semelhante ao pH gástrico (pH 4,0- o mais ácido no qual houve multiplicação microbiana em testes prévios), ao pH médio de cortes cárneos de frango (pH 5,8) e ao pH intestinal humano (pH 8,0). As proteínas foram extraídas pela técnica *single spot* (SP3) e a separação, identificação e quantificação das proteínas, foi realizada por cromatografia líquida acoplada à espectrometria de massas. Observou-se que houve diferença significativa na síntese de proteínas, nas diferentes condições de pH testadas. Foi constatado que o pH 5,8 foi o que mais favoreceu a síntese de proteínas relacionadas aos mecanismos de defesa de *C. jejuni*, sendo esse um achado interessante visto que é o pH encontrado nos cortes cárneos, podendo ser um fator que beneficie a sobrevivência do patógeno e consequentemente proporcione a disseminação da campilobacteriose. Além disso, foi identificado aumento na abundância de duas proteínas responsáveis pela geração de energia via respiração anaeróbica (NapA e FrdA) quando em pH 8,0, sugerindo que o pH intestinal induz ao aumento da atividade da cadeia transportadora de elétrons, provavelmente a fim de garantir a homeostase citoplasmática de *C. jejuni*. A partir dos resultados obtidos no presente estudo, foi possível comprovar que de alguma forma as proteínas de resposta ao estresse (térmico e oxidativo) também estão envolvidas na resposta ao estresse ácido de *C. jejuni*. Além disso, foi possível verificar que dentre os pH avaliados, o pH 5,8 foi o mais expressivo em termos de respostas ao estresse ácido, sendo uma boa condição a ser trabalhada em pesquisas futuras, a fim de se ter um melhor conhecimento sobre a interferência do pH na patogênese de *C. jejuni*.

Palavras-chave: *Campylobacter* termofílicos, estresse ácido, pH intestinal, campilobacteriose, cortes cárneos de frango.

ABSTRACT

Ramires, Tassiana. **Global proteomics of *Campylobacter jejuni* NCTC11168 under different pH conditions.** 2022. 81f. Thesis (Doctorate) - Postgraduate Program in Food Science and Technology. Federal University of Pelotas, Pelotas.

Thermophilic *Campylobacter* is a subgroup within the genus *Campylobacter*, formed by the species *C. jejuni*, *C. coli*, *C. lari* and *C. upsaliensis*, being so named because of its optimal multiplication temperature oscillating between 42 °C. Thermophilic *Campylobacter* are currently the leading foodborne disease-causing bacteria worldwide, as well as being among the top four global causes of diarrheal disease. Among the thermophilic *Campylobacter* species, the most related to campylobacteriosis is *C. jejuni*, followed by *C. coli*. The main reservoir of *C. jejuni* are birds, mainly poultry, possibly because the body temperature of these animals is similar to the optimal temperature for their multiplication. Despite the importance of this pathogen for public health, little is known about the survival and infection mechanisms of *C. jejuni*. Thus, the objective of this study was to evaluate the global proteomics of *C. jejuni* NCTC11168 when subjected to different pH conditions, simulating the barriers found in the human organism and in chicken meat cuts. For this, the standard strain *C. jejuni* NCTC 11168 was submitted to different pH values, in order to make the in vitro environment similar to gastric pH (pH 4.0 - the most acidic in which there was microbial multiplication in previous tests), to the average pH of chicken meat cuts (pH 5.8) and to the human intestinal pH (pH 8.0). Proteins were extracted by the single spot technique (SP3) and the separation, identification and quantification of proteins was performed by liquid chromatography coupled to mass spectrometry. It was observed that there was a significant difference in the synthesis of proteins, in the different pH conditions tested. It was found that pH 5.8 was the one that most favored the synthesis of proteins related to the defense mechanisms of *C. jejuni*, which is an interesting finding since it is the pH found in chicken meat cuts, which may be a factor that benefits the pathogen survival and consequently provide the campylobacteriosis spread. In addition, increase in the abundance of two proteins responsible for energy generation via anaerobic respiration (NapA and FrdA) was identified when at pH 8.0, suggesting that intestinal pH induces an increase in the activity of the electron transport chain, probably in order to to guarantee the cytoplasmic homeostasis of *C. jejuni*. From the results obtained in the present study, it was possible to prove that somehow the stress response proteins (thermal and oxidative) are also involved in the *C. jejuni* acid stress response. In addition, it was possible to verify that among the evaluated pH, pH 5.8 was the most expressive in terms of responses to acid stress, being a good condition to be worked on in future research, in order to have a better knowledge about of the interference of pH in the *C. jejuni* pathogenesis.

Keywords: Thermophilic *Campylobacter*, acid stress, intestinal pH, campylobacteriosis, chicken meat cuts.

SUMÁRIO

1. Introdução

Atualmente, existem 57 espécies que pertencem ao gênero *Campylobacter* (http://www.bacterio.net/campylobacter.html - acesso em 13/07/2022). *Campylobacter jejuni*, *C. coli*, *C. lari* e *C. upsaliensis* representam o grupo denominado termofílico, devido à sua temperatura ótima de multiplicação oscilar entre 42 °C e 43 °C (Lopes et al., 2021; Ramires et al., 2020a; Scheik et al., 2021). Dentre os representantes deste grupo, *C. jejuni* e *C. coli,* são de maior importância em saúde pública, sendo *C. jejuni* a espécie mais envolvida em casos de infecções humanas e também a mais prevalente em aves. *Campylobacter* termofílicos não são formadores de esporos e, ao contrário de outros patógenos transmitidos por alimentos, como *Salmonella* spp. e *Shigella* spp., são fastidiosos e requerem um ambiente de microaerofilia, apresentando multiplicação máxima em atmosfera contendo aproximadamente 5% de O_2, 10% de CO_2 e 85% de N_2 (Garénaux et al., 2008; Levin, 2007; Silva et al., 2011; Yan et al., 2005).

Desde 2005, esses patógenos têm sido apontados como os principais agentes zoonóticos responsáveis por gastroenterites em humanos na União Europeia, registrando mais de 120 mil casos em 2020 (EFSA and ECDC, 2021). No Brasil, assim como em outros países em desenvolvimento, poucos casos de doenças de origem alimentar são atribuídos a essa bactéria, principalmente devido a falhas no processo de notificação e por não existirem programas nacionais de vigilância destinados ao acompanhamento de campilobacteriose. Segundo a Organização Mundial da Saúde (WHO, 2020) a enfermidade é normalmente manifestada após 2 a 5 dias do início da infecção, mas pode variar de 1 a 10 dias. Os sintomas clínicos mais comuns dessa doença incluem diarreia (frequentemente sanguinolenta), dor abdominal, febre, dor de cabeça, náuseas e / ou vômitos, os quais geralmente duram de 3 a 6 dias. Algumas complicações pós-infecção podem incluir artrite reativa (inflamação dolorosa das articulações que pode durar vários meses) e a síndrome de Guillain-Barré, que resulta em paralisia muscular, podendo ocasionar disfunção respiratória ou morte encefálica.

As aves são os reservatórios primários de *C. jejuni*, possivelmente por propiciarem uma temperatura ótima de multiplicação para as espécies termofílicas (Hald et al., 2016), sendo a carne de frango, crua ou insuficientemente cozida, a principal fonte de transmissão por *C. jejuni,* responsáveis por cerca de 80% dos casos de campilobacteriose (EFSA and ECDC, 2010). Além disso, normalmente os frangos são portadores assintomáticos desse patógeno. Ressalta-se que *C. jejuni* é submetido a diferentes condições de pH durante as etapas da cadeia produtiva de frangos de corte: passagem pelo estômago das aves; adesão e colonização do intestino das aves; armazenamento e estocagem dos cortes cárneos; passagem pelo trato gastrointestinal humano; adesão e colonização do intestino humano (Hanning et al., 2008). No entanto, ao contrário de outras bactérias, como *Salmonella* spp. e *Escherichia coli*, pouco se sabe sobre os mecanismos de sobrevivência de *C. jejuni* (Bolton, 2015; Guccione et al., 2017; Lopes et al., 2021; Repérant et al., 2016), dificultando a compreensão de como essas bactérias conseguem se manter viáveis ao longo da cadeia produtiva de frangos de corte e durante as etapas de sua patogenia. É importante salientar que as reações bioquímicas que ocorrem em um organismo são extremamente sensíveis a mudanças na acidez ou alcalinidade (Tortora, Funke, & Case, 2012) sendo interessante ter o conhecimento sobre a resposta em nível proteíco que é gerada frente a essas oscilações.

Uma forma de compreender o processo de patogenia e como o patógeno responde frente às diferentes condições as quais é submetido, é analisando a síntese global de proteínas (Karlsson et al., 2015). Inicialmente, é preciso conhecer qual a função das diferentes proteínas sintetizadas por um micro-organismo e como elas variam em abundância em função das condições ambientais, para então se ter um melhor entendimento dos mecanismos de virulência e posterior tratamento e/ou prevenção da doença. O termo proteoma significa o conjunto de proteínas codificadas pelo genoma de um organismo, já proteômica é definida como a compreensão da estrutura, função e interações das proteínas (Haynes & Yates, 2000). Técnicas de proteômica têm sido aplicadas a *C. jejuni* para entender melhor como as mudanças na composição genética, limitação de nutrientes e mudança ambiental afetam esses patógenos em nível proteico (Cain et al., 2019; Taheri et al., 2019). O desenvolvimento

destas técnicas está trazendo um melhor entendimento das vias bioquímicas e os papéis de interações das proteínas.

A preparação de amostras de proteínas para análise de proteoma depende da exploração das propriedades químicas específicas das proteínas, ou de outros fatores, como a localização celular. Todas as análises abrangentes de proteoma requerem várias estratégias para a preparação de amostras devido à grande variedade de propriedades físico-químicas das proteínas. Duas considerações devem ser observadas ao escolher qualquer técnica de extração de proteínas: em primeiro lugar, o uso de agentes químicos específicos durante a preparação da amostra pode limitar as etapas de separação e análise; a segunda consideração, é a minimização de compostos contaminantes dentro da mistura de proteínas, como DNA, polissacarídeos, sais e proteínas não derivadas de *C. jejuni*. Se a remoção de tais contaminantes for possível, por meio de tratamento enzimático, diálise ou precipitação de proteínas, poderão ser gerados resultados aprimorados, mas também deve ser observado que cada etapa adicional da preparação pode estar associada a algumas perdas proteicas (Scott & Cordwell, 2009).

Atendendo a essas necessidades, a tecnologia de preparação de amostras aprimorada em fase sólida de pote único (SP3 - *single spot*) é uma abordagem baseada em esferas paramagnéticas para processamento rápido, robusto e eficiente de amostras de proteínas para análise proteômica. A SP3 usa um mecanismo de interação hidrofílica para troca ou remoção de componentes que são comumente usados para facilitar a lise de células, solubilização de proteínas e digestão enzimática, antes da análise de proteômica (Figura 1). A SP3 fornece uma plataforma simplificada para o processamento de amostras de proteínas antes da análise proteômica. A finalidade é realizar a purificação da amostra, a fim de remover contaminantes indesejados de uma mistura de proteínas. As proteínas são ligadas às esferas magnéticas por meio de um mecanismo de interação hidrofílica. Em contraste com abordagens alternativas, o SP3 combina compatibilidade com uma coleção de aditivos de solução com recuperação praticamente sem perdas de proteínas, independentemente da quantidade de entrada, tudo em um protocolo simplificado, em um único microtubo (Hughes et al., 2019).

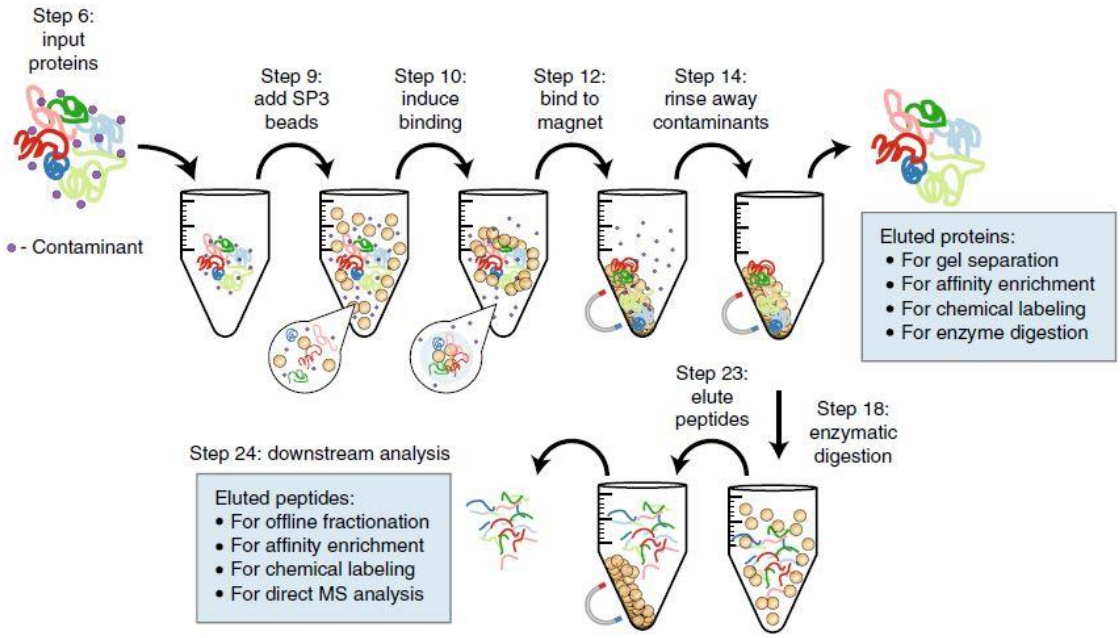

Figura 1. Fluxo do SP3 na purificação das amostras de proteínas, removendo possíveis contaminantes indesejáveis. Fonte: Hughes et al. (2019).

Existem estudos em que foi avaliada a síntese global de proteínas por *C. jejuni,* levando em consideração a atmosfera (Rodrigues et al., 2016), a temperatura (Taheri et al., 2019) ou simplesmente o mapeamento geral da proteômica desse patógeno (Shi et al., 2014). No entanto, são necessários estudos avaliando a proteômica de *C. jejuni* quando submetido a diferentes valores de pH aos quais esse micro-organismo é exposto, desde o seu estabelecimento nas aves, permanecendo viável nos cortes cárneos comercializados, e transpondo os obstáculos no organismo humano, para assim, compreendermos qual a relação entre o pH e a patogenia de *C. jejuni*.

Desta forma, este estudo teve o objetivo de avaliar a proteômica de *C. jejuni* NCTC11168 submetido a diferentes condições de pH (4, 5.8 e 8). Os resultados deste estudo contribuirão para um melhor entendimento do potencial de virulência e do mecanismo de patogenicidade de *C. jejuni*.

2. Objetivos

2.1 Objetivo Geral

Avaliar a proteômica de *C. jejuni* NCTC11168 submetido a diferentes condições de pH.

2.2 Objetivos Específicos

Objetivo 1. Realizar a padronização e a extração de proteínas de *C. jejuni* NCTC 11168, utilizando o protocolo SP3.

Objetivo 2. Determinar o perfil proteico de *C. jejuni* NCTC 11168 simulando as condições de pH encontradas em cortes cárneos de frangos e no estômago e intestino humanos.

Objetivo 3. Separar, identificar e quantificar as proteínas através da cromatografia líquida acoplada à espectrometria de massas.

Objetivo 4. Analisar e comparar o perfil proteico, caracterizando o proteoma obtido nas diferentes condições de pH avaliadas.

3. Capítulo I

3.1 Manuscrito 1- Response of *Campylobacter jejuni* NCTC 11168 to acidic and alkaline stress when in the stationary growth phase as revealed by targeted proteomics

Manuscrito a ser submetido ao periódico *Food Research International* - Fator de Impacto 7.425 e Qualis A1 na Área de Ciência de Alimentos

Tassiana Ramires[a*], Richard Wilson[b], Wladimir da Silva[a], John P. Bowman[c*]

[a]Federal University of Pelotas, Pelotas, Brazil
[b]Central Science Laboratory, University of Tasmania, Hobart, Tasmania, Australia
[c]Tasmanian Institute of Agriculture, University of Tasmania, Hobart, Tasmania, Australia

*Corresponding authors

ABSTRACT

Campylobacter jejuni is one of the major causes of food-borne infections world-wide. The species is strictly host associated and tolerates acidity and alkalinity. The ability to survive pH challenges is one of the key aspects of the ability of *C. jejuni* to survive in food, stomach transit and enables host gastrointestinal tract colonisation. In this study we exposed *C. jejuni* reference strain NCTC 11168 to growth permissive pH stress (growth at pH 5.8 and 8.0) and exposure to pH 4.0 shock, relative to a pH 7.0 control. Cells were grown to early stationary growth phase before being exposed to acid shock and harvested for proteomic analysis. Proteins extracted from biomass were quantified using a targeted data dependent acquisition label-free based approach. This was done to identify pH dependent proteins that respond in a growth phase independent manner. It was discovered that gluconate 2-dehydrogenase (Cj0414, Cj0415), NssR-regulated haemoglobin-like proteins Cgb and Ctb and uncharacterised protein Cj0761, a cytochrome c (Cj0037), and phosphate-binding transporter protein PstB all show acid pH dependent abundance increases but are not activated by acid shock. We did not discover any proteins that definitively showed abundance increases in an alkaline pH dependent manner. Protein abundances otherwise increased only for small sets of proteins specific to the given pH treatment. These responses mainly seem to be oriented to bolstering respiration for acid pH treatments while for pH 8.0 treatments the observed changes potentially act to maintain intracytoplasmic pH homeostasis. Global protein abundance reduction of proteins linked to growth and survival also occurred for all treatments and could be associated with energy conservation, linked to the

494 physiological environment, and in the case of acid stress also when abrupt pH
495 reduction occurs.
496
497 **Introduction**
498 *Campylobacter jejuni* is an animal host associated bacterial species of the
499 phylum *Campylobacterota*. The species is a major foodborne pathogen world-
500 wide and causes most of the human disease, termed campylobacteriosis, in both
501 developing and developed nations (Kaakoush et al. 2015). Campylobacteriosis
502 is mainly acquired during preparation and consumption of poultry meat. This is
503 due to the high population of *C. jejuni* in poultry caeca. *C. jejuni* can also colonise
504 the huma GI tract creating asymptomatic carriers. In Brazil stricter regulations
505 placed on poultry farms and meat processing facilities was able to reduce the
506 incidence of disease (Melo et al. 2019) indicating that preventing colonisation of
507 *C. jejuni* in food animals is key to controlling campylobacteriosis (Fodda et al.
508 2021).
509 *C. jejuni* strains has, like its relatives, a stream-lined genome of about 1.7
510 megabases coding for approximately 1600 proteins. This streamlining reduces
511 the adaptability of the species both in terms of environmental survival and ability
512 to dominate a given econiche. The ability of *C. jejuni* to cause disease mainly
513 relates to survival on poultry carcasses and the ability to survive sanitation
514 occurring after slaughter. It was found *C. jejuni* can survive peracetic acid (PAA)
515 disinfectant during processing. PAA is both an oxidising and acidic solution. PAA
516 exposure survival was higher following scalding as compared to direct chilling
517 and PAA exposure (Chen et al. 2020). This is despite other findings that report
518 *C. jejuni* does not acquire stress cross-protection from either heat or cold stress

519 (Iohanni et al. 2013). Quantitative proteomics analysis suggested the main
520 response to a sub-lethal 45 minute PAA exposure was a “peroxide shock”
521 response (Chen et al. 2022) but no obvious response could be linked to acidity,
522 even though the PAA solution was pH 4.3.

523 *C. jejuni* appears to only initiate a primitive acid tolerance response (Murphy
524 et al. 2003) compared to the elaborate responses found in other food-borne
525 pathogens such as *Listeria monocytogenes* (Bowman et al. 2012). Acid exposure
526 has been shown to activate the PerR peroxide regulator (Pesci et al. 1994), which
527 also controls iron homeostasis. It is likely an acidic PAA solution disturbs the
528 redox homeostasis of the cytoplasm and survival is linked to the ability in coping
529 with peroxide ion influx. The effect of a concomitant H^+ flux seems to be dealt
530 with simultaneously though the exact mechanisms used are not clear.

531 *Campylobacter jejuni* strains can grow between pH 5 to pH 9 at the optimal
532 temperature for growth of 42°C and survive acidic shock (Reid et al. 2008a). In
533 addition the species can manage oxidative stress by a range of conserved and
534 strain-dependent regulatory processes (Kim et al., 2015; Gundogdu et al. 2016)
535 and as mentioned above oxidative stress protective proteins can be induced,
536 such as by PAA. The species, however, lacks tolerance to osmotic stress and
537 low temperature conditions due to the inability to synthesise de novo compatible
538 solutes as well as lacking dedicated transport systems for such (Cameron et al.
539 2012). *C. jejuni* strains also lack master regulators and specific enzymatic
540 processes (such as the glutamate decarboxylase system) that can buffer the
541 intracytoplasmic pH against acidity as found in other food-borne pathogens (Horn
542 & Bhunia 2018). Mechanisms found to be important with acid stress survival by
543 *C. jejuni*, especially when considering a host content are likely linked to

544 management of physiology, in particular catabolic and respiration-based energy
545 generation (Reid et al. 2008b).

546 The goal of the research presented here is to assess responses of *C. jejuni*
547 reference strain NCTC 11168 to pH stress, both alkaline and acidic. Both alkaline
548 and acidic conditions are relevant to food and the GI tract environment. Many
549 foods and stomach transit require the ability to tolerate acidic stress, caused
550 either by mineral acids or by organic acids. Furthermore, alkaline stress can be
551 encountered in the lower parts of the small intestine due to secretion of bile and
552 accumulation of bicarbonate ions (Fallingborg 1999).

553 To investigate the responses of *C. jejuni* to pH stress label free proteomics
554 was utilised. Since the proteome of *C. jejuni* NCTC 11168 is available a targeted
555 data dependent acquisition approach was used to quantify proteins. The
556 proteomic based quantitation was applied to cells grown at pH 8.0, pH 5.8 and
557 pH 7.0 to early stationary growth phase. Having the cells in this phase specifically
558 targets pH-dependent responses since growth rate reduction due to pH may
559 invoke more global cross-protective responses. In addition an acid shock at pH
560 4.0 for 1 hour was performed to determine if protein responses are similar to that
561 of growth at pH 5.8 which in effect involves some level of possible acid
562 habituation even though the ATR is not very prominent in *C. jejuni*. The
563 hypothesis is presented that growth at either acid or alkaline pH involves proteins
564 that are pH-dependent, that could provide beneficial functions to cells when
565 encountering sub-optimal pH conditions. Another goal was to determine if acid
566 shock affects the abundances of the same proteins as found when growing *C.*
567 *jejuni* under either acid or alkaline stress
568

Materials and Methods

Sample preparation

Protein concentrations were estimated using the E-Z Quant assay (Thermo Fisher Scientific) and aliquots of 50 mg protein were sequentially reduced and alkylated using standard methods. Protein samples were digested using the SP3 method (Hughes et al, 2019) and peptide samples were desalted using ZipTips (Merck Millipore) according to manufacturer's instructions.

LC/MS analysis

Aliquots of peptides equivalent to 1 mg were first concentrated on a 20 mm x 75 µm PepMap 100 trapping column (3 µm C18) (Thermo Fisher Scientific) for 5 minutes then separated on a 250 mm x 75 µm PepMap 100 RSLC column (2µm C18) held at 45 °C using a flow rate of 300 nL/min. Peptides were eluted over a 70- minute segmented gradient from 98% mobile phase A (0.1% formic acid in water) to 45% mobile phase B, (0.08% formic acid in 80% acetonitrile and 20 % water) followed a column wash in 95% B (5 min) and re-equilibration in 2% B (15 min). The RSLCnano system was coupled to a Q-Exactive HF mass spectrometer equipped with nanospray Flex ion source (Thermo Fisher Scientific, MA, USA) and controlled using Xcalibur 4.1 software. Spray voltage was set to 2.0 kV, heated capillary temperature set at 250 °C and S-lens RF level set to 50. MS1 scans (370-1500 m/z) were acquired at 60,000 resolution with an AGC target of 3 x 10e6 and a maximum fill time of 100 ms. MS2 scans (200-2000 m/z) were acquired at 15,000 resolution in data-dependent mode using a Top15 method, with an AGC target of 2 x 10e5 and a maximum fill time of 28 ms. An isolation width of 1.4 m/z was used, and normalized collision energy for HCD set to 27eV.

Database searching and protein quantitation

Raw data files were imported into MaxQuant version 1.6.5.0 (http://maxquant.org/) (Cox & Mann, 2008) and MS2 spectra were searched using the Andromeda search engine against protein database for *Campylobacter jejuni* NCTC 11168 comprising 1625 entries (downloaded from UniProt on 5/11/2018). Default settings for protein identification by Orbitrap MS/MS were used, with the match-between-runs function enabled. Mass error tolerances were set to 20 ppm then 4.5 ppm for initial and main peptide searches, respectively, and 0.5 Da tolerance was used for fragment ions. Carbamidomethyl modification of cysteine, variable methionine oxidation and a maximum of two missed cleavages were allowed. A false discovery rate of 0.01 was used for both peptide-spectrum matches and protein identification. The MaxQuant proteinGroups.txt output file is presented in Supplemental Table XXX. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier XXXXXXX. [Reviewer account details: reviewerXXXXXXX@ebi.ac.uk; password]

Statistical analysis

Spectronaut protein quantitation data, uniport protein designations and NCBI accession numbers, were uploaded into Perseus software version 1.6.14.0 (Tyanova et al 2016) for further data processing and statistical analysis. Label-free quantitative (LFQ) protein values were first log2(x) transformed then protein groups were filtered to include proteins detected in a minimum of 70% of the samples, with remaining missing values imputed from the normal distribution of LFQ values according to Perseus default settings. ANOVA was conducted with an S0 of 0.1 and an FDR of 0.05, to select for proteins that were significantly

different between treatment groups. Differences between treatment groups were assessed using principal component ordination analysis (PCO) in Primer 7 software (Primer-E, Auckland, New Zealand). For this the intensity values for individual proteins across the replicates were deployed in a matrix and Euclidean distances calculated. Venn diagrams were generated using InteractiVenn (Heberle et al. 2016).

Results

Campylobacter jejuni NCTC 11168 grown to early stationary growth phase and under O_2 limitation was assessed for pH tolerance-associated responses using proteome analysis. The incubation time used for biomass generation place the cells in the early stationary growth phase as a result responses measured here mostly link to pH stress and the consequences of physiological adjustments above and beyond that obtained already by stationary growth adaptation. A total of 885 proteins were utilised for further analysis (Table S1). This represented 56% of the total potential proteome of strain NCTC 11168. The protein abundance dynamic range achieved was approximately 10^4. From calculations (Wiśniewski et al. 2014) based on the ratios of the MS spectral intensities the limit of detection in the study was 15-25 proteins per cell assuming cell volumes were on average 1 μm^3 and protein content was 17%.

The abundances of proteins provide a conceptualisation of NCTC 11168 energy and carbon acquisition, expressed levels of stress responsive mechanisms and virulence proteins. These are all relevant for survival in extremes of pH and for host colonisation and survival. The data suggests multiple

644 energy acquisition approaches are active simultaneously, including in particular
645 catabolism of L-aspartate (AspA) and to a lesser extent L-glutamate, L-glutamine,
646 L-serine and acetate. Besides microaerobic respiration, reduction of fumarate to
647 succinate; and nitrate reduction are also possibly operating simultaneously.
648 Based on the protein abundances respiration is coupled to a range of donors
649 including mainly H_2 (hydrogenase complex), 2-oxoglutarate (OorABC), and
650 gluconate (via gluconate 2-dehydrogenase Cj0415/416) though enzymes for
651 other utilisable donors are all relatively abundant i.e. succinate, lactate, malate,
652 and formate (van der Stel & Wösten, 2019). The protein abundances also
653 indicate motility seems active due to high presence of flagellin FlaA while
654 oxidative growth is supported by several protective and peroxide remediative
655 proteins including Tpx, Rbr, Dps, CosR, SodB, DnaK, and PEB4. Most virulence
656 proteins are detectable regardless of the pH treatment.
657

658 **Proteomic landscape of *C. jejuni* NCTC 11168 grown under alkaline**
659 **conditions.**

660 The proteome level responses of *C. jejuni* NCTC 11168 between biomass
661 samples cultured under growth permissive alkaline stress of pH 8.0 versus pH
662 7.0 were examined. An overall comparison of proteomes using PCO analysis(Fig.
663 1) suggests the pH 8.0 proteome has some responses distinctive to acid stress
664 (Fig. 1, PCO2 - 21.3%) though the individual biological replicates show some
665 dispersion suggesting a degree of physiological variation within the early stages
666 of stationary growth phase for some proteins. Most of the variance (PCO1 - 42%)
667 occurs between the proteome datasets occurs for the pH 7.0 and acid stressed
668 proteomes (Fig. 1).

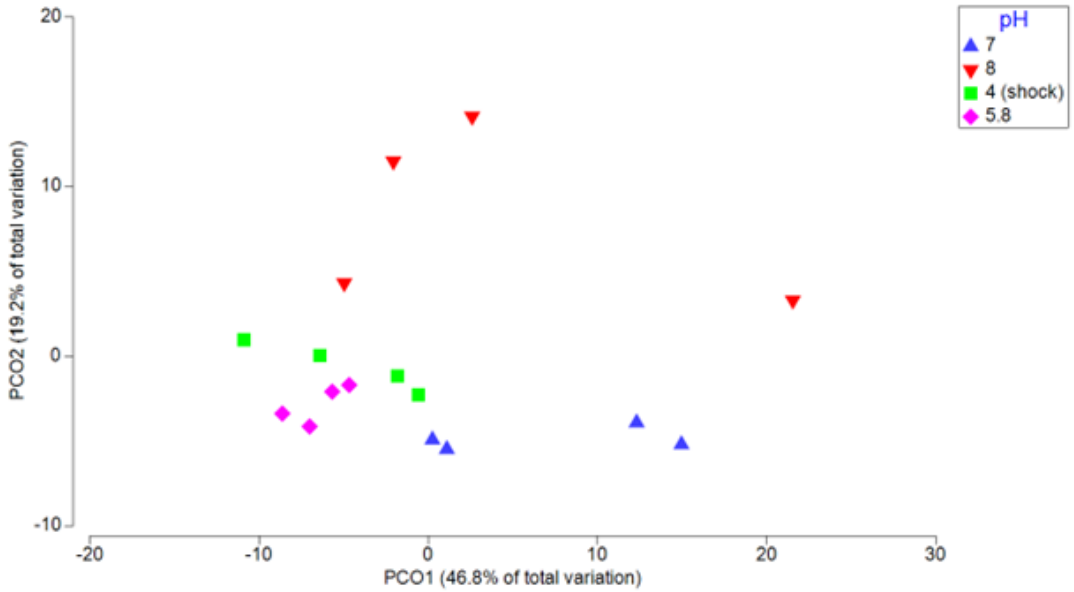

Figure 1. Principal coordinates ordination plot showing clustering patterns of proteomes of the early stationary growth phase of *Campylobacter jejuni* NCTC 11168 grown at pH 5.8, 7.0 and 8.0 and also exposed to pH 4.0 for 1 h.

Changes in protein abundance due to alkaline pH were generally modest ranging from 1.5-3.0-fold (0.5->1.5 log2). Reduction of protein abundance was much greater in expanse than that found for proteins increasing in abundance (Fig.2).

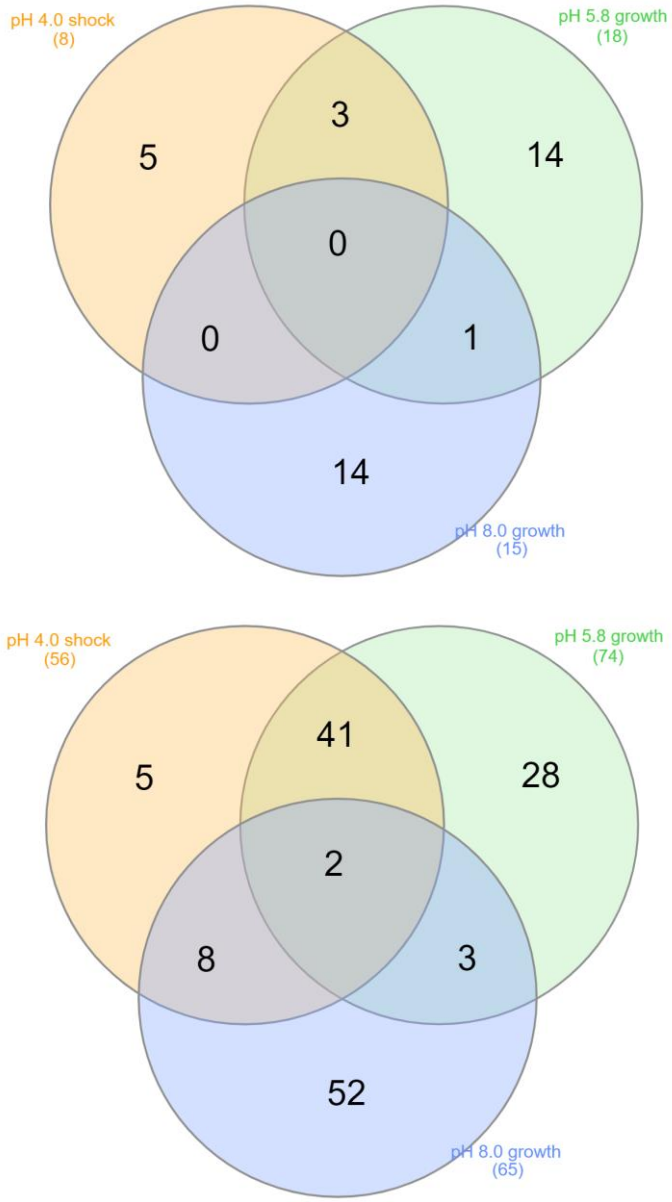

Figure 2. Venn diagram showing the number of common and different differentially abundant proteins (>1.5 fold, $p<0.05$, FDR) from the early stationary growth phase of *Campylobacter jejuni* NCTC 11168 grown at pH 5.8 and 8.0 and also exposed to pH 4.0 for 1 h compared to cells harvested from media at pH 7.0. The top Venn diagram shows proteins that have significant ($p<0.05$, FDR) increases in abundance while the bottom Venn diagram shows the number of shared and unshared proteins that have significantly reduced protein abundance.

719

720 It was discerned 15 proteins had >1.5 fold increases (FDR, $p<0.05$). By
721 comparison 70 proteins show >1.5-fold reduction in abundance. Of the proteins
722 showing increased abundance (Table 1) two proteins are involved in respiration
723 (HydA2, NapL) while subunits of methylmenaquinol:fumarate reductase MfrABC
724 (Cj0437-439) also exhibited overall greater abundance. Furthermore, several
725 amino synthesis enzymes became more abundant including those for glutamate
726 (GltDB), glutamine (GlnA) and methionine (MetE and both the N-terminal and C-
727 terminal regions of MetC). Most other responding proteins have unknown
728 function though PurB (adenylosuccinase) and a RecG-like regulator MloB
729 (Cj1552c) both showed some abundance increase. The lion's share of reduced
730 protein abundance responses are linked to critical functions required for rapid
731 growth and fitness including respiration, catabolism, the TCA cycle, cytoplasmic
732 homeostasis and proteostasis, trace metal and phosphorous compound uptake,
733 signalling, motility, adhesion and virulence (Table 1).

734

735 **Comparison of acid habituation (growth at pH 5.8) versus acid shock (pH**
736 **4.0).**

737 The results suggest acid habituation and acid shock, which was established
738 by growth at pH 5.8 and by exposure to media set at pH 4.0, respectively, lead
739 to relatively similar proteomes as shown in the PCO plot (Fig. 1). Most of this
740 similarity was related to proteins with decreased abundance between the two
741 treatments (Fig. 2, Table 1)

742 When grown at pH 5.8 NCTC11168 exhibited 2.3-2.7-fold increases in the
743 abundance of the two subunits of gluconate 2-dehydrogenase (Cj0415, Cj0416),

744 however this change was not observed in the acid shock proteome. This highly
745 abundant enzyme enables gluconate to be an electron donor during respiration.
746 Other proteins that also showed greater abundances (1.6 to 2.1-fold) included
747 phosphate uptake subunit proteins PstB and PstS; hydrogenase carbamoyl
748 dehydrogenase HypF required for hydrogenase enzyme maturation (Rowlett et
749 al. 2012), methionine aminopeptidase Map and nitric oxide-inducible globin Cgb.
750 Of these only HypF was found to be significantly more abundant in acid shocked
751 cells. Two putative periplasmic proteins of unknown function Cj0425 and Cj1169c
752 increased in abundance 2.6 and 3.1-fold in acid shocked cells, respectively.
753 Nether of these proteins showed substantial changes in the pH 5.8 treatment.
754 Cj1169c expression has been previously associated with pH stress (both acidic
755 and alkaline) and is at maximal levels in the logarithmic phase at 42°C (Soumeya
756 et al. 2016). As found with pH 8.0 grown cells proteins showing reduced
757 abundance are linked mainly to catabolic, synthetic and survival processes
758 needed to achieve rapid growth rate and biomass accumulation (in a host
759 context).
760
761 **Proteins showing opposing responses between alkaline and acidic**
762 **stress.**
763 The comparisons made above highlighted proteins showing opposite trends
764 in differential abundances between growth at pH 5.8 and pH 8.0 relative to pH
765 7.0 controls. These proteins included gluconate 2-dehydrogenase, cytochrome c
766 protein Cj0037c, cupin family protein Cj0761, NO-inducible globins Cgb and Ctb,
767 and phosphate transport protein PstB. As shown in Fig. 3 the abundance patterns
768 of these proteins suggests these proteins are acid pH-dependent but at the same

time are not induced by acid shock for cells in the stationary growth phase. Alkaline pH dependent proteins which have significantly greater expression at pH 8.0 and also exhibited reduced expression at pH 5.8 compared to pH 7.0 were not detected

Discussion

The pH treatments applied in this study had a minor reductive to no effect on cyclopropane-fatty-acyl-phospholipid synthase (Cfa, Cj1183c). This simply could mean the conversion has reached a maximum in stationary growth phase and is not pH stress dependent. Cfa converts C_{18} fatty acids to the cyclopropane form, which are then located within lysophospholipid and other polar lipids. Lysophospholipids containing cyclopropane fatty acids are required by *C. jejuni* for optimal motility under low O_2 conditions (Cao et al. 2020).

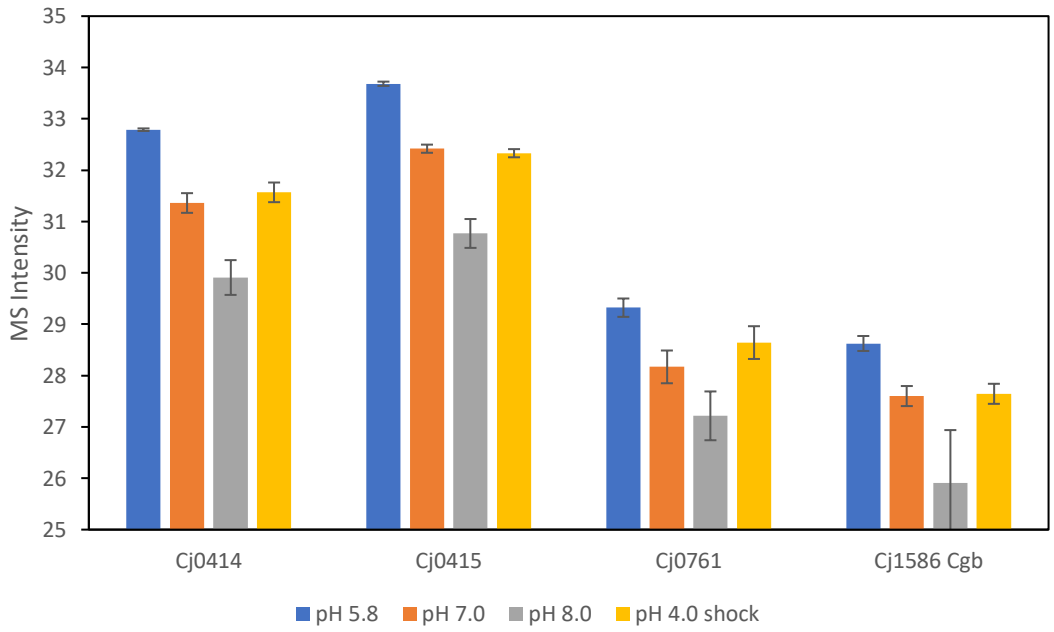

Figure 3. A comparison of the MS intensities of proteins exhibiting an acid pH dependent response in Campylobacter jejuni NCTC 11168 grown to the stationary phase in media adjusted to different pH and also exposed to acid shock. The data is based on 4 biological replicates. Error bars are standard deviations.

A membrane rich in cyclopropane fatty acids may naturally impede proton diffusion into cells but have been shown to protect against multiple external stressors (Poger and Mark, 2015) and the protection could be more focused on preventing toxic metabolites (such as solvents, organic acids) entering the cytoplasm than protons (Pini et al. 2009). Further analysis is needed to determine to what extent cyclopropane fatty acids aids *C. jejuni* survival against either pH stress or toxic compound stresses.

Extrusion of H^+ at the expense of ATP is a common mechanism to maintain intracytoplasmic pH homeostasis. Lacking ATP leads to more rapid inactivation by non-thermal stress, especially at higher growth temperatures (Ross et al. 2008). Acid stress increases the abundance of the ATPase protein complex in some food-borne pathogens, such as *Listeria monocytogenes* (Bowman et al. 2012). However in this study, growth permissive acid stress reduced the levels of ATP subunits or had no effect suggesting ATPase extrusion is not being activated further in stationary growth phase. Reversal of hydrogenase can like ATPase extrude H^+ out of the cells. Acid shock was shown to stimulate hydrogenase gene expression transiently in-vitro and in-vivo in *C. jejuni* NCTC 11168 (Reid et al. 2008a). However, in this study the catalytic subunits HydAB showed no change in expression and indeed are highly abundant under all growth conditions. Again this suggest that hydrogenase levels are not further enhanced in stationary growth phase. Some hydrogenase maturation proteins were reduced under acid conditions. HypF and HydA2 did become more abundant (1.7-fold) at ph5.8/pH4.0 shock and pH 8.0, respectively. No evidence exists that hydrogenase assists *Campylobacter* in maintaining intracytoplasmic pH stability further in stationary growth phase. The increased abundance of HypF

and HydA2 is potentially only compensatory, maintaining overall hydrogenase activity in cells.

Many genes are transiently expressed in exponentially growing *C. jejuni* when exposed to sudden acid shock (Reid et al. 2008a). It is likely most of these responses are not acid stress specific but more a response to ATP draw down due to the pH change affecting the chemiosmotic gradient and thus electron transport activity. Few genes seem specifically linked to acid stress survival in *C. jejuni*. Reid et al. (2008b) found only gluconate 2-dehydrogenase Cj0414 and Cj0415 transposon mutants had reduced growth over a range of acidic pH (pH 5.0, 5.5, 6.5). Other mutants only generated non-specific responses. It is probable many proteins make indirect contributions to low pH growth fitness and are either expressed constitutively while others attain maximum expression in the early stationary growth phase.

Specifically, gluconate 2-dehydrogenase allows *C. jejuni* to use gluconate as an electron donor during respiration and is necessary for good chicken colonisation (Panjaniappan et al. 2008) possibly by enabling good growth under hypoxic conditions. Cells under hypoxia utilising O_2 likely also use or are at least in a readied state to use nitrate, fumarate, nitrite and other available compounds as electron acceptors. Though the level of O_2 does not seem to affect gluconate dehydrogenase abundance (Guccione et al. 2017) greater expression occurs at 42°C (compared to 37°C) and it is also observed to be induced when cells are exposed to poultry and human digesta extracts (Liu et al. 2018). This study confirms a strong acid pH dependent response occurs for Cj0415 and Cj0416 protein with abundance about 7.5-fold lower at pH 8.0 as compared to pH 5.8 (Figure 3, Table 2). There was no evidence of acid shock affecting abundance

levels suggesting the regulatory pathway for Cj0414 and Cj0415 genes is potentially responding to a signal separate to acidity. Other proteins showing acid dependent responses detected in this study could be also relevant but remain to be linked. The haemoglobin-like proteins Cgb and Ctb, both regulated by NssR (Elvers et al. 2005), were found here to have an acid pH dependent expression. Cgb is known to provide nitrosative stress protection converting NO to nitrate (Shepherd et al. 2010) . The role of Ctb remains less clear and functionality could be impaired due it being truncated; the abundance of Cgb protein was 10.5-fold higher at pH 5.8 and 8-fold higher pH 7.0. These proteins were also found to be more abundant in *C. jejuni* cells exposed to the oxidising sanitiser peroxyacetic acid (Chen et al. 2022) suggesting they may help manage NO and bind O_2 simultaneously improving fitness at low pH under hypoxia where O_2 may have to be concentrated to better conserve energy via respiration. Interestingly, the regulator NssR was at near detection limits and did not show a pH dependent response suggesting Cgb and Ctb expression could be influenced by other regulatory units. Another member of the NssR regulon, Cj0761, a cupin_RmlC family protein, was also found to have acid pH-dependent expression however other potential members of the NssR regulon were not detected. Cj0761 remains uncharacterised. The *Helicobacter pylori* homolog HP0902 to Cj0761 has been shown to be linked to host colonisation success (Sim et al. 2016) though specific functionality in *H. pylori* also remains to be determined. All of the non-detected proteins are membrane proteins including two of unknown function (Cj0830 and Cj0851c) while the other is the nickel ion permease NikX (Cj01582c, Howlett et al. 2012). NikZ (Cj1584c), the cognate nickel binding protein also has an acid-pH dependency that is statistically significant though the change is small ($p<0.01$,

FDR; 1.27-fold increase at pH 5.0, 0.67-fold change at pH 8.). Since the *nik* operon is immediately adjacent to Cgb a possible regulatory and/or function connection is possible. Other potentially acid pH-dependent proteins (Table 2) were found but the degree of difference between treatments was smaller and more uncertain.

Though intestinal pH can be relatively alkaline (up to pH 8.5) , alkaline pH adaptation has not been studied specifically in *Campylobacter* (Kim et al. 2020). The classic active mechanisms for protecting intracytoplasmic pH from increasing to growth preventative levels includes: 1) ATPase proton uptake, 2) synthesis and/or uptake of acidic metabolites especially organic acids and acidic amino acids, and 3) proton uptake via an exchange antiporter typically with export of small ions such as Na^+, K^+, Li^+ or Ca^{2+} (Padan et al. 2005). In this study there was no observed increases of ATPase subunit proteins at pH 8.0. Again this may simply suggest the ATPase complex has reached a maximum abundance in stationary growth phase and H^+ uptake could be actually occurring. NhaA homologs (Cj1654c, Cj1655c), which is used in *E. coli* as a rapid response against alkaline shock, were instead undetectable. There was no evidence transporters taking up acidic organic solutes, L-glutamate or L-aspartate were more abundant, several were below detection limits, for example DcuA and DcuB. Several amino acid uptake systems were decreased at pH 8.0 (Table 1). It was determined L-glutamate (GltDB), L-glutamine (GlnA) and L-methionine (MetE, MetC') synthesis enzymes had a 1.7-2.0-fold increased expression in cells grown at pH 8.0. Under alkaline conditions the production of sulfide is also enhanced in *Campylobacter hyointestinalis* (Ma et al. 2007), possibly due to MetE/MetC activity. Whether the same occurs in *C. jejuni* requires confirmation

but this activity could also reduce intracytoplasmic pH due to sulfide being a weak acid (pK_a 7.0). Glutamate accumulation may offset consumption of L-aspartate since AspA is highly abundant and would result in production of ammonia while the other product fumarate could be accumulated before being respired and converted to succinate and other acidic metabolites in the TCA cycle. There was an observed modest increase in the abundance of HydA2 (1.8-fold) an alternative small subunit for hydrogenase. It is possible this increase is compensatory for the overall hydrogenase activity but increased conversion of molecular hydrogen to protons could also offset encroaching alkalinity, especially in locations such as the human small intestine where hydrogen-producing (and consuming) bacteria are common (Smith et al. 2018). Hydrogenase abundance is very high so may act in a capacity like that of ATPase. Further analysis of a range of alkaline conditions relevant to host systems seems warranted to deepen knowledge of how *C. jejuni* survives long term in avian and mammalian GI tracts.

Conclusions

The results obtained confirmed the presence of a set of proteins that respond specifically to acidic stress in C. jejuni NCTC 11168. Though alkaline stress responses seem limited further research, especially focusing on the metabolism of *C. jejuni*, including what amino acids and organic acids are preferentially accumulated and degraded would be useful in better understanding responses to alkaline pH. This was suggested by the possible adjustments to amino acid accumulation and fumarate respiration associated with growth at pH 8.0. Under growth permissive acidic stress proteins in the NssR regulon may help bolster access to O_2 in hypoxic conditions and guard against host defences in particular

NO, thus promoting GI tract colonisation. Gluconate also appears to be an important potential electron donor in the GI tract due to acid pH- and growth-dependent expression of gluconate 2-hydrogenase. Gluconate has been suggested as a prebiotic, either added directly (Tsukahara et al. 2002) or indirectly (Zhao et al. 2022) but there is the potential increased levels of gluconate availability may promote *C. jejuni* survival and colonisation. Further work is needed to link gluconate availability and *C. jejuni in vivo* survival and determine whether this connection can be exploited to reduce *C. jejuni* populations in poultry.

References

Bowman JP, Hages E, Nilsson RE, Kocharunchitt C, Ross T. 2012. Investigation of the Listeria monocytogenes Scott A acid tolerance response and associated physiological and phenotypic features via whole proteome analysis. Journal of Proteome Research 11, 2409–2426.

Cameron A, Frirdich E, Huynh S, Parker CT, Gaynor EC. 2012. Hyperosmotic stress response of Campylobacter jejuni. Journal of Bacteriology 194, 6116–6130.

Cao X, Brouwers JFHM, va Dijk L, va de Lest CHA, Parker CT, Huynh S, van Putten JPM, Kelly DJ, Wösten MMSM. 2020. The unique phospholipidome of the enteric pathogen *Campylobacter jejuni*: lysophosholipids are required for motility at low oxygen availability. Journal of Molecular Biology 432, 5244-5258.

Chen HS, Bose U, Broadbent JA, Fegan N, Wilson R, Kocharunchitt C, Bowman JP, Colgrave ML, Duffy LL. 2022. Proteome analysis of chlorine

937 resistant *Campylobacter jejuni* strain 2704 survival during 45 min treatment of
938 sublethal peracetic acid. International Journal of Food Microbiology. In review.
939 Chen SH, Fegan N, Kocharunchitt C, Bowman JP, Duffy LL. 2020. Effect of
940 peracetic acid on *Campylobacter* in food matrices mimicking commercial poultry
941 processing. Food Control 113, 107185.
942 Cox J, Mann M. 2008. MaxQuant enables high peptide identification rates,
943 individualized p.p.b.-range mass accuracies and proteome-wide protein
944 quantification. Nature Biotechnology 26, 1367-1372.
945 Elvers KT, Turner SM, Wainwright LM, Marsden G, Hinds J, Cole JA, Poole
946 RK, Penn CW, Park SF: NssR, a member of the Crp-Fnr superfamily from
947 *Campylobacter jejuni*, regulates a nitrosative stress-response regulon that
948 includes both a single-domain and a truncated haemoglobin. Molecular
949 Microbiology 57, 735-750.
950 Fallingborg J. 1999. Intraluminal pH of the human gastrointestinal tract.
951 Danish Medical Bulletin 46, 183-196.
952 Fodda A, Nauta M, Ellis-Iversen J. 2021. Risk-based control of
953 *Campylobacter* spp. in broiler farms and slaughtered flocks to mitigate risk of
954 human campylobacteriosis – A One Health approach. Microbial Risk Analysis.
955 21, 100190.
956 Guccione EJ, Kendall JJ, Hitchcock A, Garg N, White MA, Mulholland F, Poole
957 RK, Kelly DJ. 2017. Transcriptome and proteome dynamics in chemostat culture
958 reveal how *Campylobacter jejuni* modulates metabolism, stress responses and
959 virulence factors upon changes in oxygen availability Environmental Microbiology
960 19, 4326-4348.

961 Gundogdu O, da Silva DT, Mohammad B, Elmi A, Wren BW, van Vliet AHM,
962 Dorrell N. The Campylobacter jejuni oxidative stress regulator RrpB is associated
963 with a genomic hypervariable region and altered oxidative stress resistance.
964 Frontiers in Microbiology 7, 2117.
965 Heberle H, Meirelles GV, da Silva FR, Telles GP, Minghim R. 2015.
966 InteractiVenn: a web-based tool for the analysis of sets through Venn diagrams.
967 BMC Bioinformatics 16, 169.
968 Hughes CS, Moggridge S, Müller T, Sorensen PH, Morin GB, Krijgsveld J.
969 2019. Single-pot, solid-phase-enhanced sample preparation for proteomics
970 experiments. Nature Protocols 14, 68–85.
971 Horn N, Bhunia AK. 2018. Food-associated stress primes foodborne
972 pathogens for the gastrointestinal phase of infection. Frontiers of Microbiology 9,
973 1962.
974 Howlett RM, Hughes BM, Hitchcock A, Kelly DJ. 2012. Hydrogenase activity
975 in the foodborne pathogen *Campylobacter jejuni* depends upon a novel ABC-type
976 nickel transporter (NikZYXWV) and is SlyD-independent. Microbiology (UK) 158,
977 1645-1655.
978 Isohanni P, Huehn S, Aho T, Alter T, Lyhs U. 2013. Heat stress adaptation
979 induces cross-protection against lethal acid stress conditions in *Arcobacter*
980 *butzleri* but not *in Campylobacter jejuni*. Food Microbiology 34, 431–435.
981 Kaakoush NO, Castaño-Rodríguez N, Mitchell HM, Man SM. 2015. Global
982 epidemiology of *Campylobacter* infection. Clinical Microbiology Reviews 28, 687-
983 720.
984 Kim S-H, Chelliah R, Ramakrishnan SR, Peruma AS, Bang W-S, Rubab M,
985 Daliri EB, Barathikannan K, Elahi F, Park E, Jo HY, Hwang S-B, Oh DH. 2020.

Review on stress tolerance in *Campylobacter jejuni*. Frontiers in Cellular and Infection Microbiology 10, 596570.

Kim J-C, Oh E, Kim J, Jeon B. 2015. Regulation of oxidative stress resistance in *Campylobacter jejuni*, a microaerophilic foodborne pathogen. Frontiers in Microbiology 6, 751

Liu MM, Boinett CJ, Chan ACK, Parkhill J, Murphy MEP, Gaynor EC. 2018. Investigating the *Campylobacter jejuni* transcriptional response to host intestinal extracts reveals the involvement of a widely conserved iron uptake system. mBio 9, 4

Ma M, Amano T, Enokimoto M, Yano T, Moe KK, Misawa N. 2007. Influence of pH of TSI medium on the detection of hydrogen sulfide production by *Campylobacter hyointestinalis*. Letters in Applied Microbiology 44, 544-549.

Melo RT, Grazziotina AL, Valadares Júnior EC, Prado RR, Mendonça EP, Monteiro GP, Peres PABM, Rossi DA. 2019. Evolution of *Campylobacter jejuni* of poultry origin in Brazil. Food Microbiology 82, 489-496.

Murphy C, Carroll C, Jordan KN. 2003. Induction of an adaptive tolerance response in the foodborne pathogen, *Campylobacter jejuni*. FEMS Microbiology Letters 223, 89–93.

Padan E, Bibi E, Ito M, Krulwich TA. 2005. Alkaline pH homeostasis in bacteria: new insights. Biochimica Biophysica Acta 1717, 67-88.

Pajaniappan M, Hall JE, Cawthraw SA, Newell DG, Gaynor EC, Fields JA, Rathbun KM, Agee WA, Burns CM, Hall SJ, Kelly DJ, Thompson SA. 2008. A temperature-regulated *Campylobacter jejuni* gluconate dehydrogenase is involved in respiration-dependent energy conservation and chicken colonization. Molecular Microbiology 68, 474-491.

1011 Pesci E.C, Cottle DL, Pickett CL. 1994. Genetic, enzymatic, and pathogenic
1012 studies of the iron superoxide dismutase of *Campylobacter jejuni*. Infection and
1013 Immunity 62, 2687–2694.
1014 Pini C-V, Bernal P, Godoy P, Ramos J-L, Segura A. 2009. Cyclopropane fatty
1015 acids are involved in organic solvent tolerance but not in acid stress resistance
1016 in Pseudomonas putida DOT-T1E. Microbial Biotechnology 2, 253–261.
1017 Poger D., Mark A.E. 2015. A ring to rule them all: the effect of cyclopropane
1018 fatty acids on the fluidity of lipid bilayers. Journal of Physical Chemistry B 119,
1019 5487–5495.
1020 Reid AE, Pandey R, Palyada K, Naikare H, Stintzi A. 2008a. Identification of
1021 *Campylobacter jejuni* genes involved in the response to acidic pH and stomach
1022 transit. Applied and Environmental Microbiology 74, 1583-1597.
1023 Reid AE, Pandey R, Palyada K, Whitworth L, Doukhanine E, Stintzi A. 2008b.
1024 Identification of *Campylobacter jejuni* Genes Contributing to Acid Adaptation by
1025 Transcriptional Profiling and Genome-Wide Mutagenesis. Applied and
1026 Environmental Microbiology 74, 1598-1612.
1027 Ross T, Zhang D, McQuestin OJ. 2008. Temperature governs the inactivation
1028 rate of vegetative bacteria under growth-preventing conditions. International
1029 Journal of Food Microbiology 30, 129-135.
1030 Shepherd M, Barynin V, Lu C, Bernhardt PV, Wu G, Yeh S-R. 2010. Novel D-
1031 helix conformation, proximal hydrogen bonding that influences ligand binding,
1032 and peroxidase-like redox properties. Protein Structure and Folding 285, 12747-
1033 12754.
1034 Sim D-W, Kim J-H, Kim H-Y, Jang J-H, Lee CL, Kim E-H, Park P-J, Lee K-H,
1035 Won H-S. 2016. Structural identification of the lipopolysaccharide-binding

capability of a cupin-family protein from *Helicobacter pylori*. FEBS Lett 590, 2997-3004.

Smith NW, Shorten PR, Altermann EH, Roy NC, McNabb WC. 2018. Hydrogen cross-feeders of the human gastrointestinal tract. Gut Microbes 10. 270-288.

Soumeya A, Pagès J-M, Bolla J-M. 2016. Cloning, expression, purification, regulation, and subcellular localization of a mini-protein from *Campylobacter jejuni*. Current Microbiology 72, 511–517.

Tsukahara T, Koyama H, Okada M, Ushida K. 2002. Stimulation of butyrate production by gluconic acid in batch culture of pig cecal digesta and identification of butyrate-producing bacteria. Journal of Nutrition 132, 2229-2234.

Tyanova S, Temu T, Sinitcyn P, Carlson A, Hein MY, Geiger T, Mann M, Cox J. 2016. The Perseus computational platform for comprehensive analysis of (prote)omics data. Nature Methods 13, 731–740.

Wiśniewski JR, Hein MY, Cox J, Mann M. 2014. A “proteomic ruler” for protein copy number and concentration estimation without spike-in standards. Molecular & Cellular Proteomics 13, 3497-3506.

van der Stel A-X & Wösten MMSM. 2019. Regulation of respiratory pathways in *Campylobacterota*: A Review. Frontiers of Microbiology 10, article 1719.

Zhao Y, Fu J, Li P, Chen N, Liu Y, Liu D, Guo Y. 2022. Effects of dietary glucose oxidase on growth performance and intestinal health of AA broilers challenged by *Clostridium perfringens*. Poultry Science 101, 101553.

Table 1. Differentially abundant proteins of *Campylobacter jejuni* NCTC 11168 determined to have differential abundances between cultures grown (or exposed) to acidic or alkaline pH relative to neutral pH (pH 7.0) controls.

Locus	Protein description	Function	pH 4.0 shock	pH 5.8	pH 8.0
			Fold change (log 2):[a]		
Cj0007	Glutamate synthase (NADPH) large subunit GltD	glutamate synthesis			+0.84
Cj0008	DUF262 domain-containing protein Cj0008	unknown			**+1.16**[b]
Cj0009	Glutamate synthase (NADPH) small subunit GltD	glutamate synthesis			**+1.01**
Cj0265c	cytochrome C-type haem-binding periplasmic protein Cj0265c	electron transport/respiration		**+0.56**	
Cj0358	Putative cytochrome C551 peroxidase Cj0358	defence, resist peroxide-induced cellular stress			+0.59
Cj0425	Putative periplasmic protein Cj0425	unknown	**+1.79**		
Cj0437	Methylmenaquinol:fumarate reductase MfrA	electron transport/respiration; fumarate reduction			**+0.96**
Cj0438	Methylmenaquinol:fumarate iron sulfur subunit MfrB	electron transport/respiration; fumarate reduction			+0.61
Cj0439	Methylmenaquinol:fumarate reductase subunit MfrC	electron transport/respiration; fumarate reduction			**+1.08**
Cj0613	ABC-type phosphate substrate binding protein PstS	phosphate uptake		**+0.76**	
Cj0622	Carbamoyltransferase HypF	electron transport/respiration, hydrogenase	**+0.85**	**+0.87**	
Cj0699c	Glutamine synthetase GlnA	glutamine synthesis			**+0.75**
Cj0712	Ribosome maturation factor RimM	ribosome maintenance/assembly/maturation		**+0.64**	
Cj0717	Putative ArsC family protein Cj0717	detoxification	**+0.70**		
Cj0784	Putative periplasmic protein NapL	electron transport/respiration, nitrate reduction			**+0.76**
Cj0879c	Putative periplasmic protein Cj0879c	unknown	**+0.55**	+0.52	
Cj1005c	membrane bound ATPase Cj1005c	unknown			**+0.92**
Cj1169c	Putative periplasmic protein Cj1169c	unknown	**+1.63**		
Cj1201	cobalamin-independent methionine synthase MetE	methionine synthesis			+0.61
Cj1224	Putative iron-binding protein Cj1224	iron uptake/carrier		+0.54	
Cj1225	RHH_3 domain-containing protein Cj1225	unknown		**+0.63**	**+1.15**
Cj1273	DNA-directed RNA polymerase subunit omega RpoZ	RNA polymerase, transcription	**+0.77**		
Cj1372	Putative periplasmic protein Cj1372c	unknown	**+0.55**	**+0.59**	
Cj1392	cystathionine beta-lyase, N-terminus MetC'	methionine synthesis			**+0.96**
Cj1393	cystathionine beta-lyase, C-terminus MetC'	methionine synthesis			**+1.08**
Cj1394	Adenylosuccinate lyase PurB	purine synthesis			**+0.90**
Cj1399c	Ni/Fe-hydrogenase small subunit HydA2	electron transport/respiration, hydrogenase			**+0.84**
Cj1419c	Putative methyltransferase Cj1419c	LOS, capsule, flagella-related carbohydrate synthesis; O-methyl phosphoramidate synthesis	**+0.70**	**+0.66**	
Cj1514c	Uncharacterized protein Cj1514c	unknown		**+0.78**	
Cj1552c	uncharacterized RecG-like transcriptional regulator MloB	regulator, role unknown			**+0.60**
Cj1651c	Methionine aminopeptidase Map	peptide metabolism, methionine provision		**+0.85**	

Cj1718c	3-isopropylmalate dehydrogenase LeuB	branched chain amino acid synthesis		**+0.55**	
Cj0017c	Disulphide bond formation protein DbsI	disulfide bond formation and maintenance	**-1.43**	**-1.36**	-0.54
Cj0019c	Putative MCP-domain signal transduction protein DocA	signalling, required for caecal colonisation	**-1.32**	**-1.36**	
Cj0020c	Cytochrome C551 peroxidase DocB	defence, resist peroxide-induced cellular stress	**-0.55**		-0.85
Cj0021c	Putative fumarylacetoacetate (FAA) hydrolase family protein	unknown			**-0.93**
Cj0073c	L-lactate dehydrogenase complex protein LldG	aerobic/anaerobic respiration; lactic acid catabolism			**-0.64**
Cj0074c	L-lactate dehydrogenase iron-sulfur protein LldF	aerobic/anaerobic respiration; lactic acid catabolism			**-0.64**
Cj0089	Putative lipoprotein Cj0089	unknown	**-1.41**	**-1.23**	-0.89
Cj0091	outer membrane lipoprotein LpoB	regulation of peptidoglycan synthesis	-1.28	**-1.24**	-0.57
Cj0103	ATP synthase subunit b AtpF	aerobic/anaerobic respiration; ATP synthesis	**-1.08**	**-1.05**	
Cj0108	ATP synthase epsilon subunit AtpC	aerobic/anaerobic respiration; ATP synthesis	**-0.59**	**-0.54**	
Cj0113	Peptidoglycan-associated protein Pal	outer membrane, cell division	-0.84	**-1.23**	
Cj0115	Peptidyl-prolyl cis-trans isomerase SlyD	protein folding, protein disaggregation/protein rescue	**-0.62**		-0.80
Cj0124c	Putative membrane protein Cj0124c	unknown	-1.39	**-1.27**	-0.75
Cj0129c	Outer membrane protein assembly factor BamA	outer membrane assembly	**-0.58**	**-0.72**	
Cj0131	Putative peptidase M23 family protein Cj0131	peptide metabolism	-1.59	**-2.27**	
Cj0144	Putative methyl-accepting chemotaxis signal transduction protein Cj0144	signalling, chemotaxis	**-1.31**	**-1.41**	-0.70
Cj0147c	Thioredoxin TrxA	disulfide bond formation and maintenance	**-0.76**		**-1.23**
Cj0152c	Putative membrane protein Cj0152c	unknown		**-1.12**	
Cj0169	Superoxide dismutase Fe-type SodB	defence, oxidative stress management			**-1.11**
Cj0183	Putative integral membrane protein with haemolysin domain Cj0183	unknown, not associated with haemolysis	**-1.79**	**-1.27**	
Cj0238	Putative mechanosensitive ion channel family protein Cj0238	osmotic pressure homeostasis	**-1.70**	**-1.86**	-0.73
Cj0239c	NifU family iron-sulfur cluster assembly scaffold protein Cj0239c	Fe-S cluster assembly	**-0.55**		**-1.13**
Cj0240c	Cysteine desulfurase IscS	Fe-S cluster assembly	-0.54		**-1.09**
Cj0262c	Putative methyl-accepting chemotaxis signal transduction protein Cj0262c	signalling, chemotaxis	**-1.63**	**-1.60**	-1.10
Cj0268c	Putative transmembrane protein Cj0268c	unknown	-0.78	**-1.02**	
Cj0287c	Transcription elongation/cleavage factor GreA	transcription related	**-0.69**	**-0.60**	-0.91
Cj0289c	Major antigenic glycoprotein Peb3	adhesion/virulence		**-0.88**	
Cj0300c	ABC-type molybdenum transport ATP-binding protein ModC	molybdate uptake	**-1.01**		**-1.26**
Cj0303c	ABC-type molybdate substrate-binding protein ModA	molybdate uptake			**-2.37**
Cj0318	Flagellar M-ring protein FliF	motility, flagella motor	**-1.66**	**-1.51**	-0.68
Cj0334	Alkyl hydroperoxide reductase C AhpC	thioredoxin-dependent peroxiredoxin, oxidative stress management	**-0.80**	-0.44	**-1.00**

Cj0365c	Outer membrane channel protein CmeC	toxic substance efflux	**-1.16**	**-1.40**	
Cj0366c	Efflux pump membrane transporter CmeB	toxic substance efflux	**-1.51**	**-1.47**	-0.53
Cj0367c	periplasmic fusion protein CmeA	toxic substance efflux	**-1.43**	**-1.42**	-0.72
Cj0371	UPF0323 family lipoprotein Cj0371	regulates chemotaxis by interacting with CheV	-0.85	**-1.57**	
Cj0393c	Malate:quinone oxidoreductase Mqo	electron transport/respiration, malate oxidation			**-0.77**
Cj0396c	Putative lipoprotein Cj0396c	unknown	-0.75	**-1.04**	
Cj0397c	Uncharacterized protein Cj0397c	unknown	-0.88	**-1.09**	
Cj0404	Putative transmembrane protein Cj0404	unknown	-0.97	**-1.20**	
Cj0406c	Putative lipoprotein Cj0406c	unknown	**-1.10**	**-1.28**	-0.66
Cj0427	Uncharacterized protein Cj0427	unknown			**-1.05**
Cj0441	Acyl carrier protein AcpP	fatty acid synthesis	-0.69		-1.22
Cj0481	Putative dihydrodipicolinate synthase DapA	L-lysine synthesis			**-1.48**
Cj0483	Putative altronate hydrolase C-terminus UxaA'	fucose utilisation?, truncated protein			**-1.49**
Cj0485	Putative oxidoreductase Cj0485	fucose utilisation		**-0.56**	**-1.92**
Cj0497	Putative lipoprotein Cj0497	unknown	**-1.65**	**-1.46**	-0.91
Cj0499	Putative histidine triad (HIT) family protein Cj0499	unknown		**-1.22**	
Cj0508	peptidoglycan transglycosylase PbpA	peptidoglycan synthesis	-1.08	**-1.78**	-1.30
Cj0515	Putative periplasmic protein Cj0515	unknown	**-1.24**	**-1.49**	-0.58
Cj0531	Isocitrate dehydrogenase [NADP] Icd	TCA cycle			**-0.71**
Cj0532	Malate dehydrogenase Mdh	TCA cycle			-0.54
Cj0533	Succinate--CoA ligase subunit beta SucC	TCA cycle			**-0.87**
Cj0534	Succinate--CoA ligase subunit alpha SucD	TCA cycle			**-0.90**
Cj0536	2-oxoglutarate:acceptor oxidoreductase OorA	TCA cycle			**-0.80**
Cj0538	2-oxoglutarate:acceptor oxidoreductase OorC	TCA cycle			-0.65
Cj0547	Flagellar protein FlaG	motility, flagella shaft			**-1.14**
Cj0554	Uncharacterized protein Cj0554	unknown			**-0.77**
Cj0556	Putative amidohydrolase family protein Cj0556	unknown			**-0.60**
Cj0559	thioredoxin reductase (NADPH) Cj0559	disulfide bond formation and maintenance			-0.64
Cj0612c	Bacterial non-heme ferritin Ftn/Cft	iron homeostasis	**-0.98**	**-0.84**	-0.92
Cj0623	Hydrogenase isoenzymes formation protein HypB	electron transport/respiration, hydrogenase			**-0.97**
Cj0626	Hydrogenase isoenzymes formation protein HypE	electron transport/respiration, hydrogenase			-0.61
Cj0650	checkpoint GTP-binding protein EngB	cell division			-0.61
Cj0703	DUF3972 domain-containing protein Cj0703	unknown	**-0.68**	**-0.67**	-1.09
Cj0726c	Magnesium transport protein CorA	magnesium uptake	-0.96	**-1.04**	
Cj0734c	ABC-type amino acid transport substrate binding protein CjaC	amino acid uptake	**-1.20**	**-1.54**	-0.55
Cj0762c	Aspartate aminotransferase AspB	L-aspartate catabolism, L-arginine synthesis			**-0.63**
Cj0779	Thiol peroxidase Tpx	thioredoxin-dependent peroxiredoxin, oxidative stress management			-0.82

Cj0817	ABC-type glutamine substrate binding protein GlnH	glutamine uptake			-0.56
Cj0835c	Aconitate hydratase B AcnB	TCA cycle			**-1.06**
Cj0898	Putative histidine triad (HIT) family protein Cj0898	unknown, signalling?	**-0.65**		-0.96
Cj0912c	Cysteine synthase B CysM	cysteine synthesis			**-0.94**
Cj0921c	polar amino acid transport system substrate-binding protein Peb1A	amino acid uptake; adhesion/virulence			**-0.73**
Cj0922c	ABC-type amino acid transporter ATP-binding protein Peb1C	amino acid uptake			**-0.72**
Cj0950c	Putative lipoprotein Cj0950c	unknown	-0.99	**-0.90**	
Cj0956c	tRNA modification GTPase MnmE	tRNA processing/modification	**-0.99**	**-1.33**	-1.17
Cj0982c	ABC-type amino-acid transporter substrate-binding protein CjaA	amino acid uptake	**-1.56**	**-1.49**	-0.95
Cj0983	Adhesion lipoprotein JlpA	adhesion/virulence	**-1.31**	**-1.11**	-0.93
Cj0996	GTP cyclohydrolase II RibA	riboflavin synthesis			**-1.03**
Cj0998c	YceI family periplasmic protein Cj0998c	unknown	**-0.70**	**-0.71**	-0.57
Cj1007c	Putative mechanosensitive ion channel family protein Cj1007c	osmotic pressure homeostasis	**-1.76**	**-1.28**	-0.74
Cj1013c	Putative cytochrome C biogenesis protein Cj1013c	electron transport/respiration, cytochrome C biogenesis	**-1.43**	**-1.15**	
Cj1026c	Putative lipoprotein Cj1026c	unknown	**-1.30**	**-1.71**	-0.94
Cj1029c	Outer membrane lipoprotein MapA	outer membrane associated	**-1.33**	**-1.74**	-1.20
Cj1033	Integral membrane component of efflux system CmeF	transport-related, efflux, unknown substance	-0.69	**-0.92**	
Cj1093c	Protein translocase subunit SecD	general secretion system	**-1.74**	**-1.17**	-0.76
Cj1106	Putative periplasmic thioredoxin Cj1106	disulfide bond formation and maintenance	**-1.10**	**-0.92**	-0.67
Cj1116c	ATP-dependent zinc metalloprotease FtsH	cell division	**-1.65**	**-1.08**	-0.50
Cj1120c	UDP-N-acetyl-alpha-D-glucosamine C6 dehydratase PglF	LOS, capsule, flagella-related carbohydrate synthesis	**-1.58**	**-1.79**	-0.90
Cj1170c	Outer-membrane tyrosine kinase CjtK/Omp50	signalling, posttranslational modification	-0.95	**-1.44**	
Cj1171c	Peptidyl-prolyl cis-trans isomerase Ppi	protein folding	**-0.55**		-0.84
Cj1176c	Sec-independent protein translocase protein TatA	Twin arginine system protein secretion		**-1.16**	
Cj1183c	Cyclopropane-fatty-acyl-phospholipid synthase Cfa	fatty acid synthesis			-0.59
Cj1184c	Ubiquinol-cytochrome C reductase cytochrome C subunit PetC	oxidative phosphorylation	**-1.13**	**-1.15**	-0.83
Cj1185c	Cytochrome b PetB	oxidative phosphorylation	**-1.41**	**-1.13**	-1.00
Cj1186c	Ubiquinol-cytochrome c reductase iron-sulfur subunit PetA	oxidative phosphorylation			**-0.64**
Cj1190c	Bipartate energy taxis response protein CetA	signalling, chemotaxis	-1.21	**-1.26**	
Cj1219c	Putative periplasmic protein Cj1219c	unknown	**-1.51**	**-2.00**	-0.60
Cj1259	Major outer membrane porin PorA	adhesion/virulence	-0.64	**-1.07**	
Cj1275c	Putative peptidase M23 family protein Cj1275c	peptide metabolism			**-0.55**
Cj1279c	fibronectin-binding lipoprotein FlpA	adhesion/virulence	**-1.04**	**-1.49**	
Cj1291c	Biotin carboxyl carrier protein of acetyl-CoA carboxylase AccB	fatty acid synthesis	**-0.78**		

Cj1304	Putative acyl carrier protein AcpP3	fatty acid synthesis			**-1.07**
Cj1308	Putative acyl carrier protein Cj1308	unknown	**-1.12**	**-0.93**	-1.52
Cj1357c	Nitrite reductase (cytochrome; ammonia-forming) NrfA	electron transport/respiration, nitrite reduction	-0.77	**-1.54**	
Cj1358c	Cytochrome c-type protein NrfH	electron transport/respiration, nitrite reduction	-1.12	**-1.57**	
Cj1382c	Flavodoxin FldA	electron transport/respiration	**-0.90**		**-1.25**
Cj1445c	Capsule polysaccharide export system inner membrane protein KpsE	LOS lipid A/sugar core synthesis	-0.78	**-0.96**	-0.52
Cj1478c	Outer membrane fibronectin-binding protein CadF	adhesion/virulence	-0.81	**-1.20**	-0.59
Cj1487c	Cbb3-type cytochrome c oxidase subunit CcoP	electron transport/respiration, Cytochrome C oxidase	**-1.53**	**-1.94**	-1.25
Cj1489c	Cb-type cytochrome C oxidase subunit II CcoO	electron transport/respiration, Cytochrome C oxidase		**-0.62**	
Cj1503c	proline dehydrogenase/delta-1-pyrroline-5-carboxylate dehydrogenase	proline metabolism (and possible catabolism)		**-0.68**	**-1.05**
Cj1506c	Putative MCP-type signal transduction protein Cj1506c	signalling, chemotaxis	**-1.54**	**-1.41**	-0.69
Cj1509c	Formate dehydrogenase, cytochrome B subunit FdhC	aerobic/anaerobic respiration	**-1.43**	**-1.59**	-1.15
Cj1523c	CRISPR-associated endonuclease Cas9	foreign DNA defence, CRISPR		**-0.73**	
Cj1534c	Ferritin-like protein Dps	iron homeostasis	**-1.13**	**-0.77**	-0.86
Cj1537c	Acetyl-coenzyme A synthetase Acs	acetate catabolism		**-0.66**	**-1.89**
Cj1540	ABC-type tungstate substrate-binding protein TupA	tungsten uptake			**-1.20**
Cj1541	5-oxoprolinase subunit A PxpA	glutathione metabolism			**-1.10**
Cj1542	Allophanate hydrolase subunit 1 Cj1542	defence, glutathione metabolism		**-0.53**	**-1.87**
Cj1543	Allophanate hydrolase subunit 2 Cj1543	defence, glutathione metabolism			**-0.95**
Cj1564	Putative methyl-accepting chemotaxis signal transduction protein Cj1564	signalling, chemotaxis	**-1.32**	**-1.38**	-0.66
Cj1571c	NADH-quinone oxidoreductase subunit I NuoI	electron transport/respiration, NADH synthesis			-0.57
Cj1584c	ABC-type system nickel binding protein NikZ	nickel uptake			**-0.57**
Cj1611	30S ribosomal protein S20 RpsT	ribosomal proteins	-1.26	**-1.41**	-0.84
Cj1626c	Putative periplasmic protein Cj1626c	unknown			-2.21
Cj1639	NifU family protein, putative FeS cluster assembly scaffold protein	Fe-S cluster assembly	**-0.81**	-0.43	-0.50
Cj1663	Putative ABC-type phosphonate transport ATP-binding protein Cj1663	phosphonate uptake			-0.71
Cj1666c	Putative periplasmic protein Cj1666c	unknown		**-1.37**	
Cj1680c	Putative periplasmic protein Cj1680c	unknown			-0.87
Cj1682c	Citrate synthase GltA	TCA cycle			**-1.25**
Cj1694c	30S ribosomal protein S14 type Z RpsZ	ribosomal proteins	**-0.82**	**-0.60**	-1.90

[a]Blank cells include data that is not significant ($p>0.05$) and below a 1.5-fold (≤0.52 log 2 ratio) change threshold.

[b]Values in bold are significant after false discovery rate adjustment ($p<0.05$, FDR). Non bold values have not passed FDR assessment.

Table 2. Proteins exhibiting acid pH-dependent responses but not induced rapidly by acid shock

locus	Protein description	Function	pH 4.0 shock	pH 5.8	pH 8.0
				Fold change (log 2):	
Cj0037c	Putative cytochrome C Cj0037c	electron transport/respiration		**0.54**	**-1.42**
Cj0414	Gluconate 2-dehydrogenase gamma chain Cj0414	electron transport/respiration, gluconate oxidation		**1.43**	**-1.45**
Cj0415	Gluconate 2-dehydrogenase alpha chain Cj0415	electron transport/respiration, gluconate oxidation		**1.26**	**-1.65**
Cj0465c	Group 3 truncated haemoglobin Ctb	oxygen flux management		**0.55**	-0.81
Cj0616	ABC-type phosphate import ATP-binding protein PstB	phosphate uptake		**0.67**	**-0.96**
Cj0761	Uncharacterized protein Cj0761	unknown		**1.15**	-0.95
Cj1586	Single domain haemoglobin Cgb	oxygen flux management		**1.02**	**-1.69**

4. CAPÍTULO II

4.1 Manuscrito 2- Alternative methods for control of thermophilic *Campylobacter* in poultry

Manuscrito de revisão a ser submetido ao periódico *Food Research International* - Fator de Impacto 7.425 e Qualis A1 na Área de Ciência de Alimentos

Tassiana Ramires[a], Natalie Rauber Kleinubing[a], Isabela Schneid Kroning[a], Ângela Maria Fiorentini[a], Wladimir Padilha da Silva [a,b]

[a] Departamento de Ciência e Tecnologia Agroindustrial (DCTA), Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas (UFPel), Pelotas, RS, Brazil

[b] Núcleo de Biotecnologia, Centro de Desenvolvimento Tecnológico, Universidade Federal de Pelotas (UFPel), Pelotas, RS, Brazil

ABSTRACT

In the last decade, *Campylobacter* has been the main pathogen associated with foodborne diseases worldwide. This is a pathogen closely related to poultry, and chicken meat consumption is estimated to be responsible for up to 69% of campylobacteriosis cases, highlighting the need to develop infection control strategies. *Campylobacter* has acquired resistance to major clinically significant antimicrobials in the last years, compromising the effectiveness of antimicrobials treatments. The rise in bacterial resistance and the demand for antibiotic-free products has led to the search to the several alternative pathogen control measures in animal reservoirs. Some of the strategies that have been researched are the use of natural compounds extracted from different plant sources, in addition to some effective measures, such as the use of probiotics and application of bacteriophages. This review explores the use of natural compounds extracted from plants, probiotics and bacteriophages in order to reduce *Campylobacter* loads both pre- and post-harvest. This review analyzed and critically discussed 87 scientific articles on alternative methods of controlling thermophilic *Campylobacter* in poultry, as well as their probable mechanisms of action.

Keywords: *Campylobacter jejuni*; control strategies; antibiotic resistance; bacteriophage; essential oil; probiotics.

Introduction

Campylobacter spp. is the mainly pathogen that cause foodborne gastroenteritis in the world (EFSA, 2021). Human campylobacteriosis is caused by the thermophilic *Campylobacter* species: *C. jejuni*, *C. coli, C. lari*, and *C. upsaliensis* (Ramires et al., 2020b). This disease is usually associated with eating raw or undercooked chicken meat and with cross-contamination of foods consumed in natura, with chicken meat being the most important source of campylobacteriosis, with an estimated 65–69% of human cases (Ravel et al., 2017). Birds, particularly poultry, are natural reservoirs of these bacteria in their intestines and are often asymptomatic (CDC, 2021; Kleinubing et al., 2021).

Antimicrobial resistance raises concerns around the world (Rodríguez-Baño et al., 2021; Watkins & Bonomo, 2020), and there are increasing reports of antimicrobial resistance in *Campylobacter* (García-Sánchez et al., 2019; Kleinubing et al., 2021; Ramires et al., 2020b; Wieczorek et al., 2020). According to the CDC (2019), drug-resistant *Campylobacter* is classified as a serious threat, highlighting the resistance to ciprofloxacin, which has doubled in the last 20 years (CDC, 2019). Another important fact is the increasing isolation in several countries of *Campylobacter* carrying the *ermB* gene, which represents a major problem as it confers resistance to macrolides, widely used in the treatment of campylobacteriosis (Chen et al., 2018; Florez-Cuadrado et al., 2016; Qin et al., 2014; Y. Wang et al., 2014). Excessive use of antimicrobials in poultry production is considered the main cause of development of resistance to these compounds in bacteria (Kousar et al., 2021). Due to the threat to public health, the use of antimicrobials in poultry production has become more restricted (CDC, 2019). With an increasing demand for antibiotic-free and organic chicken, novel antimicrobial approaches are desired, that are safe, effective, and environmentally friendly.

Natural antimicrobial products are isolated from different sources: animal sources such as lysozyme (egg, milk), lactoperoxidase (milk), lactoferrin (milk), chitosan (crustaceans); plant sources and their essential oils (EOs) and extracts; and microbial sources such as natamycin, nisin, other bacteriocins, fermentation metabolites, protective cultures, bacteriophages (Davidson et al., 2015; Možina et al., 2018). Taking into account the vast potential offered by natural antimicrobial compounds, there is increasing research on the effectiveness of constituents derived from plants and animals, as well as from bacteria and fungi (Silvan et al., 2019). Nonetheless, studies with this approach in *Campylobacter* are scarce and difficult to find in the literature, probably due to the specific requirements needed for work with this pathogen (Silvan et al., 2019).

Since the decrease in *Campylobacter* colonization in poultry is able to reduce human exposure to the pathogen (Richards et al., 2019). It is estimated that it would be possible to reduce the risk of campilobacteriosis in 90% by reducing the load of *Campylobacter* spp. in poultry intestines by 3 logs, and even a 1 log reduction in carcasses would reduce the risk between 50 and 90% (EFSA, 2011). Therefore, this review will emphasize the control of thermophilic *Campylobacter* through natural compounds: EOs and plants extracts, probiotics and bacteriophages.

2. Natural compounds derived from plants with activity to inhibit thermophilic *Campylobacter*

Compounds that act by inhibiting the inflammatory action of pathogens, collaborate to the maintaining of the integrity of the host's intestinal epithelium and downregulating some bacterial virulence factors (Balta et al., 2021). In recent years numerous studies have shown that *Campylobacter* species are susceptible to a wide variety of EOs (Table 1). The potential of antibacterial activity of natural compounds against *Campylobacter*

spp. it is not new (Friedman et al., 2002; Smith-Palmer et al., 1998). On the other hand, the knowledge about which stages of the pathogenesis are affected and especially which genes are down regulated is still little known.

Kovács et al. (2019) verified, for the first time, the anti-*Campylobacter* effect of Peppermint Essential Oil (PEO), evaluating 190 *C. jejuni* clinical isolates from humans and four reference strains (NCTC 11168, RM1221, 81-176, 81116). After 24 h of microaerobic incubation, untreated cells showed a turbidity area of 32.67 ± 3.21 mm, while *C. jejuni* cells treated with a sublethal 50 μg.mL^{-1} PEO concentration had an area of 13.33 ± 4.04 mm. No swarming was observed in cells inoculated into Soft agar medium containing 150 µg.mL^{-1} PEO.

Table 1 . Studies reported in the literature evaluating the use of essential oils to control *Campylobacter* spp.

Essential Oils	References
Thyme	Agrimonti et al. (2019); Clemente et al. (2020); Lin et al. (2018); Navarro et al. (2015); Thanissery et al. (2014)
Clove	Kovács et al. (2016); Navarro et al. (2015); Thanissery et al. (2014)
Garlic	Clemente et al. (2020); Navarro et al. (2015)
Lemon	Clemente et al. (2020); Fisher & Phillips (2006); Navarro et al. (2015)
Lemon Grass	Clemente et al. (2020); Navarro et al. (2015)
Lemon Myrtle	Kurekci et al. (2013, 2014)
Cardamom	Mutlu-Ingok (2018)
Onion	Clemente et al. (2020)
Oregano	Agrimonti et al. (2019); Aslim et al. (2007); Clemente et al. (2020); Navarro et al. (2015)
Rosemary	Clemente et al. (2020); Navarro et al. (2015); Piskernik et al. (2011); Thanissery et al. (2014)
Sage	Navarro et al. (2015)
Eugenol	Johny et al. (2008); Navarro et al. (2015); Wagle et al. (2019)
Carvacrol	Johny et al. (2008); Nair et al. (2015); Navarro et al. (2015); Shrestha et al. (2019); Wagle et al. (2019); Windiasti et al. (2019)
Thymol	Epps et al. (2015); Johny et al. (2008); Navarro et al. (2015)
Orange	Fisher & Phillips (2006); Nannapaneni et al. (2009); Thanissery et al. (2014)
Cinnamon	Agrimonti et al. (2019); Ahmed et al. (2018); Cui et al. (2021)
Coriander	Duarte et al. (2016)
Linalool	Duarte et al. (2016)
Bergamot	Fischer & Phillips (2006)

The bacterial adhesion process in *Campylobacter* spp. play a fundamental role in the colonization and maintenance of the pathogen in the environment, through the production

of biofilm (Klančnik et al., 2018). A recent study showed the potential of using bioactive juniper extracts as a new antimicrobial and anti-adhesion alternative against *C. jejuni* (Klančnik et al., 2018). It is noteworthy that the juniper fruit crude extract was very effective in inhibiting *C. jejuni* adhesion at concentrations that do not affect *C. jejuni* growth. These authors report that this may limit the *C. jejuni* biofilm formation, its persistence in food processing areas and its transmission through the food chain. Klančnik et al. (2018) also still demonstrated the potential activiy of the juniper fruit crude extract in reducing the adhesion of *C. jejuni* in co-cultures with *Listeria monocytogenes,* another important foodborne pathogen.

An interesting study evaluated the potential for inhibiting the adhesion and invasion of *C. jejuni* by a wild strain of *Lactobacillus casei* and a genetically modified *L. casei* strain (LC^{+mcra}), which produced large amounts of conjugated linoleic acid (CLA). In addition, the authors evaluated the interference of adding a prebiotic (peanut flour) (Tabashsum et al., 2018). *Lactobacillus casei* with peanut flour or LC^{+mcra} alone significantly reduced the *C. jejuni* adhesion and invasion capacity in both cell lines evaluated, HD-11 and HeLa.

Salaheen et al. (2014), evaluated the interference of peanut fractions (prebiotic) on the growth of *L. casei* in co-culture with *C. jejuni*. The author described that, probably, the high content of oleic acid found in peanuts stimulated the growth of *L. casei* causing the probiotic to competitively exclude adhesion of *C. jejuni* to INT407 cells. The decrease in the adesion rates of *C. jejuni* was significant when the ratio of *L. casei* and *C. jejuni* was 1:1 in the co-infection assay. The authors suggest that this reduction in the adhesion capacity of *C. jejuni* to INT407 cells occurs by competition for fibronectin receptors, present on the surface of the host cells, since both *L. casei* and *C. jejuni* express proteins on the surface of their cells membranes, capable of binding to fibronectin.

Oregano EO has shown potential as an antimicrobial against *C. jejuni*. Clemente et al. (2020) evaluated nine EOS and verified that the oregano EO showed the highest inhibitory activity (MIC 62.5 µg/mL). In the agar disk-diffusion test, the oregano EO promoted complete inhibition of growth of all four *C. jejuni* strains tested (Clemente et al., 2020). A similar result was obtained by Navarro et al. (2015), with the oregano EO showing the best anti-*Campylobacter* activity. Clemente et al. (2020) also studied the effect of the combination between oregano EO and pulsed electric fields (PEF), however, no synergic effect was observed with this association. Another study tested the ability of oregano EO to inhibit ciprofloxacin-resistant *Campylobacter* isolates (*C. jejuni*, *C. coli* and *C.lari*), with MIC results varying among the isolates (MIC ranging from 7.8 to 800 µg/mL) (Aslim & Yucel, 2008) emphasizing the anti-*Campylobacter* action of oregano EO.

The anti-*Campylobacter* activity of thyme EO was evaluated by Navarro et al. (2015), which presented MIC values of 0.006%. Clemente et al. (2020) had tested the thyme EO action against *Campylobacter* strains evaluating the MIC, MBC and the agar diffusion assay. The results obtained by Clemente et al. (2020) indicate the anti-*Campylobacter* potential of thyme EO, with MIC (for the four *C. jejuni* tested in the study) of 250 µg/mL, MBC of 250 µg/mL (for three *C. jejuni* isolates) and 500 µg/mL (for only one strain tested). The results were in agreement with those obtained in the agar diffusion assay, where there was 100% inhibition of *Campylobacter* growth for all strains tested.

It is important to emphasize that not always when there is a reduction in the capacity of adhesion and invasion in vitro, there will necessarily be a reduction in the expression of genes involved in these processes. In a study that assessed the capacity of blackberry and blueberry pomace extract to inhibit the adhesion and invasion of *C. jejuni* in cell lines, it was observed that the reduction in the ability of *C. jejuni* to invade DF1 cells was >75% and >30%, respectively. In INT407 cells, the invasion ability of *C. jejuni* was also reduced

similarly, nearly 79% in the presence of blackberry, and nearly 52% in the presence of blueberry pomace extracts.

2.1 Mode of action of essential oils and plant extracts against thermophilic *Campylobacter*

The use of EOs as an alternative to synthetic antimicrobials is an increasing trend nowadays. Plant EOs are aromatic oily liquids which can be obtained by expression, fermentation, enfleurage, extraction, or steam distillation from different parts of plants (Burt, 2004). It has been reported that the antimicrobial activity of EOs is generally due to phenolic and terpenoid as well as aliphatic compounds (Bendiabdellah et al., 2013; Lv et al., 2011). It is generally believed that EOs act mainly against the microbial cytoplasmic cell membrane. The hydrophobicity is an important characteristic of EOs and their components which enables them to accumulate in cell membrane, disturbing the structures and causing an increase in permeability. Leakage of intracellular constituents and impairment of microbial enzyme systems can then occur (Bajpai et al., 2013), and extensive loss of cellular content will cause microbial cell death (Lv et al., 2011).

The increase in relative conductivity demonstrate that the bacterial cell membrane became permeable at different levels after treatment with EOs, indicating a complete release of electrolytes out of the cell by cellular leakage. Likewise, a significant increase in optical density at 260 nm is observed when cellular constituents are released, revealing loss of cell membrane integrity. Irreversible damage to cytoplasmic membranes is indicated by detecting cellular constituents, like proteins and some essential molecules (Lv et al., 2011; Mutlu-Ingok et al., 2019).

ATP is used for many cell functions including transport of substances across cell membranes. The increase in extracellular ATP levels after treatment with OEs indicates

that these compounds released ATP out of cells, probably due to damage to the cellular envelope induced by Eos (Turgis et al., 2009). Depletion of ATP levels causes impairment of essential processes in the cell, which can lead to cell death, as ATP has several cellular functions that are necessary for growth, replication, and survival in living organisms (Mutlu-Ingok & Karbancioglu-Guler, 2017).

Kovács et al. (2019) verified that when *C. jejuni* was exposed to PEO, the stress response that was generated was more similar to a general stress response rather than an oxidative stress response, suggesting that cytoplasmic membrane disruption and leakage were not the major antibacterial mode of action of this EO. The response observed was an impaired ability to swarm, downregulation of certain virulence-associated genes, and elongated cell morphology, in contrast to the rounded cell morphology typically observed under oxidative stress conditions.

In addition to the use of EOs, active components of EOs have also been studied. For example, carvacrol is the active component of oregano oil. A recent study in vitro aiming to verify the mechanism of action of carvacrol against *C. jejuni,* demonstrated that this compound attenuates *C. jejuni* by decreasing motility, attachment, quorum sensing, and tolerance to stress. In addition, liquid chromatography tandem mass spectrometry analysis revealed modulation of selected proteins that could potentially contribute to impaired colonization factor function in *C. jejuni* (Wagle et al., 2020).

The antimicrobial effect of plant extracts depends on several variables such as extract composition, concentration of bioactive compounds, and the solvent used in the extraction process. The mechanisms of action have been mainly associated to membrane damage and enzymatic inhibition, i.e., ATP synthase activity, provoking the inhibition of energy metabolism (Bezek et al., 2016; Silvan et al., 2017, 2019). The antibacterial activity of a grape seed extract (GSE) was evaluated against different *Campylobacter*

strains, demonstrating the strong capacity of the GSE to inhibit *Campylobacter* growth. The phenolic profile of GSE mainly consisted on flavonols, phenolic acids, catechins, proanthocyanidins, and anthocyanins. The analysis of the antibacterial activity of the collected fractions against *C. jejuni* showed that phenolic acids, catechins and proanthocyanidins were the main responsible for the antimicrobial activity (Silván et al., 2013). Blackberry and blueberry pomace extracts significantly reduced the growth of *C. jejuni* and altered the bacterial physicochemical properties such as cell surface hydrophobicity and auto-aggregation capacity of this bacterial pathogen (Salaheen et al., 2014). There are several studies evaluating the anti-*Campylobacter* activity of EOs and plant extracts, however more studies on their mechanism of action are required to understand how these compounds work and their potential use for the control of *Campylobacter* spp. by the food industry.

3. Probiotics for the control of thermophilic *Campylobacter* in poultry

Probiotics, especially lactic acid bacteria (LAB), are important in reducing zoonotic bacterial pathogens from the gastrointestinal tract of poultry and may also improve overall health and prevent disease in these animals (Abd El-Hack et al., 2022)(Abd El-Hack et al., 2022). The main mechanisms against colonization and infections by pathogen are to stimulate host immune responses and to secrete peptides, proteins and other metabolites, as well as to competitively block receptor-mediated attachment of pathogens to the epithelial cell surface (Tabashsum et al., 2018). Therefore, the aim of many studies is to reduce the infectious load in chickens, thus also decreasing the pathogens load, and consequently, reducing the impact on consumers health (Baffoni et al., 2017). Most research focuses on the action of probiotic bacteria as competitors of *Campylobacter* (Baffoni et al., 2017; Mortada et al., 2020; Saint-Cyr et al., 2017; Smialek et al., 2018; C.

Wang et al., 2020), preventing the adhesion, and consecutively the invasion of these pathogens.

The inhibitory effect of six *Lactobacillus* spp. against *C. jejuni* was studied in vitro by Taha-Abdelaziz et al. (2019). Both the neutralized cell-free supernatant and *Lactobacilli* spp. cell culture inhibited the growth of *C. jejuni*. Additional experiments demonstrated that when *C. jejuni* was exposed to *Lactobacilli* spp. exhibited down-regulation of genes responsible for motility (*flaA*, *flaB* and *flhA*) and invasion (*ciaB*), as well as reduced production of quorum sensing detection molecule AI-2. However, one species, *L. reuteri*, was ineffective in down-regulating the *C. jejuni* virulence genes, showing that not all *Lactobacilli* spp. act in the same way. Baffoni et al. (2017), evaluated the administration of *Bifidobacterium longum* subsp. *longum* PCB133 combined with a prebiotic (xylooligosaccharide) in an in vivo broiler experiment, with two different administration times: starting from the first day of life and from the 14th day of life of the broilers. These authors concluded that the administration of probiotic+prebiotic was able to reduce cecal colonization of *Campylobacter* spp.. Furthermore, they found that the earlier the administration, the more effective it is. The most controversial issue concerns the economic aspect, as lifetime supplementation is undoubtedly more expensive, and farmers may be discouraged from using these additives. On the other hand, Saint-Cyr et al. (2017), artificially contaminated 30 broilers with *C. jejuni* by oral gavage added with a bacterial suspension of *Lactobacillus salivarius* SMXD51, at 14 and 35 days of age. On day 14, the comparison between the control and treated groups showed a significant reduction of 0.82 log in *Campylobacter* loads, while after 35 days, a significant reduction of 2.81 log was obtained.

Smialek et al. (2018) evaluated the feasibility of reducing the infection rate of *Campylobacter* spp. in broilers by the addition of multispecies probiotics (*Lactococcus*

lactis, *Carnobacterium divergens*, *Lactobacillus casei*, *Lactobacillus plantarum* and *Saccharomyces cerevisae*) to the broilers feed. The authors observed that the multispecies probiotic product was able to reduce the extent of invasion of the intestinal tract of broilers by *Campylobacter* spp. and, consequently, reduce the level of contamination in the environment of the broilers, which ended up contributing to the improvement of the hygienic parameters of the carcasses of the analyzed broilers.

In another study, Mortada et al. (2020) performed in vitro and in vivo experiments to evaluate the effectiveness of commercial feed additives based on probiotics and organic acids (OA) for the reduction of *C. coli*. Probiotic strains (*Lactobacillus reuteri*, *Pediococcus acidilactici*, *Bifidobacterium animalis*, and *Enterococcus faecium*) significantly inhibited the growth of *C. coli* in vitro, while in vivo there was no significant reduction (Mortada et al., 2020). More research are needed on *Campylobacter* spp. and control strategies using probiotics still need to be thoroughly studied and standardized. The use of probiotics is a potential alternative to reduce the infectious load of thermophilic *Campylobacter* in poultry, having applications in different production conditions and diets, as well as providing some benefits for alternative poultry management results (Jeni et al., 2021).

3.1 Mode of action of probiotics against thermophilic *Campylobacter* in poultry

Probiotics are beneficial microorganisms that have many health-promoting functions for a host (Tang & Lu, 2019). One of the strategies aimed at reducing the carriage of *Campylobacter* spp. among poultries includes the use of probiotic microorganisms that compete with pathogenic bacteria for colonization of the gut. The administration of probiotics is advantageous as compared to other strategies that aim to eliminate unwanted microbiota (e.g., vaccination, antimicrobial treatment, or chemical disinfection),

as they are easy to administer and inexpensive to produce, as well as because they may persist in the animal (Nowaczek et al., 2019).

Probiotic may eliminate unfavorable microbiota by several possible mechanisms, including production of inhibitory substances, such as organic acids, bacteriocins, blocking of adhesion sites on intestinal epithelial surfaces, competition for nutrients, and stimulation of immunity. These beneficial properties are largely dependent on their prolonged residence in the gastrointestinal tract and are dictated by their adherence to the intestinal mucosa (Lebeer et al., 2008).

The lactic acid disrupts the membrane of *C. jejuni* and is responsible for inhibiting the growth of these bacteria *in vitro* and for reducing their intestinal colonization in chickens (Bratz et al., 2015; Neal-McKinney et al., 2012; Nowaczek et al., 2019; Y. Wang et al., 2014). Some probiotics are known to produce bacteriocins that act antagonistically against intestinal pathogens. Bacteriocins are small peptides of bacterial origin that exhibit anti-bacterial activities by disrupting bacterial membrane.

Anti-*Campylobacter* bacteriocin treatment could be an effective strategy to reduce the *C. jejuni* load in broilers. Probiotic bacteria isolated from intestinal tract of poultry have already shown an inhibitory effect against thermophilic *Campylobacter*, some of which had their bacteriocins purified and characterized, among them *Lactobacillus salivarius* SMXD51 (Messaoudi et al., 2012), *L. salivarius* NRRL B-30514 (Stern et al., 2006; Stern et al., 2008), and *L. curvatus* DN317 (Zommiti et al., 2016).

The control of *Campylobacter* by probiotic strains depends not only on production of antimicrobial substances, but also on the ability of these strains to adhere to the intestinal epithelium. The adhesion mechanism involves passive forces, electrostatic and hydrophobic interactions, as well as specific bindings dependent on bacterial surface adhesins. Several studies have reported a positive correlation between hydrophobicity of

probiotic strains and their adhesion to epithelial cells, as well as the reduction of adhesion and invasion efficacy of *C. jejuni* in these cells (Baffoni et al., 2017; Mortada et al., 2020; Nowaczek et al., 2019; Tabashsum et al., 2018; Wang et al., 2014).

Research shows that probiotics are usually administered in broilers in order to colonize the intestine and maintain intestinal microbial balance promoting a competitive exclusion of pathogenic bacteria, through competition by attachment sites and nutrients, causing reduction and inhibition of *Campylobacter* growth (Baffoni et al., 2017; Mortada et al., 2020; Smialek et al., 2018). Different *Lactobacillus* strains can modulate the immune system by increasing serum pro-inflammatory cytokine levels, antibodies and chicken macrophage phagocytosis (Brisbin et al., 2015; Saint-Cyr et al., 2017). Saint-Cyr et al. (2017) administered *L. salivarius* SMXD51 in 30 broilers artificially contaminated with *C. jejuni* by oral gavage and verified slight effects on the gene expression of pro-inflammatory cytokines in the cecal tonsils of these animals. These authors showed that a 4 h treatment with *L. salivarius* SMXD51 enhanced IL-8 and K60 gene expression in avian LMH cells. The application of probiotics in anti-*Campylobacter* therapy in the poultry production system is a promising and growing field capable of reducing food contamination by this important pathogen.

4. Bacteriophages as a control of thermophilic *Campylobacter* in poultry

Due to the rising concern with antimicrobial resistance, phage therapy has attracted renewed attention as a potential therapy to combat pathogens including *Campylobacter* species (Kittler et al., 2013; Nafarrate et al., 2021; Richards et al., 2019). It is known that phage isolation is more efficient in samples where the host bacterium is incident, and the broiler production chain and the broilers themselves are the most suitable type of sample for phage isolation against thermophilic *Campylobacter*, because there is high level of

contamination of this pathogen (Furuta et al., 2017). Phage cocktail is the name for a mixture of several phages and has the advantage of broadening the general range of bacteria susceptible to phage infection (Haines et al., 2021; Steffan et al., 2022). There is still a need for research and advances in the characterization and selection of *Campylobacter* phages, because there is no active phages against all *Campylobacter* spp. strains. The use of phage cocktails is necessary for effective control of thermophilic *Campylobacter* in poultry and consequently in food and its derivatives. It is known that the combination of phage cocktails prevents bacterial resistance to phages, increasing the range of susceptible *Campylobacter* strains (Furuta et al., 2017; Steffan et al., 2022). Besides, Kittler et al. (2013) showed the positive effects of phage administration to broilers via drinking water 1 to 4 days before slaughter. This treatment led to a reduction of *Campylobacter* spp. counts up to 3.2 log CFU in cecal content compared to the control. In another study, a reduction of 1.68 log CFU/g of *C. jejuni* in samples of artificially contaminated chicken meat was obtained, after 48 h of storage at refrigeration temperature (4 °C), suggesting that bacteriophages also can be used as a postharvest biocontrol agent (Thung et al., 2020). Richards et al. (2019), used a mixture of two *Campylobacter* phages to treat chickens experimentally infected with *C. jejuni* and observed considerable reduction in *Campylobacter* counts in the intestinal tract throughout the 5-day treatment period, however the most obvious difference was seen 2 days after starting treatment. The use of bacteriophages against *Campylobacter* spp. is an effective alternative, due to its specificity and promising results, since a number of commercial phage-based products are now available to apply on animal products and ready-to-eat foods (Richards et al., 2019).

4.1 Mode of action of Bacteriophages against thermophilic *Campylobacter* in poultry

Bacteriophages (phages) are bacterial viruses that can infect and lyse bacterial cells. Phages depend on bacteria to replicate, so it is essential that they survive in the environment until they find their bacterial host. The mechanism of action of bacteriophages against *Campylobacter* is common to all bacterial cells, however, it is noteworthy that bacterial phage infection is determined by specific receptors on bacterial surfaces (Janež & Loc-Carrillo, 2013).

Campylobacter phages specifically infect *Campylobacter* strains and do not affect the natural intestinal microbiota of poultry (Richards et al., 2019). Most *Campylobacter* phages have a contractile tail and belong to the *Myoviridae* family (Nowaczek et al., 2019). Based on DNA sequence analysis *Campylobacter* phages are further subdivided into the genera *Firehammerviruses* and *Fletcherviruses* (Javed et al., 2014). Phages belonging to the genus *Firehammerviruses* infect *C. coli* and *C. jejuni* and recognize their hosts through the flagellum, while phages of the genus *Fletcherviruses* infect only *C. jejuni* and bind to the host's capsular polysaccharides (Zampara et al., 2017). According to Carvalho et al. (2010), when the aim is to control thermophilic *Campylobacter* in general, without identifying the species (*C. jejuni* or *C. coli*), the use of a phage cocktail containing both genera is considered the best choice. Steffan et al. (2022), showed that a mixture of phages from the two genera is better for practical applications against *C. coli* and *C. jejuni* than using only just one phage genus.

After finding the bacterial host, the absorption phase begins, which occurs initially by a reversible binding, followed by irreversible bacteriophage binding and transfer of the bacteriophage genome to the host, which typically take place rapidly after collision between a bacteriophage particle and a bacteriophage-susceptible bacterium.

Bacteriophage replication within the bacterial cell and release of bacteriophage progeny, are dependent on the metabolic status of the bacterial cell (EFSA, 2009).

Conclusion

It is evident the importance of controlling thermophilic *Campylobacter* in the broiler production chain in order to obtain a significant reduction in cases of human campylobacteriosis. The use of natural compounds is a potential tool for this control and also to combat one of the biggest concerns worldwide, which is bacterial resistance to antimicrobials. Despite the existence of a wide range of studies that prove the effectiveness of natural compounds against thermophilic *Campylobacter*, more studies are still needed in order to better understand how these compounds actually act to inhibit the growth of the pathogen. In addition, it is also necessary to invest in improvements for the use of these compounds in the control not only in broiler production systems, but also in the steps of food processing.

References

Abd El-Hack, M. E., El-Saadony, M. T., Salem, H. M., El-Tahan, A. M., Soliman, M. M., Youssef, G. B. A., Taha, A. E., Soliman, S. M., Ahmed, A. E., El-kott, A. F., Al Syaad, K. M., & Swelum, A. A. (2022). Alternatives to antibiotics for organic poultry production: types, modes of action and impacts on bird's health and production. *Poultry Science*, *101*(4), 101696. https://doi.org/10.1016/j.psj.2022.101696

Agrimonti, C., White, J. C., Tonetti, S., & Marmiroli, N. (2019). Antimicrobial activity of cellulosic pads amended with emulsions of essential oils of oregano, thyme and cinnamon against microorganisms in minced beef meat. *International Journal of Food Microbiology*, *305*(May), 108246. https://doi.org/10.1016/j.ijfoodmicro.2019.108246

Ahmed, J., Mulla, M., Arfat, Y. A., Bher, A., Jacob, H., & Auras, R. (2018). Compression molded LLDPE films loaded with bimetallic (Ag-Cu) nanoparticles and cinnamon essential oil for chicken meat packaging applications. *Lwt*, *93*, 329–338. https://doi.org/10.1016/j.lwt.2018.03.051

Aslim, B., & Yucel, N. (2008). In vitro antimicrobial activity of essential oil from endemic Origanum minutiflorum on ciprofloxacin-resistant Campylobacter spp. *Food Chemistry*, *107*(2), 602–606. https://doi.org/10.1016/j.foodchem.2007.08.048

Baffoni, L., Gaggìa, F., Garofolo, G., Di Serafino, G., Buglione, E., Di Giannatale, E., & Di Gioia, D. (2017). Evidence of Campylobacter jejuni reduction in broilers with early synbiotic administration. *International Journal of Food Microbiology*, *251*(April), 41–

47. https://doi.org/10.1016/j.ijfoodmicro.2017.04.001

Bajpai, V. K., Sharma, A., & Baek, K. H. (2013). Antibacterial mode of action of Cudrania tricuspidata fruit essential oil, affecting membrane permeability and surface characteristics of food-borne pathogens. *Food Control*, *32*(2), 582–590. https://doi.org/10.1016/j.foodcont.2013.01.032

Balta, I., Marcu, A., Linton, M., Kelly, C., Gundogdu, O., Stef, L., Pet, I., Ward, P., Deshaies, M., Callaway, T., Sopharat, P., Gradisteanu-Pircalabioru, G., & Corcionivoschi, N. (2021). Mixtures of natural antimicrobials can reduce Campylobacter jejuni, Salmonella enterica and Clostridium perfringens infections and cellular inflammatory response in MDCK cells. *Gut Pathogens*, *13*(1), 1–13. https://doi.org/10.1186/s13099-021-00433-5

Barlow, S., Chesson, A., Collins, J. D., Flynn, A., Hardy, A., Knaap, A., Kuiper, H., Larsen, J. C., Neindre, P. Le, Schans, J., Silano, V., Skerfving, S., Vannier, P., EFSA Panel on Biological Hazards (BIOHAZ), Andreoletti, O., Budka, H., Buncic, S., Colin, P., Collins, J. D., … European Food Safety Authorty. (2009). The use and mode of action of bacteriophages in food production 1 Scientific Opinion of the Panel on Biological Hazards Adopted on 22 April 2009. *EFSA Journal*, *10*(388), 1–9. www.efsa.europa.eu/efsajournal

Bendiabdellah, A., Dib, M. E. A., Meliani, N., Muselli, A., Nassim, D., Tabti, B., & Costa, J. (2013). Antibacterial activity of Daucus crinitus essential oils along the vegetative life of the plant. *Journal of Chemistry*, *2013*. https://doi.org/10.1155/2013/149502

Bezek, K., Kurinčič, M., Knauder, E., Klančnik, A., Raspor, P., Bucar, F., & Smole Možina, S. (2016). Attenuation of Adhesion, Biofilm Formation and Quorum Sensing of Campylobacter jejuni by Euodia ruticarpa. *Phytotherapy Research*, *February*, 1527–1532. https://doi.org/10.1002/ptr.5658

Bratz, K., Gölz, G., Janczyk, P., Nöckler, K., & Alter, T. (2015). Analysis of in vitro and in vivo effects of probiotics against Campylobacter spp. *Berliner Und Münchener Tierärztliche Wochenschrift*, *128*(3–4), 155–162. https://doi.org/10.2376/0005-9366-128-155

Brisbin, J. T., Davidge, L., Roshdieh, A., & Sharif, S. (2015). Characterization of the effects of three Lactobacillus species on the function of chicken macrophages. *Research in Veterinary Science*, *100*, 39–44. https://doi.org/10.1016/j.rvsc.2015.03.003

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods - A review. *International Journal of Food Microbiology*, *94*(3), 223–253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

Carvalho, C. M., Gannon, B. W., Halfhide, D. E., Santos, S. B., Hayes, C. M., Roe, J. M., & Azeredo, J. (2010). The in vivo efficacy of two administration routes of a phage cocktail to reduce numbers of Campylobacter coli and Campylobacter jejuni in chickens. *BMC Microbiology*, *10*. https://doi.org/10.1186/1471-2180-10-232

CDC. (2019). *Campylobacter (Campylobacteriosis)*. CDC Web Page. https://www.cdc.gov/campylobacter/index.html

Chen, J. C., Tagg, K. A., Joung, Y. J., Bennett, C., Watkins, L. F., Eikmeier, D., & Folster, J. P. (2018). Report of erm(B) campylobacter jejuni in the United States. *Antimicrobial Agents and Chemotherapy*, *62*(6), 1–7. https://doi.org/10.1128/AAC.02615-17

Clemente, I., Condón-Abanto, S., Pedrós-Garrido, S., Whyte, P., & Lyng, J. G. (2020). Efficacy of pulsed electric fields and antimicrobial compounds used alone and in combination for the inactivation of Campylobacter jejuni in liquids and raw chicken. *Food Control*, *107*. https://doi.org/10.1016/j.foodcont.2019.01.017

Cui, H., Yang, H., Abdel-Samie, M. A., Siva, S., & Lin, L. (2021). Controlled-release casein/cinnamon essential oil nanospheres for the inactivation of Campylobacter jejuni in duck. *International Journal of Food Microbiology*, *341*(July 2020), 109074. https://doi.org/10.1016/j.ijfoodmicro.2021.109074

Davidson, P. M., Cekmer, H. B., Monu, E. A., & Techathuvanan, C. (2015). The use of natural antimicrobials in food: An overview. In *Handbook of Natural Antimicrobials for Food Safety and Quality* (Issue 3). Elsevier Ltd. https://doi.org/10.1016/B978-1-78242-034-7.00001-3

Duarte, A., Luís, Â., Oleastro, M., & Domingues, F. C. (2016). Antioxidant properties of coriander essential oil and linalool and their potential to control Campylobacter spp. *Food Control*, *61*, 115–122. https://doi.org/10.1016/j.foodcont.2015.09.033

EFSA. (2021). The European Union One Health 2019 Zoonoses Report. *EFSA Journal*, *19*(2). https://doi.org/10.2903/j.efsa.2021.6406

Epps, S. V. R., PetrujkicÍ, B. T., Sedej, I., Krueger, N. A., Harvey, R. B., Beier, R. C., Stanton, T. B., Phillips, T. D., Anderson, R. C., & Nisbet, D. J. (2015). Comparison of anti-Campylobacter activity of free thymol and thymol-β-d-glucopyranoside in absence or presence of β-glycoside-hydrolysing gut bacteria. *Food Chemistry*, *173*, 92–98. https://doi.org/10.1016/j.foodchem.2014.10.007

Fisher, K., & Phillips, C. A. (2006). The effect of lemon, orange and bergamot essential oils and their components on the survival of Campylobacter jejuni, Escherichia coli O157, Listeria monocytogenes, Bacillus cereus and Staphylococcus aureus in vitro and in food systems. *Journal of Applied Microbiology*, *101*(6), 1232–1240. https://doi.org/10.1111/j.1365-2672.2006.03035.x

Florez-Cuadrado, D., Ugarte-Ruiz, M., Quesada, A., Palomo, G., Domínguez, L., & Porrero, M. C. (2016). Description of an erm(B)-carrying Campylobacter coli isolate in Europe. *Journal of Antimicrobial Chemotherapy*, *71*(3), 841–843. https://doi.org/10.1093/jac/dkv383

Friedman, M., Henika, P. R., & Mandrell, R. E. (2002). *Bactericidal Activities of Plant Essential Oils and Some of Their Isolated Constituents against Campylobacter jejuni , Escherichia coli , Listeria monocytogenes , and Salmonella enterica*. *65*(10), 1545–1560.

Furuta, M., Nasu, T., Umeki, K., Minh, D. H., Honjoh, K. I., & Miyamoto, T. (2017). Characterization and application of lytic bacteriophages against campylobacter jejuni isolated from poultry in Japan. *Biocontrol Science*, *22*(4), 213–221. https://doi.org/10.4265/bio.22.213

García-Sánchez, L., Melero, B., Jaime, I., Rossi, M., Ortega, I., & Rovira, J. (2019). Biofilm formation, virulence and antimicrobial resistance of different Campylobacter jejuni isolates from a poultry slaughterhouse. *Food Microbiology*, *83*(May), 193–199. https://doi.org/10.1016/j.fm.2019.05.016

Haines, M. E. K., Hodges, F. E., Nale, J. Y., Mahony, J., van Sinderen, D., Kaczorowska, J., Alrashid, B., Akter, M., Brown, N., Sauvageau, D., Sicheritz-Pontén, T., Thanki, A. M., Millard, A. D., Galyov, E. E., & Clokie, M. R. J. (2021). Analysis of Selection Methods to Develop Novel Phage Therapy Cocktails Against Antimicrobial Resistant Clinical Isolates of Bacteria. *Frontiers in Microbiology*, *12*(March), 1–15. https://doi.org/10.3389/fmicb.2021.613529

Janež, N., & Loc-Carrillo, C. (2013). Use of phages to control campylobacter spp. *Journal of Microbiological Methods*, *95*(1), 68–75. https://doi.org/10.1016/j.mimet.2013.06.024

Javed, M. A., Ackermann, H. W., Azeredo, J., Carvalho, C. M., Connerton, I., Evoy, S., Hammerl, J. A., Hertwig, S., Lavigne, R., Singh, A., Szymanski, C. M., Timms, A., &

Kropinski, A. M. (2014). A suggested classification for two groups of Campylobacter myoviruses. *Archives of Virology*, *159*(1), 181–190. https://doi.org/10.1007/s00705-013-1788-2

Jeni, R. El, Dittoe, D. K., Olson, E. G., Lourenco, J., Corcionivoschi, N., Ricke, S. C., & Callaway, T. R. (2021). Probiotics and potential applications for alternative poultry production systems. *Poultry Science*, *100*(7), 101156. https://doi.org/10.1016/j.psj.2021.101156

Kittler, S., Fischer, S., Abdulmawjood, A., Glünder, G., & Kleina, G. (2013). Effect of bacteriophage application on Campylobacter jejuni loads in commercial broiler flocks. *Applied and Environmental Microbiology*, *79*(23), 7525–7533. https://doi.org/10.1128/AEM.02703-13

Klančnik, A., Zorko, Š., Toplak, N., Kovač, M., Bucar, F., Jeršek, B., & Smole Možina, S. (2018). Antiadhesion activity of juniper (Juniperus communis L.) preparations against Campylobacter jejuni evaluated with PCR-based methods. *Phytotherapy Research*, *32*(3), 542–550. https://doi.org/10.1002/ptr.6005

Kleinubing, N. R., Ramires, T., Würfel, S. de F. R., Haubert, L., Scheik, L. K., Kremer, F. S., Lopes, G. V., & Silva, W. P. da. (2021). Antimicrobial resistance genes and plasmids in Campylobacter jejuni from broiler production chain in Southern Brazil. *Lwt*, *144*(January). https://doi.org/10.1016/j.lwt.2021.111202

Kollanoor Johny, A., Darre, M. J., Hoagland, T. A., Schreiber, D. T., Donoghue, A. M., Donoghue, D. J., & Venkitanarayanan, K. (2008). Antibacterial effect of trans-cinnamaldehyde on Salmonella enteritidis and Campylobacter jejuni in chicken drinking water. *Journal of Applied Poultry Research*, *17*(4), 490–497. https://doi.org/10.3382/japr.2008-00051

Kousar, S., Rehman, N., Javed, A., Hussain, A., Naeem, M., Masood, S., Ali, H. A., Manzoor, A., Khan, A. A., Akrem, A., Iqbal, F., Zulfiqar, A., Jamshaid, M. B., Waqas, M., Waseem, A., & Saeed, M. Q. (2021). Intensive poultry farming practices influence antibiotic resistance profiles in pseudomonas aeruginosa inhabiting nearby soils. *Infection and Drug Resistance*, *14*(June), 4511–4516. https://doi.org/10.2147/IDR.S324055

Kovács, J. K., Felső, P., Horváth, G., Schmidt, J., Dorn, A., Ábrahám, H., Cox, A., Márk, L., Emődy, L., Kovács, T., & Schneider, G. (2019). Stress Response and Virulence Potential Modulating Effect of Peppermint Essential Oil in Campylobacter jejuni. *BioMed Research International*, *2019*. https://doi.org/10.1155/2019/2971741

Kovács, Judit K., Felso, P., Makszin, L., Pápai, Z., Horváth, G., Ábrahám, H., Palkovics, T., Böszörményi, A., Emody, L., & Schneider, G. (2016). Antimicrobial and virulence-modulating effects of clove essential oil on the foodborne pathogen Campylobacter jejuni. *Applied and Environmental Microbiology*, *82*(20), 6158–6166. https://doi.org/10.1128/AEM.01221-16

Kurekci, C., Jassim, R. Al, Hassan, E., Bishop-Hurley, S. L., Padmanabha, J., & McSweeney, C. S. (2014). Effects of feeding plant-derived agents on the colonization of Campylobacter jejuni in broiler chickens. *Poultry Science*, *93*(9), 2337–2346. https://doi.org/10.3382/ps.2014-03950

Kurekci, C., Padmanabha, J., Bishop-Hurley, S. L., Hassan, E., Al Jassim, R. A. M., & McSweeney, C. S. (2013). Antimicrobial activity of essential oils and five terpenoid compounds against Campylobacter jejuni in pure and mixed culture experiments. *International Journal of Food Microbiology*, *166*(3), 450–457. https://doi.org/10.1016/j.ijfoodmicro.2013.08.014

Lebeer, S., Vanderleyden, J., & De Keersmaecker, S. C. J. (2008). Genes and Molecules of Lactobacilli Supporting Probiotic Action. *Microbiology and Molecular Biology*

Reviews, *72*(4), 728–764. https://doi.org/10.1128/mmbr.00017-08

Lin, L., Zhu, Y., & Cui, H. (2018). Electrospun thyme essential oil/gelatin nanofibers for active packaging against Campylobacter jejuni in chicken. *Lwt*, *97*(April), 711–718. https://doi.org/10.1016/j.lwt.2018.08.015

Lv, F., Liang, H., Yuan, Q., & Li, C. (2011). In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. *Food Research International*, *44*(9), 3057–3064. https://doi.org/10.1016/j.foodres.2011.07.030

Messaoudi, S., Madi, A., Prévost, H., Feuilloley, M., Manai, M., Dousset, X., & Connil, N. (2012). In vitro evaluation of the probiotic potential of Lactobacillus salivarius SMXD51. *Anaerobe*, *18*(6), 584–589. https://doi.org/10.1016/j.anaerobe.2012.10.004

Mortada, M., Cosby, D. E., Shanmugasundaram, R., & Selvaraj, R. K. (2020). In vivo and in vitro assessment of commercial probiotic and organic acid feed additives in broilers challenged with Campylobacter coli. *Journal of Applied Poultry Research*, *29*(2), 435–446. https://doi.org/10.1016/j.japr.2020.02.001

Možina, S. S., Klančnik, A., Kovac, J., Jeršek, B., & Bucar, F. (2018). *Antimicrobial Natural Products Against Campylobacter*. 3–30. https://doi.org/10.1007/978-3-319-67045-4_1

Mutlu-Ingok, A., & Karbancioglu-Guler, F. (2017). Cardamom, Cumin, and Dill Weed Essential Oils: Chemical Compositions, Antimicrobial Activities, and Mechanisms of Action against Campylobacter spp. *Molecules (Basel, Switzerland)*, *22*(7). https://doi.org/10.3390/molecules22071191

Mutlu-Ingok, A., Tasir, S., Seven, A., Akgun, N., & Karbancioglu-Guler, F. (2019). Evaluation of the single and combined antibacterial efficiency of essential oils for controlling Campylobacter coli, Campylobacter jejuni, Escherichia coli, Staphylococcus aureus, and mixed cultures. *Flavour and Fragrance Journal*, *34*(4), 280–287. https://doi.org/10.1002/ffj.3501

Nafarrate, I., Mateo, E., Miranda-Cadena, K., & Lasagabaster, A. (2021). Isolation, host specificity and genetic characterization of Campylobacter specific bacteriophages from poultry and swine sources. *Food Microbiology*, *97*(September 2020). https://doi.org/10.1016/j.fm.2021.103742

Nair, D. V. T., Kiess, A., Nannapaneni, R., Schilling, W., & Sharma, C. S. (2015). The combined efficacy of carvacrol and modified atmosphere packaging on the survival of Salmonella, Campylobacter jejuni and lactic acid bacteria on Turkey breast cutlets. *Food Microbiology*, *49*, 134–141. https://doi.org/10.1016/j.fm.2015.01.010

Nannapaneni, R., Chalova, V. I., Crandall, P. G., Ricke, S. C., Johnson, M. G., & O'Bryan, C. A. (2009). Campylobacter and Arcobacter species sensitivity to commercial orange oil fractions. *International Journal of Food Microbiology*, *129*(1), 43–49. https://doi.org/10.1016/j.ijfoodmicro.2008.11.008

Navarro, M., Stanley, R., Cusack, A., & Sultanbawa, Y. (2015). *Combinations of plant-derived compounds against Campylobacter in vitro*.

Neal-McKinney, J. M., Lu, X., Duong, T., Larson, C. L., Call, D. R., Shah, D. H., & Konkel, M. E. (2012). Production of Organic Acids by Probiotic Lactobacilli Can Be Used to Reduce Pathogen Load in Poultry. *PLoS ONE*, *7*(9). https://doi.org/10.1371/journal.pone.0043928

Nowaczek, A., Urban-Chmiel, R., Dec, M., Puchalski, A., Stępień-Pyśniak, D., Marek, A., & Pyzik, E. (2019). Campylobacter spp. and bacteriophages from broiler chickens: Characterization of antibiotic susceptibility profiles and lytic bacteriophages. *MicrobiologyOpen*, *8*(7), 1–10. https://doi.org/10.1002/mbo3.784

Opinion, S. (2011). Scientific Opinion on Campylobacter in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. *EFSA Journal*, *9*(4), 1–141. https://doi.org/10.2903/j.efsa.2011.2105

Piskernik, S., Klančnik, A., Riedel, C. T., Brøndsted, L., & Možina, S. S. (2011). Reduction of Campylobacter jejuni by natural antimicrobials in chicken meat-related conditions. *Food Control*, *22*(5), 718–724. https://doi.org/10.1016/j.foodcont.2010.11.002

Qin, S., Wang, Y., Zhang, Q., Zhang, M., Deng, F., Shen, Z., Wu, C., Wang, S., Zhang, J., & Shen, J. (2014). Report of ribosomal RNA methylase gene erm(B) in multidrug-resistant Campylobacter coli. *Journal of Antimicrobial Chemotherapy*, *69*(4), 964–968. https://doi.org/10.1093/jac/dkt492

Ramires, T., de Oliveira, M. G., Kleinubing, N. R., de Fátima Rauber Würfel, S., Mata, M. M., Iglesias, M. A., Lopes, G. V., Dellagostin, O. A., & da Silva, W. P. (2020). Genetic diversity, antimicrobial resistance, and virulence genes of thermophilic Campylobacter isolated from broiler production chain. *Brazilian Journal of Microbiology*, *51*(4), 2021–2032. https://doi.org/10.1007/s42770-020-00314-0

Ravel, A., Hurst, M., Petrica, N., David, J., Mutschall, S. K., Pintar, K., Taboada, E. N., & Pollari, F. (2017). Source attribution of human campylobacteriosis at the point of exposure by combining comparative exposure assessment and subtype comparison based on comparative genomic fingerprinting. *PLoS ONE*, *12*(8), 1–21. https://doi.org/10.1371/journal.pone.0183790

Richards, P. J., Connerton, P. L., & Connerton, I. F. (2019). Phage biocontrol of campylobacter jejuni in chickens does not produce collateral effects on the gut microbiota. *Frontiers in Microbiology*, *10*(MAR), 1–10. https://doi.org/10.3389/fmicb.2019.00476

Rodríguez-Baño, J., Rossolini, G. M., Schultsz, C., Tacconelli, E., Murthy, S., Ohmagari, N., Holmes, A., Bachmann, T., Goossens, H., Canton, R., Roberts, A. P., Henriques-Normark, B., Clancy, C. J., Huttner, B., Fagerstedt, P., Lahiri, S., Kaushic, C., Hoffman, S. J., Warren, M., … Plant, L. (2021). Antimicrobial resistance research in a post-pandemic world: Insights on antimicrobial resistance research in the COVID-19 pandemic. *Journal of Global Antimicrobial Resistance*, *25*, 5–7. https://doi.org/10.1016/j.jgar.2021.02.013

Saint-Cyr, M. J., Haddad, N., Taminiau, B., Poezevara, T., Quesne, S., Amelot, M., Daube, G., Chemaly, M., Dousset, X., & Guyard-Nicodème, M. (2017). Use of the potential probiotic strain Lactobacillus salivarius SMXD51 to control Campylobacter jejuni in broilers. *International Journal of Food Microbiology*, *247*, 9–17. https://doi.org/10.1016/j.ijfoodmicro.2016.07.003

Salaheen, S., White, B., Bequette, B. J., & Biswas, D. (2014). Peanut fractions boost the growth of Lactobacillus casei that alters the interactions between Campylobacter jejuni and host epithelial cells. *Food Research International*, *62*, 1141–1146. https://doi.org/10.1016/j.foodres.2014.05.061

Shrestha, S., Wagle, B. R., Upadhyay, A., Arsi, K., Donoghue, D. J., & Donoghue, A. M. (2019). Carvacrol antimicrobial wash treatments reduce Campylobacter jejuni and aerobic bacteria on broiler chicken skin. *Poultry Science*, *98*(9), 4073–4083. https://doi.org/10.3382/ps/pez198

Silván, J. M., Mingo, E., Hidalgo, M., de Pascual-Teresa, S., Carrascosa, A. V., & Martinez-Rodriguez, A. J. (2013). Antibacterial activity of a grape seed extract and its fractions against Campylobacter spp. *Food Control*, *29*(1), 25–31. https://doi.org/10.1016/j.foodcont.2012.05.063

Silvan, J. M., Mingo, E., & Martinez-Rodriguez, A. J. (2017). Grape seed extract (GSE) modulates campylobacter pro-inflammatory response in human intestinal epithelial

cell lines. *Food and Agricultural Immunology*, *28*(5), 739–753. https://doi.org/10.1080/09540105.2017.1312292

Silvan, J. M., Pinto-Bustillos, M. A., Vásquez-Ponce, P., Prodanov, M., & Martinez-Rodriguez, A. J. (2019). Olive mill wastewater as a potential source of antibacterial and anti-inflammatory compounds against the food-borne pathogen Campylobacter. *Innovative Food Science and Emerging Technologies*, *51*, 177–185. https://doi.org/10.1016/j.ifset.2018.05.013

Smialek, M., Burchardt, S., & Koncicki, A. (2018). The influence of probiotic supplementation in broiler chickens on population and carcass contamination with Campylobacter spp. - Field study. *Research in Veterinary Science*, *118*(April 2017), 312–316. https://doi.org/10.1016/j.rvsc.2018.03.009

Smith-Palmer, A., Stewart, J., & Fyfe, L. (1998). Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Letters in Applied Microbiology*, *1987*, 118–122.

Steffan, S. M., Shakeri, G., Kehrenberg, C., Peh, E., Rohde, M., Plötz, M., & Kittler, S. (2022). Campylobacter Bacteriophage Cocktail Design Based on an Advanced Selection Scheme. *Antibiotics*, *11*(2), 1–16. https://doi.org/10.3390/antibiotics11020228

Stern, N. J., Svetoch, E. A., Eruslanov, B. V., Perelygin, V. V., Mitsevich, E. V., Mitsevich, I. P., Pokhilenko, V. D., Levchuk, V. P., Svetoch, O. E., & Seal, B. S. (2006). Isolation of a Lactobacillus salivarius strain and purification of its bacteriocin, which is inhibitory to Campylobacter jejuni in the chicken gastrointestinal system. *Antimicrobial Agents and Chemotherapy*, *50*(9), 3111–3116. https://doi.org/10.1128/AAC.00259-06

Stern, Norman J., Eruslanov, B. V., Pokhilenko, V. D., Kovalev, Y. N., Volodina, L. L., Perelygin, V. V., Mitsevich, E. V., Mitsevich, I. P., Borzenkov, V. N., Levchuk, V. P., Svetoch, O. E., Stepanshin, Y. G., & Svetoch, E. A. (2008). Bacteriocins reduce Campylobacter jejuni colonization while bacteria producing bacteriocins are ineffective. *Microbial Ecology in Health and Disease*, *20*(2), 74–79. https://doi.org/10.1080/08910600802030196

Tabashsum, Z., Peng, M., Salaheen, S., Comis, C., & Biswas, D. (2018). Competitive elimination and virulence property alteration of Campylobacter jejuni by genetically engineered Lactobacillus casei. In *Food Control* (Vol. 85). Elsevier Ltd. https://doi.org/10.1016/j.foodcont.2017.10.010

Taha-Abdelaziz, K., Astill, J., Kulkarni, R. R., Read, L. R., Najarian, A., Farber, J. M., & Sharif, S. (2019). In vitro assessment of immunomodulatory and anti-Campylobacter activities of probiotic lactobacilli. *Scientific Reports*, *9*(1), 1–15. https://doi.org/10.1038/s41598-019-54494-3

Tang, C., & Lu, Z. (2019). Health promoting activities of probiotics. *Journal of Food Biochemistry*, *43*(8), 1–16. https://doi.org/10.1111/jfbc.12944

Thanissery, R., Kathariou, S., & Smith, D. P. (2014). Rosemary oil, clove oil, and a mix of thyme-orange essential oils inhibit Salmonella and Campylobacterin vitro. *Journal of Applied Poultry Research*, *23*(2), 221–227. https://doi.org/10.3382/japr.2013-00888

Thung, T. Y., Lee, E., Mahyudin, N. A., Wan Mohamed Radzi, C. W. J., Mazlan, N., Tan, C. W., & Radu, S. (2020). Partial characterization and in vitro evaluation of a lytic bacteriophage for biocontrol of Campylobacter jejuni in mutton and chicken meat. *Journal of Food Safety*, *40*(2). https://doi.org/10.1111/jfs.12770

Turgis, M., Han, J., Caillet, S., & Lacroix, M. (2009). Antimicrobial activity of mustard essential oil against Escherichia coli O157:H7 and Salmonella typhi. *Food Control*, *20*(12), 1073–1079. https://doi.org/10.1016/j.foodcont.2009.02.001

Wagle, B. R., Donoghue, A. M., Shrestha, S., Upadhyaya, I., Arsi, K., Gupta, A., Liyanage, R., Rath, N. C., Donoghue, D. J., & Upadhyay, A. (2020). Carvacrol attenuates Campylobacter jejuni colonization factors and proteome critical for persistence in the chicken gut. *Poultry Science*, *99*(9), 4566–4577. https://doi.org/10.1016/j.psj.2020.06.020

Wagle, B. R., Upadhyay, A., Shrestha, S., Arsi, K., Upadhyaya, I., Donoghue, A. M., & Donoghue, D. J. (2019). Pectin or chitosan coating fortified with eugenol reduces Campylobacter jejuni on chicken wingettes and modulates expression of critical survival genes. *Poultry Science*, *98*(3), 1461–1471. https://doi.org/10.3382/ps/pey505

Wang, C., Zhou, H., Guo, F., Yang, B., Su, X., Lin, J., & Xu, F. (2020). Oral Immunization of Chickens with Lactococcus lactis Expressing cjaA Temporarily Reduces Campylobacter jejuni Colonization. *Foodborne Pathogens and Disease*, *17*(6), 366–372. https://doi.org/10.1089/fpd.2019.2727

Wang, Y., Zhang, M., Deng, F., Shen, Z., Wu, C., Zhang, J., Zhang, Q., & Shen, J. (2014). Emergence of multidrug-resistant Campylobacter species isolates with a horizontally acquired rRNA methylase. *Antimicrobial Agents and Chemotherapy*, *58*(9), 5405–5412. https://doi.org/10.1128/AAC.03039-14

Watkins, R. R., & Bonomo, R. A. (2020). Overview: The Ongoing Threat of Antimicrobial Resistance. *Infectious Disease Clinics of North America*, *34*(4), 649–658. https://doi.org/10.1016/j.idc.2020.04.002

Wieczorek, K., Bocian, Ł., & Osek, J. (2020). Prevalence and antimicrobial resistance of Campylobacter isolated from carcasses of chickens slaughtered in Poland – a retrospective study. *Food Control*, *112*, 107159. https://doi.org/10.1016/j.foodcont.2020.107159

Windiasti, G., Feng, J., Ma, L., Hu, Y., Hakeem, M. J., Amoako, K., Delaquis, P., & Lu, X. (2019). Investigating the synergistic antimicrobial effect of carvacrol and zinc oxide nanoparticles against Campylobacter jejuni. *Food Control*, *96*(May 2018), 39–46. https://doi.org/10.1016/j.foodcont.2018.08.028

Zampara, A., Sørensen, M. C. H., Elsser-Gravesen, A., & Brøndsted, L. (2017). Significance of phage-host interactions for biocontrol of Campylobacter jejuni in food. *Food Control*, *73*, 1169–1175. https://doi.org/10.1016/j.foodcont.2016.10.033

Zommiti, M., Almohammed, H., & Ferchichi, M. (2016). Purification and Characterization of a Novel Anti-Campylobacter Bacteriocin Produced by Lactobacillus curvatus DN317. *Probiotics and Antimicrobial Proteins*, *8*(4), 191–201. https://doi.org/10.1007/s12602-016-9237-7

5. Perspectivas Futuras

Em função dos resultados obtidos na proteômica, observou-se a importância de se avaliar a expressão de alguns genes relacionados à virulência de *C. jejuni*, sob diferentes condições de estresse ácido. Os resultados referentes a esses experimentos ainda não foram concluídos e analisados, muito em função da pandemia de Covid-19, que dificultou o acesso aos laboratórios e atrasou o andamento dos experimentos. Após a análise e interpretação criteriosa dos resultados obtidos, o manuscrito será submetido a um periódico de alto fator de impacto na área de Ciência e Tecnologia de Alimentos.

5.1 Expressão gênica de isolados de *Campylobacter jejuni* representativos da cadeia produtiva de frangos de corte do sul do Rio Grande do Sul

5.1.1 Seleção dos isolados

No total, a bacterioteca do Laboratório de Microbiologia de Alimentos da FAEM/UFPel, conta com 29 isolados de *C. jejuni*, distintos genotipicamente, provenientes de diferentes etapas da cadeira produtiva de frangos de corte. A fim de se obter um isolado representativo de cada etapa da cadeia produtiva (granja, abatedouro e cortes cárneos de frango), foram realizados experimentos para verificar quais isolados suportariam melhor o estresse ácido. Os isolados selecionados constam na Tabela 2.

Tabela 1. Identificação dos isolados de *Campylobacter jejuni* utilizados nos experimentos de estresse ácido

Isolado	Etapa da Cadeia
100	Cortes cárneos de frango
198	Abatedouro
230	Granja

5.1.2 Expressão Gênica

Os experimentos visam avaliar a transcrição dos genes *ciaB*, *cdtA*, *cdtB* e *cdtC*, sob interferência de diferentes valores de pH ácido. O RNA dos três isolados selecionados e da cepa *C. jejuni* ATCC 33291 foi extraído e quantificado. No total, obteve-se o RNA para sintetizar o cDNA (*cdtC)* de 16 amostras (3 isolados + ATCC, submetidos a 4 valores de pH), os quais foram submetidos à qPCR e os dados relativos a esse gene estão prontos para serem analisados (Figura 2).

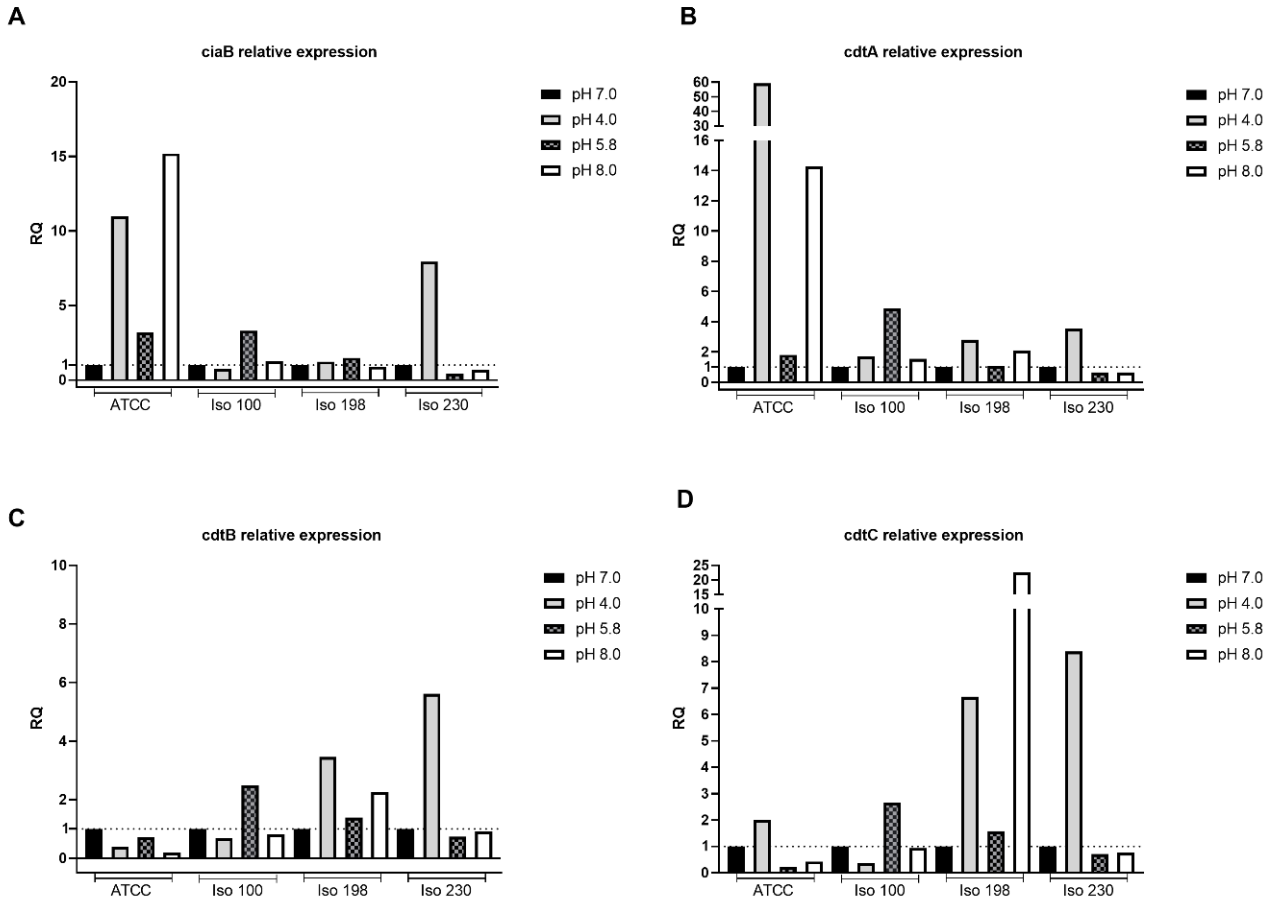

Figura 2. Gráficos gerados após a reação em cadeia da polimerase em tempo real, relativos às 16 amostras (3 isolados + controle, submetidos a 4 valores de pH), referentes à amplificação dos genes ciaB (A), cdtA (B), cdtB (C) e cdtC (D)

6. Considerações Finais

Os resultados obtidos no presente estudo confirmam a presença de um conjunto de proteínas que respondem especificamente ao estresse ácido em *C. jejuni* NCTC 11168. Embora as respostas ao estresse alcalino pareçam limitadas, pesquisas futuras, especialmente com foco no metabolismo de *C. jejuni*, incluindo quais aminoácidos e ácidos orgânicos são preferencialmente acumulados e degradados seria útil para entender melhor as respostas ao pH alcalino. De acordo com os dados obtidos na literatura, fica evidente o potencial antimicrobiano de óleos essenciais frente a *Campylobacter* termofílicos, sendo necessários mais estudos em busca de aplicações para o controle do patógeno ao longo da cadeia produtiva de frangos de corte.

REFERÊNCIAS BIBLIOGRÁFICAS

ABUOUN, M.; MANNING, G.; CAWTHRAW, S. A.; RIDLEY, A.; AHMED, I. H.; ASSENAAR, T. M.; NEWELL, D. G. Cytolethal distending toxin (CDT)- negative *Campylobacter jejuni* strains and anti-CDT neutralizing antibodies are induced during human infection but not during colonization in chickens. **Infection and Immunity**, v. 73, p. 3053-3062, 2005.

ALEMKA, A.; CLYNE, M.; SHANAHAN, F.; TOMPKINS, T.; CORCIONIVOSCHI, N.; BOURKE, B. Probiotic Colonization of the Adherent Mucus Layer of HT29MTXE12 Cells Attenuates *Campylobacter jejuni* Virulence Properties. **Infection and Immunity**, v. 78(6), p. 2812–2822, 2010.

ASAKURA, M.; SAMOSORNSUK, W.; TAGUCHI, M.; KOBAYASHI, K.; MISAWA, N.; KUSUMOTO, M.; NISHIMURA, K.; MATSUHISA, A.; YAMASAKI, S. Comparative analysis of cytolethal distending toxin (cdt) genes among *Campylobacter jejuni*, *C. coli* and *C. fetus* strains. **Microbiology Pathogenesis**, v. 42, p.174-183, 2007.

ASKOURA, M.; SARVAN, S.; COUTURE, J-F.; STINTZI, A. The *Campylobacter jejuni* Ferric Uptake Regulator Promotes Acid Survival and Cross-Protection against Oxidative Stress. s. **Infection and Immunity**, v. 84, p. 1287–1300, 2016.

ASKOURAA, M.; YOUNSB, M.; HEGAZY, W.A.H. Investigating the influence of iron on *Campylobacter jejuni* transcriptome in response to acid stress. **Microbial Pathogenesis**, v. 138, 103777, 2020.

BAILLON, M.L.; VAN VLIET, A.H.; KETLEY, J.M.; CONSTANTINIDOU, C.; PENN, C.W. An iron regulated alkyl hydroperoxide reductase (AhpC) confers aerotolerance and oxidative stress resistance to the microaerophilic pathogen *Campylobacter jejuni*. **Journal of Bacteriology**, v. 181, p. 4798-4804, 1999.

BANG, D. D.; NIELSEN, E. M.; SCHEUTZ, F.; PEDERSEN, K.; HANDBERG, K.; MADSEN, M. PCR Detection of seven virulence and toxin genes of *Campylobacter jejuni* isolates from Danish pigs and cattle and cytolethal distending toxin production of the isolates. **Journal of Applied Microbiology**, v. 94, p.1003-1014, 2003.

BEARSON, S.; BEARSON, B.; FOSTER, J.W. Acid stress responses in enterobacteria. **FEMS Microbiology Letters**, v. 147, p. 173-180, 1997.

BIRK, T.; KNOCHEL, S. Fate of food-associated bacteria in pork as affected by marinade, temperature, and ultrasound. **Journal of Food Protection**, v. 72, p. 549-555, 2009.

BOLTON, D. J. *Campylobacter* virulence and survival factors. **Food Microbiology**, v. 48, p. 99-108, 2015.

CAIN, J.A.; DALE, A.L.; NIEWOLD, P.; KLARE, W.P.; MAN, L.; WHITE, M.Y.; SCOTT, N.E.; CORDWELL, S.J. Proteomics reveals multiple phenotypes associated with *N*-linked glycosylation in *Campylobacter jejuni*. **Molecular & Cellular Proteomics**, v.18 (4), p. 715-734, 2019.

CHAISOWWONG, W.; KUSUMOTO, A.; HASHIMOTO, M.; HARADA, T.; MAKLON, K.; KAWAMOTO, K. Physiological characterization of *Campylobacter jejuni* under cold stresses conditions: its potential for public threat. Journal of **Veterinary Medical Science**, v. 74, p. 43-50, 2012.

EFSA. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. **EFSA Journal**, 2018.

EFSA (European Food Safety Authority). Analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses in the EU, 2008. **EFSA Journal**, v. 8, p. 1503-1602, 2010.

FOSTER, J.W. *Salmonella* acid shock proteins are required for the adaptive acid tolerance response. **Journal of Bacteriology**, v. 173, p. 6896–6902, 1991.

GANJI, L.; ALEBOUYEH, M.; SHIRAZI, M.H.; ZALI, M.R. Comparative transcriptional analysis for Toll-like receptors, inflammatory cytokines, and apoptotic genes in response to different cytolethal-encoding and noncoding isolates of *Salmonella enterica* and *Campylobacter jejuni* from food and human stool. **Microbial Pathogenesis**, v. 133, 103550, 2019.

GARÉNAUX, A.; JUGIAU, F.; JORGE, R.; DENIS, M.; FEDERIGHI, M.; RITZ, M. *Campylobacter jejuni* strains from different origins under oxidative stress conditions: effect of temperature. **Current Microbiology**, v.56, p. 293-297, 2008.

GUCCIONE, E.; HITCHCOCK, A.; HALL, S.; MULHOLLAND, F.; SHEARER, N.; VAN VLIET, A.H.; KELLY, D.J. Reduction of fumarate, mesaconate and crotonate by Mfr, a novel oxygen-regulated periplasmic reductase in *Campylobacter jejuni*. **Environmental Microbiology**, v. 12, p. 576–591, 2010.

GUCCIONE, E.J.; KENDALL, J.J.; HITCHCOCK, A.; GARG, N.; WHITE, M.A.; MULHOLLAND, F.; POOLE, R.K.; KELLY, D.J. Transcriptome and proteome dynamics in chemostat culture reveal how *Campylobacter jejuni* modulates metabolism, stress

responses and virulence factors upon changes in oxygen availability. **Environmental Microbiology**, v. 19(10), p. 4326–4348, 2017.

HALD, B.; SKOV, M.N.; NIELSEN, E.M.; RAHBEK, C.; MADSEN, J.J.; WAINØ, M.; CHRIÉL, M.; NORDENTOFT, S.; BAGGESEN, D.L.; MADSEN, M. *Campylobacter jejuni* and *Campylobacter coli* in wild birds on Danish livestock farms. **Acta Veterinaria Scandinavica**, v. 58, 2016.

HÄNEL, I.; MULLER, J.; MULLER, W.; SCHULZE, E. Correlation between invasion of Caco-2 eukaryotic cells and colonization ability in the chick gut in *Campylobacter jejuni*. **Veterinary Microbiology**, v. 101, p. 75–82, 2004.

HANNING, I.; JARQUIN, R.; SLAVIK, M. *Campylobacter jejuni* as a secondary colonizer of poultry biofilms. **Journal of Applied Microbiology**, v. 105, p. 1199-1208, 2008.

HU, L.; KOPECKO, D.J. Interactions of *Campylobacter* with eukaryotic cells: gut luminal colonisation and mucosal invasion mechanisms. In: Nachamkin, I., Blaser, M.J. (Eds.), Campylobacter, 2nd ed. **American Society for Microbiology Press**, Washington, p. 191–215, 2000.

HUGHES, C.S.; MOGGRIDGE, S.; MÜLLER, T.; SORENSEN, P.H.; MORIN, G.B.; KRIJGSVELD, J. Single-pot, solid-phase-enhanced sample preparation for proteomics experiments. **Nature Protocols**, v. 14, p. 68-85, 2019.

JANSSEN, R.; KROGFELT, K.A.; CAWTHRAW, S.A.; VAN PELT, W.; WAGENAAR, J.A.; OWEN, R.J. Host pathogen interactions in *Campylobacter* infections: the host perspective. **Clinical Microbiology Reviews**, v. 21, p. 505–518, 2008.

JOHN, D.A., WILLIAMS, L.K., KANAMARLAPUDI, V., HUMPHREY, T.J., WILKINSON, T.S. The bacterial species *Campylobacter jejuni* induce diverse innate immune responses in human and avian intestinal epithelial cells. **Frontiers in Microbiology**, v. 8, p. 1840, 2017.

KARLSSON, R.; SILES, L.G.; BOULUND, F.; STADLER, L.S.; SKOVBJERG, S.; KARLSSON, A.; DAVIDSON, M.; HULTH, S.; KRISTIANSSON, E.; MOORE, E.R.B. Review Proteotyping: Proteomic characterization, classification and identification of microorganisms – A prospectus. **Systematic and Applied Microbiology**, 2015.

KONKEL, M. E.; GRAY, S. A.; KIM, B. J.; GARVIS, S. G.; YOON, J. Identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* based on the *cadF* virulence gene and its product. **Journal Clinical Microbiology***,* v. 37, p. 510-517, 1999.

KOOLMAN, L.; WHYTE, P.; BURGESS, C.; BOLTON, D. Virulence gene expression, adhesion and invasion of *Campylobacter jejuni* exposed to oxidative stress (H_2O_2). **International Journal of Food Microbiology**, v. 220, p. 33-38, 2016.

LEVIN, R.E. *Campylobacter jejuni*: a review of its characteristics, pathogenicity, ecology, distribution, subspecies characterization and molecular methods of detection. **Food Biotechnology**, v.21, p. 271-347, 2007.

LIN, J.; LEE, I.S.; FREY, J.; SLONCZEWSKI, J.L.; FOSTER, J.W. Comparative analysis of extreme acid survival in *Salmonella* Typhimurium, *Shigella flexneri*, and *Escherichia coli*. **Journal of Bacteriology**, v. 177, p. 4097-4104, 1995.

LIVAK, K.J.; SCHMITTGEN, T.D. Analysis of Relative Gene Expression Data Using RealTime Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. **Methods**, v. 25, p. 402-408, 2001.

MACCALLUM, A.J.; HARRIS, D.; HADDOCK, G.; EVEREST, P.H. *Campylobacter jejuni* infected human epithelial cell lines vary in their ability to secrete interleukin-8 compared to in vitro-infected primary human intestinal tissue. **Microbiology**, v. 152, p. 3661–3665, 2006.

MINTMIER, B.; MCGARRY, J.M.; SPARACINO-WATKINS, C.E.; SALLMEN, J.; FISCHER-SCHRADER, K.; MAGALON, A.; MCCORMICK, J.R.; STOLZ, J.F.; SCHWARZ, G.; BAIN, D.J.; BASU, P. Molecular cloning, expression and biochemical characterization of periplasmic nitrate reductase from *Campylobacter jejuni*. **FEMS Microbiology Letters**, v. 365(16), 2018.

MONTEVILLE, M. R.; YOON, J. E.; KONKEL, M. E. Maximal adherence and invasion of INT 407 cells by *Campylobacter jejuni* requires the CadF outer membrane protein and microfilament reorganisation. **Microbiology**, v. 149, p. 153-165, 2002.

MOORE, J. E.; CORCORAN, D.; DOOLEY, J. S. G.; FANNING, S.; LUCEY, B.; MATSUDA, McDOWELL, D. A.; MÉGRAUD, F.; MILLAR, B. C.; O' MAHONY, R.; O'RIODAN, L; O' ROURKE, M.; RAO, J. R.; ROONEY, P. J.; SAILS, A.; WHYTE, P. *Campylobacter*. **Veterinary Research**, v. 36, p. 351-382, 2005.

PARKHILL, J.; WREN, B.W.; MUNGALL, K.; KETLEY, J.M.; CHURCHER, C.; BASHAM, D.; CHILLINGWORTH, T.; DAVIES, R.M.; FELTWELL, T.; HOLROYD, S.; JAGELS, K.; KARLYSHEV, A.V.; MOULE, S.; PALLEN, M.J.; PENN, C.W.; QUAIL, M.A.; RAJANDREAM, M-A.; RUTHERFORD, K.M.; VAN VLIET, A.H.M.; WHITEHEAD. S.; BARRELL, B.G. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. **Nature**, v. 403, p. 665-668, 2000.

PITTMAN, M.S.; ELVERS, K.T.; LEE, L.; JONES, M.A.; POOLE, R.K.; PARK, S.F.; KELLY, D.J. Growth of *Campylobacter jejuni* on nitrate and nitrite: electron transport to NapA and NrfA via NrfH and distinct roles for NrfA and the globin Cgb in protection against nitrosative stress. **Molecular Microbiology**, v. 63(2), p. 575–590, 2007.

POLI, V.F.S.; THORSEN, L.; OLESEN, I.; WIK, M.T.; JESPERSEN, L. Differentiation of the virulence potential of *Campylobacter jejuni* strains by use of gene transcription analysis and a Caco-2 assay. **International Journal of Food Microbiology**, v. 155, p. 60-68, 2012.

REID, A.N.; PANDEY, R.; PALYADA, K.; NAIKARE, H.; STINTZI, A. Identification of *Campylobacter jejuni* genes involved in the response to acidic pH and stomach transit. **Applied Environmental Microbiology**, v. 74, p. 1583–1597, 2008.

REPÉRANT, E.; LAISNEY, M.J.; NAGARD, B.; QUESNE, S.; ROUXEL, S.; LE GALL, F.; CHEMALY, M.; DENIS, M. Influence of enrichment and isolation media on the detection of *Campylobacter* spp. in naturally contaminated chicken samples. **Journal of Microbiological Methods**, v.128, p. 42–47, 2016.

RIVERA-AMILL, V.; KIM, B. J.; SESHU, J.; KONKEL, M. E. Secretion of the virulence associated *Campylobacter* invasion antigens from *Campylobacter jejuni* requires a stimulatory signal. **The Journal of Infectious Diseases**, v. 183, p. 1607-1616, 2001.

RODRIGUES, R.C.; BRONNEC, V.; TRESSE, O.; CAPPELIER, J.-M.; HADDAD, N. A review of *Campylobacter jejuni* pathogenesis: main virulence factors and their use as biomarkers. In: Bertucci, B.A. (Ed.), ***Campylobacter*** **Infections - Epidemiology, Clinical Management and Prevention**, 1sted. Nova Science Publishers, New York, p. 1–28, 2015.

RODRIGUES, R.C.; POCHERON, A-L.; CAPPELIER, J-M.; TRESSE, O.; HADDAD, N. An adapted *in vitro* assay to assess *Campylobacter jejuni* interaction with intestinal epithelial cells: Taking into stimulation with TNFα**. Journal of Microbiological Methods**, v. 149, p. 67-72, 2018.

ROMERO, M.R.; D'AGOSTINO, M.; ARIAS, A.P.; ROBLES., S.; CASADO., C.F.; ITURBE, L.O.; LERMA., O.G.; ANDREOU, M.; COOK, N. An immunomagnetic separation/loop-mediated isothermal amplification method for rapid direct detection of thermotolerant *Campylobacter* spp. during poultry production. **Journal of Applied Microbiology**, 120, 469-477, 2016.

SAINT-CYR, M.J.; HADDAD, N.; TAMINIAU, B.; POEZEVARA, T.; QUESNE, S.; AMELOT, M.; DAUBE, G.; CHEMALY, M.; DOUSSET, X.; GUYARD-NICODÈME, M. Use of the

potential probiotic strain *Lactobacillus salivarius* SMXD51 to control *Campylobacter jejuni* in broilers. **International Journal of Food Microbiology**, v. 247, p. 9-17, 2017.

SAMBROOK, J.; RUSSELL, D.W. **Molecular Cloning: A Laboratory Manual**. 3ª ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2001.

SELLARS, M.J.; HALL, S.J.; KELLY, D.J. Growth of *Campylobacter jejuni* supported by respiration of fumarate, nitrate, nitrite, trimethylamine-N-oxide, or dimethyl sulfoxide requires oxygen. **Journal of Bacteriology**, v. 184, p. 4187–4196, 2002.

SILVA, J.; LEITE, D.; FERNANDES, M.; MENA, C.; GIBBS, P.A.; TEIXEIRA, P. *Campylobacter* spp. as foodborne pathogen: a review. **Frontiers in Microbiology**, v. 2, p. 1-12, 2011.

SIMON, J.; KLOTZ, M.G. Diversity and evolution of bioenergetic systems involved in microbial nitrogen compound transformations. **Biochimica et Biophysica Acta BBA – Bioenergetics**, v. 1827, p. 114–135, 2013.

SMITH, J. L.; BAYLES, D. O. The contribution of cytolethal distending toxin to bacterial pathogenesis. **Critical Reviews in Microbiology**, v. 32, p. 227- 248, 2006.

TAHERI, N.; FÄLLMAN, M.; WAI, S.N.; FAHLGREN, A. Accumulation of virulence-associated proteins in *Campylobacter jejuni* Outer Membrane Vesicles at human body temperature. **Journal of Proteomics**, v. 195, p. 33–40, 2019.

TORTORA, G.J.; FUNKE, B.R.; CASE, CL. Microbiologia. 10. ed., Porto Alegre: Artmed, 2012.

URWIN, R.; MAIDEN, M. C. J. Multi-locus sequence typing: a tool for global epidemiology. **Trends in Microbiology**, v. 11, n. 10, p. 479-487, 2003.

VAN-DEUN, K.; HAESEBROUCK, F.; HENDRICKX, M.; FAVOREEL, H.; DEWULF, J.; CEELEN, L.; DUMEZ, L.; MESSENS, W.; LELEU, S.; VAN IMMERSAL, F.; DUCATELLE R.; PASMANS, F. Virulence properties of *Campylobacter jejuni* isolates of poultry and human origin. **Journal of Medical Microbiology**, v. 56, p. 1284-1289, 2007.

WEINGARTEN, R.A.; TAVEIRNE, M.E.; OLSON, J.W. The dual-functioning fumarate reductase is the sole succinate: quinone reductase in *Campylobacter jejuni* and is required for full host colonization. **Journal of Bacteriology**, v. 191, 5293–5300, 2009.

WINTER, J.; JAKOB, U. Beyond transcription – new mechanisms for the regulation of molecular chaperones. **Critical Reviews in Biochemistry and Molecular Biology**, v. 39, p. 297–317, 2004.

WHO (WORLD HEALTH ORGANIZATION). **The global view of campylobacteriosis: report of an expert consulation**. 2013. Disponível em: < https://apps.who.int/iris/handle/10665/80751 > Acesso em: 15 de jul. 2022.

WHO (WORLD HEALTH ORGANIZATION). ***Campylobacter***. 2020. Disponível em: http://www.who.int/mediacentre/factsheets/fs255/en/ Acesso em: 15 de jul. 2022.

YAN, S.S.; PENDRAK, M.L.; FOLEY, S.L.; POWERS, J.H. *Campylobacter* infection and Guillain-Barré syndrome: public health concerns from a microbial food safety perspective. **Clinical and Applied Immunology Reviews**, v. 5, p. 285-305, 2005.

YOUNG, K.T.; DAVIS, L.M.; DIRITA, V.J. *Campylobacter jejuni*: molecular biology and pathogenesis. **Nature Reviews Microbiology**, v. 5, p. 665–679, 2007.