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Volatile organic compounds produced by thermophilic and non-thermophilic *Campylobacter* spp.: Influence of growth phase and nutrient composition

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Abstract

Bacteria produce a plethora of volatile organic compounds (VOCs) into the atmosphere as a means of interacting with their habitats, as well as for intra- and interkingdom communication, and to survive and thrive in nature and inside their hosts. *Campylobacter*, which is commonly found in poultry and ruminants, has demonstrated remarkable endurance in aquatic settings, making it one of the world's most hazardous foodborne pathogens that kill thousands of humans every year. In the present work, the VOCs released by both thermophilic (*C. jejuni*, *C. coli*, and *C. lari*) and non-thermophilic (*C. fetus*) *Campylobacter* of clinical significance, which were influenced by nutrient composition (media) and growth phase, were profiled using an active sampling approach, with active charcoal adsorbent and gas chromatography-mass spectrometry. Alcohols and ketones were detected only in the thermophilic *Campylobacter* strains, *C. jejuni* subsp. *jejuni* (1-heptadecanol; 1,8-nonadien-3-ol; 3,7,11-trimethyl-3-dodecanol; 1s,4R,7R,11R-1,3,4,7-tetramethyltricyclo [5.3.1.0(4,11)] undec-2-en-8-one; and isophorone), *C. coli* (isophorone and 3,5-bis(1,1-dimethylethyl)-phenol), and *C. lari* (3,7,11-trimethyl-3-dodecanol and 1s,4R,7R,11R-1,3,4,7-tetramethyltricyclo [5.3.1.0(4,11)] undec-2-en-8-one) between early stationary and stationary growth phases. The non-thermophilic *C. fetus* produced only a range of unidentified compounds that need to be investigated further in the future. The results from the principal component analysis showed distinctive clustering of VOC markers between the four bacteria taxa and within the strains of *C. jejuni*, including distinguishable groupings of VOCs throughout the growth phase of each bacterium and between different culture media. This demonstrates the influence of bacterial growth and media composition on the volatilome of the studied *Campylobacter* spp. Overall, the VOC profiling of these foodborne pathogens, under influencing factors of growth phase and media, paves the way for future rapid identification of *Campylobacter* spp. in food matrices.

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Introduction

The genus *Campylobacter* is known for the challenge in its detection, isolation, and identification in the laboratory due to its physiological characteristics such as its non-glycolytic diet, the low oxygen required to grow (obligate microaerophile), and ability to enter a coccoid form during non-favourable growth conditions, known as a viable but non-culturable (VBNC) state (Chlebicz and Slizewska, 2018; Ricke *et al*., 2019). Despite the constraints of culturing this bacterium *in vitro*, *Campylobacter* spp. are ubiquitous and colonise the intestinal tracts of many wild and domesticated animals (Gundogdu and Wren, 2020). These foodborne pathogens only require a small number of bacterial cells to cause human infection (Gundogdu and Wren, 2020), which is primarily transmitted through the consumption of poultry meat, particularly contaminated broiler chicken (*Gallus gallus*) (Chlebicz and Slizewska, 2018; Tang *et al.*, 2020).

Campylobacter jejuni subsp. *jejuni* is the major species of the taxon, and recognised as the leading cause of human gastroenteritis worldwide (WHO, 2013; Bessa *et al.*, 2020). Kaakoush *et al.* (2015) predicted that infections caused by *Campylobacter* (mainly *C. jejuni*) will likely pose a threat to public health worldwide in the years to come. Another

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review article on human campylobacteriosis (Igwaran and Okoh, 2019) noted reports of an increase in *Campylobacter* infections globally, as well as the increase in some *Campylobacter* species as one of the primary diarrhoea-causing organisms and susceptibility to antimicrobial agent resistance.

The infection triggered by *C. jejuni* is mainly characterised by mild to severe symptoms, including watery to bloody diarrhoea, abdominal pain, fever, vomiting, and dehydration (WHO, 2013; Chlebicz and Slizewska, 2018). However, the symptoms only last about five to seven days (Chlebicz and Slizewska, 2018), which could contribute to numerous unreported cases (Bessa *et al.*, 2020). Nonetheless, severe post-infection illnesses have been associated with *C. jejuni*, such as Guillain-Barré syndrome (GBS), which is an autoimmune disease characterised by weakness of the limbs and paralysis. Other post-infection diseases associated with *Campylobacter* include Miller-Fisher syndrome, which is a variant of GBS; musculoskeletal conditions such as reactive arthritis (ReA); and chronic gastrointestinal sequelae such as irritable bowel syndrome (IBS) (WHO, 2013; Igwaran and Okoh, 2019).

Campylobacter coli is the second-most-reported species of human campylobacteriosis. It is very similar to *C. jejuni* and is frequently isolated together with *C. jejuni* from contaminated poultry and faeces samples (Igwaran and Okoh, 2019; Mortada *et al.*, 2020). The third-most clinically significant *Campylobacter* species is *C. lari*. Although *C. lari* is infrequently associated with human infections, it has been isolated from patients with diarrhoea (Mikulic *et al.*, 2016; Igwaran and Okoh, 2019). Another clinically important species is *C. fetus* subsp. *fetus*, which is an opportunistic human pathogen that primarily affects patients with weakened immune systems (Wagenaar *et al.*, 2014; Igwaran and Okoh, 2019).

The standard test for food safety mostly utilises culturing, polymerase chain reaction (PCR), and enzyme-linked immunosorbent assay (ELISA) methods, which are time-consuming, labour-intensive, and prone to false negative results (Buss *et al.*, 2019; Ricke *et al.*, 2019; Ferone *et al.*, 2020). This is particularly true for *Campylobacter* detection in food samples, due to the nature of VBNC, and its low concentration in food, which causes a high false-negative detection rate when using the standard culturing method in a laboratory (Chai *et al*., 2007; Chlebicz and Slizewska, 2018; Ricke *et al*., 2019). Therefore, in order to ensure food quality and safety, and in considering the seriousness of the risks posed by *Campylobacter* spp. (WHO, 2013), as well as the limitations of the current culturing methods to detect these pathogens in food samples, particularly for time-sensitive goods such as fresh meat and dairy (Ferone *et al.*, 2020), a new methodology for *Campylobacter* detection in food which is simpler, rapid, highly sensitive, and non-culture-dependent is urgently required (WHO, 2013; Ricke *et al.*, 2019). In this regard, several studies have demonstrated that bacteria produce distinctive volatile organic compounds (VOCs) during culturing, which creates a single VOC-emission pattern that could potentially be developed into a unique metabolic biomarker for bacterial identification with high specificity and simplicity in real-time (Chen *et al.*, 2017; Smart *et al.*, 2019; Kos *et al.*, 2022).

The bacterial VOCs are detected using gas chromatographic techniques, particularly gas chromatography-mass spectrometry (GC-MS), which is generally preceded by sample preparation (Chen *et al.*, 2017). For instance, solid-phase microextraction (SPME) has been largely used to sample microbial VOCs (Chen *et al.*, 2017; Garcia-Alcega *et al.*, 2017). However, owing to the lack of standardised methods, the nature of the targeted sample matrix (*i.e*., the composition and the analytes' properties), and current experimental requirements and constraints (*i.e*., costs and instrumental issues, *etc*.), the selection of the most suitable sampling procedure to serve the purpose of VOC fingerprinting is challenging (Wang *et al.*, 2016; Franchina *et al.*, 2019). Nevertheless, the sorption tube approach seems to be a suitable strategy that is widely adopted for gas sampling, as it could retain a range of compatible VOCs at ambient temperature that is subjected to the interaction between the analytes and the sorbent material, where the retained content could be desorbed for subsequent measurement (Wang *et al.*, 2016; Franchina *et al.*, 2019). This technique is simple, inexpensive, and more appropriate for thermally unstable compounds when desorption *via* a compatible solvent system is applied (Matysik *et al.*, 2009; Ramirez *et al.*, 2010; Garcia-Alcega *et al.*, 2017).

Despite the recent research interests drawn by bacterial VOCs (Chen *et al.*, 2017; Fang *et al.*, 2021), only a few studies regarding VOC emissions of *Campylobacter* spp. have been published. In one study, the whole-cell fatty acids of *C. fetus* subsp. *venerealis* were investigated, and revealed

hexadecanoic acid as the most significant volatile emitted by the bacterium (Brondz and Olsen, 1991). Additionally, the profile of VOCs emitted by *C. jejuni* in contaminated human stools, and by *C. jejuni* and *C. coli* in chicken faeces, have also been previously explored (Probert *et al.*, 2004; Garner *et al.*, 2008). Furthermore, the prevalence of alcohols was observed in broth inoculated with *C. jejuni* after 20 h of incubation (Nunez-Carmona *et al.*, 2019). However, the VOCs emitted by a particular bacterial species vary considerably when subjected to different types of media, growth phases, and bacterial strains (Wang *et al.*, 2016; Chen *et al.*, 2017; Reese *et al*., 2020). Therefore, the present work attempted to investigate the variation of VOCs emitted by the four clinically significant species of *Campylobacter* (*C. jejuni*, *C. coli*, *C. lari*, and *C. fetus*) in different culture media and growth phases. The present work would be a cornerstone for further development of VOC-based sensing technology for the detection of foodborne *Campylobacter* spp. in foods.

Materials and methods

Strains, culture media, and laboratory maintenance

Five bacterial strains of *Campylobacter* were targeted: *C. jejuni* subsp. *jejuni* (ATCC 33291 and ATCC 29428), *C. coli* (ATCC 43478), *C. fetus* subsp. *fetus* (ATCC 27374), and *C. lari* (ATCC 35221). These strains were acquired from Microbiologics (Minnesota, USA), and revived by inoculating the swab into sterile Bolton broth (BB; Oxoid, UK) supplemented with 5% of lysed horse blood. The lysed horse blood was prepared from fresh horse blood, which was purchased from the Faculty of Veterinary Medicine, Universiti Putra Malaysia. The fresh horse blood was subjected to multiple rounds of freezing and thawing until no separation into two layers was observed (Chai *et al.*, 2007). The inoculated media were incubated at 37°C for 48 h in a sealed universal bottle with 20% headspace, creating the microaerophilic condition.

After 48 h of incubation, the turbid broth was aliquoted into sterilised Eppendorf tubes (1.5 mL volume), and centrifuged at 6,500 rpm for 2 min. The supernatant was discarded, and 0.5 mL of double-strength Brain Heart Infusion broth (BHI; Himedia, India) and 0.5 mL of glycerol were added into the vials, then thoroughly mixed by pipetting. The glycerol stocks were then divided into two parts to be maintained in freezers at -20 and -80°C.

The strains of *Campylobacter* were revived from glycerol stocks for the headspace VOC sampling as follows: 100 µL were transferred from the glycerol vial to a sterile Bolton broth tube supplemented with 5% of lysed horse blood, and incubated for 48 h at 37°C in a sealed universal bottle with 20% headspace. After growth, the inoculum was streaked onto modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA; Oxoid, UK) in triplicate, and incubated under a microaerophilic atmosphere generated by Campygen 3.5 L (Oxoid, Basingstoke Hampshire, UK) at 37°C for 48 h. Five to ten colonies were then transferred to a test tube containing BB, and gently mixed. After that, 1 mL of the bacterial suspension was pipetted into a 100-mL screw-cap bottle (Duran, Mainz, Germany) containing 80 mL of culture media, leaving 20 mL of headspace volume for headspace VOC sampling.

Headspace-volatile organic compound sampling and experimental conditions

Briefly, the VOCs were collected by forcing the headspace gas in the bacterial culture bottle to pass through an ORBO-activated coconut charcoal (100/50 mg) sorbent tube (6 × 70 mm) at a fixed rate *via* a pump. The sorbent tube was removed, and sealed pending solvent extraction and subsequent GC-MS analysis. Preliminary tests were performed to optimise the volume of headspace air that needed to pass through the adsorbent matrix to ascertain detectable signals for subsequent investigations. The apparatus used for the sampling was assembled inside an anaerobic jar with a lit candle to generate the microaerophilic condition. The sample bottle was equipped with a GL 45 screw cap with two ports (Duran, Mainz, Germany). Both inlet and outlet ports were connected by Tygon tubes (4.0 mm internal diameter). The inlet tube was connected to a portable battery air pump DC-900 (Xilong, China) that operated at a flow rate of approximately 2 L/min, and the sorbent tube was inserted in the outlet tube. The duration of the headspace-air sampling was based on the length of each incubation period.

The *C. jejuni* strains (ATCC 29428 and 33291) were inoculated individually in two different high-protein media: BB was supplemented with 5% of lysed horse blood, and double-strength BHI with an initial concentration of approximately 10^4 MPN/mL. All the samples were incubated at 37°C, and the headspace was sampled at three time points: 12, 24, and 48 h. These time points were selected to sample

the VOCs produced by exponential (12 h) and stationary cells (24 and 48 h), as indicated by previous work on *Campylobacter* sp. growth rate in culture broth (Wright *et al*., 2009; Battersby *et al*., 2016). The other *Campylobacter* species, *C. coli*, *C. fetus* subsp. *fetus*, and *C. lari*, were inoculated only in BB supplemented with 5% of lysed horse blood and with an initial bacterial concentration of approximately 10^5, 10^3, and 10^3 MPN/mL, respectively. They were also incubated at 37°C with continuous active headspace sampling for 12, 24, and 48 h.

Solvent desorption and gas chromatography-mass spectrometry

After the headspace air sampling, the sorbent tube was removed, and the sorbent content was transferred into a 1.5 mL vial. Then, 1 mL of carbon disulphide (Merck, Darmstadt, Germany) was added to the vial for the desorption of the VOCs. The vial was thoroughly shaken using a vortex mixer, and the extract was further transferred into another clean GC vial before it was sent for immediate GC-MS analysis (Matysik *et al.*, 2009; Ramirez *et al.*, 2010).

The carbon disulphide extract was analysed *via* GC-MS (Brand: Shimadzu; Model: QP2010 ULTRA 7890A GC/MS Agilent 5975) using the capillary column RTX-5 MS (length: 30 m; internal diameter: 0.25 mm; and film thickness: 0.25 µm). Preliminary tests were conducted to identify the optimal temperature program, owing to the complexity of chromatographic separations that depend on influencing factors such as the analyte characteristics (Wang *et al.*, 2016; Franchina *et al.*, 2019). As a result by using a splitless mode throughout the process, the GC operated under the following settings: the temperature of the injection port was 230°C; the oven was held at 40°C for 1 min; then, the temperature was raised to 280°C at a rate of 6°C/min; the final temperature was held for 1 min; a solvent cut of 3 min was performed; and the length of the analysis was 25 min per sample.

For the samples containing BHI broth, the temperature program was refined due to noisy chromatographic resolutions produced during initial trials using the same program for Bolton broth samples. Hence, after a series of trial-and-error tests, the following program was applied to BHI samples: the temperature of the injection port was increased to 250°C; the oven was held at 40°C for 1 min; the temperature was then raised to 250°C at a rate of 6°C/min; and the final temperature was held for 11 min, leading to a 46-min run time. For both settings, a full scan mode was used for the mass spectrometry analysis.

For reference purposes, a series of *n*-alkane standards (Merck, Darmstadt, Germany) was run under the same GC-MS operating conditions; the retention index (RI) calculated from the alkane runs was an independent system, thus was reproducible (Jahiddin and Low, 2020).

Enumeration of bacterial count

Before the incubation periods (12, 24, and 48 h) and after the headspace sampling, 1 mL of the enrichment broth was collected from the sample for a three-tube Most Probable Number (MPN) analysis. The aliquot was serially diluted using fresh BB with 5% of lysed horse blood in a 10-fold dilution until a maximum of 10^{-9}, depending on the expected growth phase, and incubated in triplicate at 37°C for 48 h under microaerophilic conditions (Chai *et al*., 2007).

The tubes were then checked for turbidity. The tubes that became turbid were plated on mCCDA, and incubated for 48 h at 37°C under the microaerophilic condition to confirm the presence of *Campylobacter* in the tubes. The concentration of bacterial cells in the tube (MPN/mL) was then calculated with an MPN calculator developed by the United States Environmental Protection Agency (USEPA, 2013). The growth curves of *Campylobacter* spp. (MPN/mL) for all replicates throughout the experiment were also plotted.

Data analysis

The VOCs were identified by taking into account both mass spectra and RI values. The mass spectrum of each compound was matched against the reference spectrum available in the National Institute of Standards and Technology (NIST) mass spectral library, where only those with a high degree of similarity (> 70%) were further considered (Koo *et al.*, 2013). In addition to library mapping, the RI provided complementary information to aid the identification of VOCs. The normalised retention time (non-isothermal Kovats retention index) was calculated according to Van Den Dool and Kratz (1963). For further identification, the calculated RIs were compared with the reference RIs of the same kind of stationary phase; those with 5% or more of a relative standard deviation were rejected (Reese *et al.*, 2020).

Moreover, two criteria were adopted to determine the putative volatiles that were most likely to be produced by the microorganism with relative consistency. The first criterion was that the compound must not be present in the background (blank media) at the same time point (incubation period) as the bacterial samples, and the second criterion was that the compound must be detected in at least two samples throughout the repetitions. Only filtered VOCs were considered for statistical comparison of bacterial strains and growth phases. The relative peak area of these selected compounds was subjected to principal component analysis (PCA) to explore the similarities/dissimilarities in VOC patterns that may be associated with the bacterial strains, the production of VOC during different growth phases, and culture media. The calculation was performed using the SAS JMP 15 software.

Results

Identification of putative bacterial volatiles produced by Campylobacter

Many peaks were detected during the GC-MS analysis of the bacterial samples and the blank media (negative control). The five *Campylobacter* strains assessed were found to produce a total of 344 identified VOCs during growth in either Bolton or Brain Heart Infusion broth, including overlapping VOCs within the strains. Of the VOCs identified, only 35.4% remained after removing the VOCs found in the blank samples (*i.e*., VOCs produced by the culture medium), and 18.8% of these VOCs were detected in at least two biological replicates (criterion 2). The VOCs that met both criteria 1 and 2 were designated as potential VOC biomarkers.

A total of 16 VOC biomarkers were linked to *Campylobacter* spp., as presented in Figure 1. These VOCs were detected and related to the following groups: alcohol (3), ketone (2), ester (1), phenyl alkene (1), phenol (1), and unidentified (8) (Figure 1). Some potential biomarkers were labelled as "not identified" due to poor matching with the mass spectrum and retention index. These unidentified chemicals, on the other hand, were labelled based on their mass spectrum and the computed RI for comparison between samples.

It is worth noting that the VOC biomarkers generated by *Campylobacter* spp. cultivated in Bolton and BHI broth did not coincide. When grown in Bolton broth, both strains of *C. jejuni* (ATCC 29428 and ATCC 33291) produced 1s,4R,7R,11R-1,3,4,7-tetramethyltricyclo [5.3.1.0(4,11)] undec-2-en-8-one (ketone); when cultured in BHI broth, they produced 1-heptadecanol (alcohol) (Figure 1).

While both *C. jejuni* strains shared only one VOC biomarker when grown in Bolton broth, *C. jejuni* ATCC 29428 shared several with *C. lari* ATCC 35221: 3,7,11-trimethyl-3-dodecanol (alcohol), 1s,4R,7R,11R-1,3,4,7-tetramethyltricyclo [5.3.1.0(4,11)] undec-2-en-8-one (ketone), undec-2-en-8-one (ketone), and unidentified compound 29. The unidentified VOC compound 29 was also discovered to be produced by *C. fetus* ATCC 27374. Isophorone (ketone) was produced by *C. jejuni* ATCC 33291 and *C. coli* 43478. In terms of VOC biomarkers, *C. coli* did not have any similarities with *C. fetus* or *C. lari* (Figure 1).

Bacterial growth

The growth curves of *Campylobacter* spp. cultured in both Bolton and BHI broths were determined by the MPN enumeration approach at 0, 12, 24, and 48 h of incubation. The growth curve was then plotted as shown in Figure 2. All but *C. jejuni* 33291 in BHI broth showed exponential growth in the first 12 h of incubation, indicating an exponential growth phase, which was then followed by a significant deceleration in the growth rate at 12 h to 24 and 48 h, indicating a late exponential phase that entered the early stage of the stationary growth phase. For *C. jejuni* ATCC 33291 grown in BHI broth, the exponential phase lasted another 12 h until the growth rate began to decline at 24 h (Figure 2d).

Volatile organic compounds emitted by Campylobacter species during different growth phases

The majority of the identified potential VOC markers were discovered after 12 h of incubation, indicating that active VOC generation occurred during the late exponential and early stationary phases of *Campylobacter* spp. in laboratory culture conditions (Table 1). In general, alcohol was detected in *C. jejuni* cultures after 24 h; 1-heptadecanol, which was exclusively detected in *C. jejuni* BHI cultures, was also detected after 12 h (Table 1). At 48 h, the stationary phase was dominated by volatile ketones, 1s,4R,7R,11R-1,3,4,7-tetramethyltricyclo [5.3.1.0(4, 11)] undec-2-en-8-one, and isophorone (Table 1).

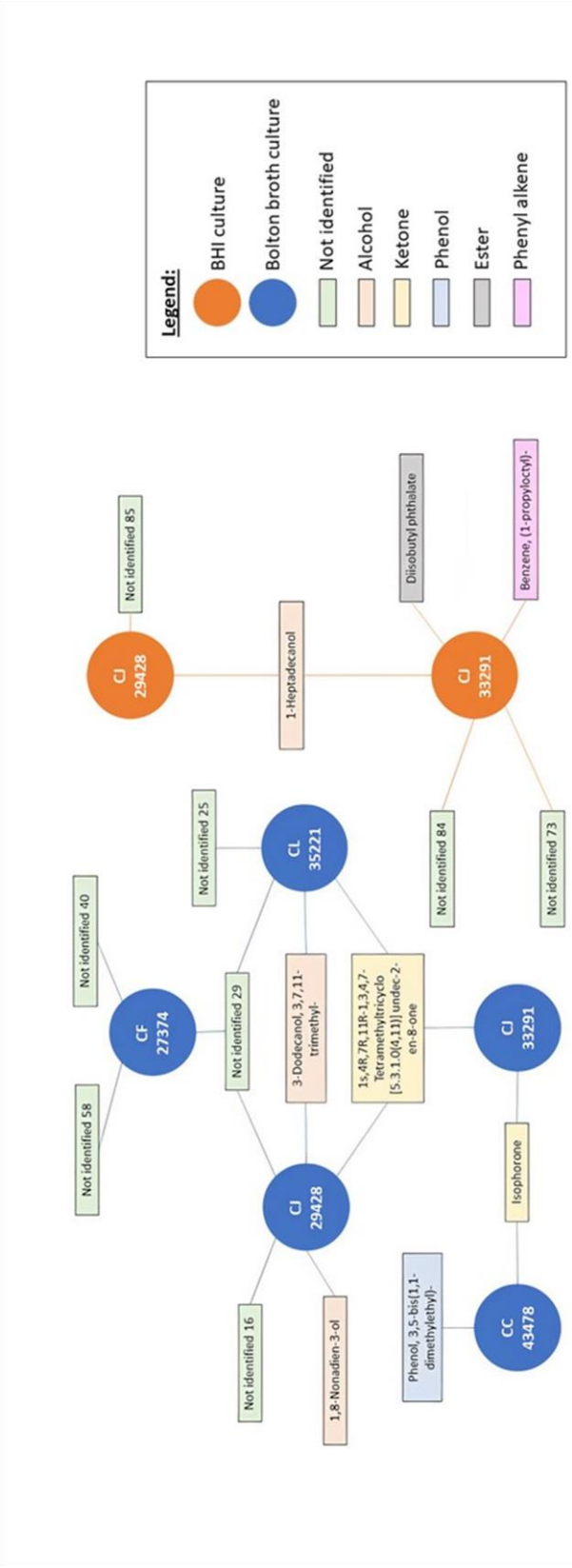

Figure 1. VOC biomarkers produced by *C. jejuni* (CJ) ATCC 29428, *C. jejuni* ATCC 33291, *C. coli* (CC) ATCC 43478, *C. lari* (CL) ATCC 35221, and *C. fetus* (CF) ATCC 27374 cultured in Bolton and BHI broths for maximum 48 h.

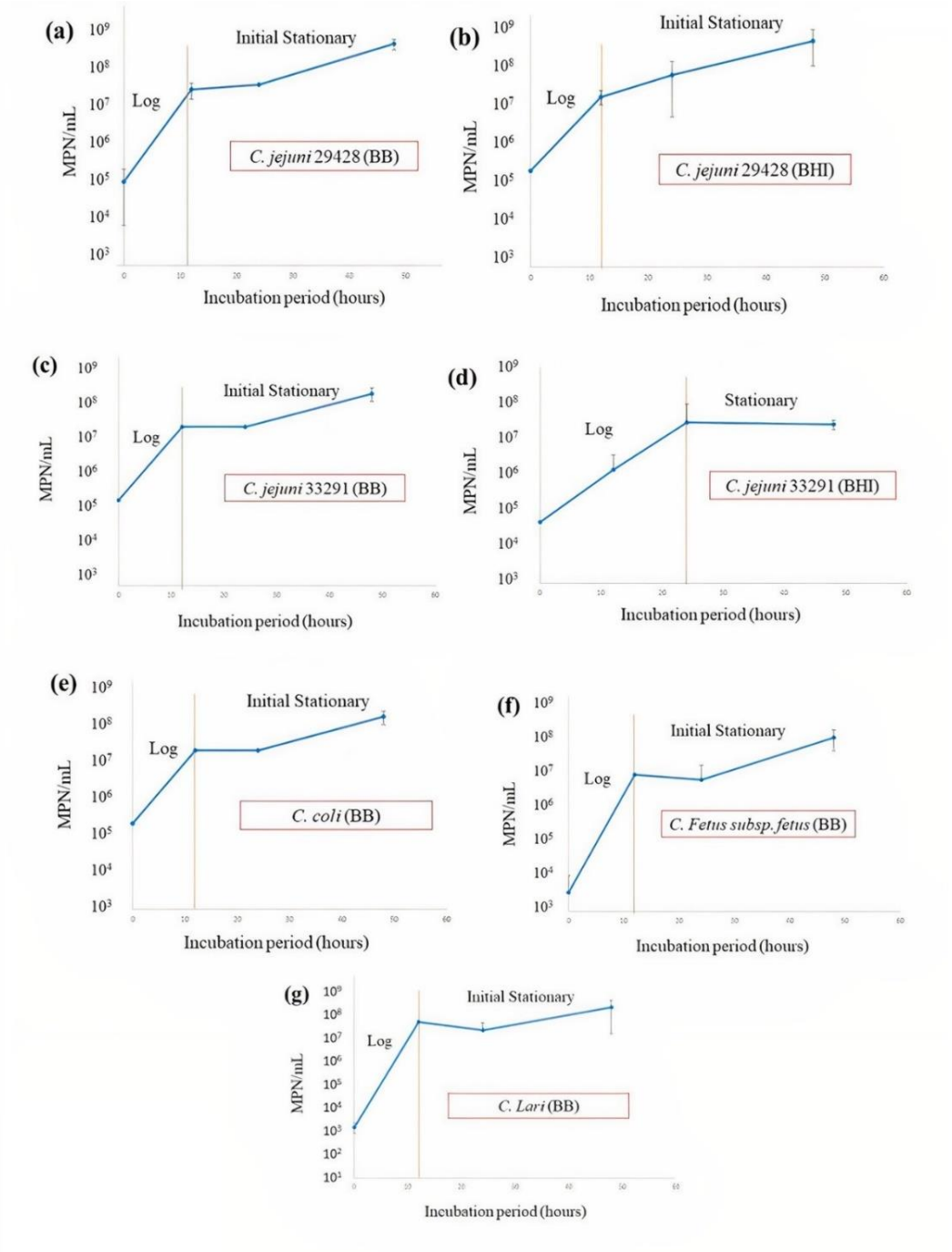

Figure 2. Growth curves in MPN (Most Probable Number) per mL of *Campylobacter* spp. **(a)** *C. jejuni* ATCC 29428 in Bolton broth (BB); **(b)** *C. jejuni* ATCC 29428 in BHI; **(c)** *C. jejuni* ATCC 33291 in BB; **(d)** *C. jejuni* ATCC 33291 in BHI; **(e)** *C. coli* ATCC 43478; **(f)** *C. fetus* subsp. *fetus* ATCC 27374; and **(g)** *C. lari* ATCC 35221, over incubation periods of 0, 12, 24, and 48 h throughout all repetitions. Error bars represent the standard deviation calculated within the MPN/mL of all five repetitions at each time point.

Table 1. Relative peak area (average) of bacterial volatile organic compounds detected at 12, 24, and 48 h of incubation at 37°C.

Strain	Media	Class	Volatile Organic Compound	Relative Peak Area		
				Exponential	Stationary	
				12 h	24 h	48 h
***C. jejuni* subsp. *jejuni* ATCC 29428**	Bolton broth	Alcohol	1,8-nonadien-3-ol	0	0.1075	0
		Alcohol	3,7,11-trimethyl-3-dodecanol	0	0.102	0.0458*
		Ketone	1s,4R,7R,11R-1,3,4,7-tetramethyltricyclo [5.3.1.0(4,11)] undec-2-en-8-one	0.0478	0	0.0592
		Unknown	not identified - 29	0	0	0.122
		Unknown	not identified - 16	1.324	0	0
	BHI broth	Alcohol	1-heptadecanol	0	0.0416	0.3796
		Ketone	isophorone	0	0	0.2128*
		Unknown	not identified - 85	0	0	0.0589
***C. jejuni* subsp. *jejuni* ATCC 33291**	Bolton broth	Ketone	isophorone	0	0	0.2319
		Ketone	1s,4R,7R,11R-1,3,4,7-tetramethyltricyclo [5.3.1.0(4,11)] undec-2-en-8-one	0	0.0345	0
	BHI broth	Alcohol	1-heptadecanol	0	0.0226	0.0314*
		Ketone	isophorone	0	0	0.2991*
		Ester	diisobutyl phthalate	0.5166*	0.0647	0.0671
		Phenyl Alkene	benzene, (1-propyloctyl)-	0	0.03625	0
		Unknown	not identified - 73	0.1580	0	0
		Unknown	not identified - 84	0.1994	0	0
Campylobacter coli	Bolton broth	Ketone	isophorone	0	0	0.2770
		Phenol	3,5-bis(1,1-dimethylethyl)- phenol	0	0	5.80
Campylobacter fetus* subsp. *fetus	Bolton broth	Unknown	not identified - 29	25.79	15.28	0
		Unknown	not identified - 40	0	16.55	0
		Unknown	not identified - 58	0	2.26	0
		Unknown	not identified - 42	0	5.07	0
Campylobacter lari	Bolton broth	Alcohol	3,7,11-trimethyl-3-dodecanol	0	0	6.25
		Ketone	1s,4R,7R,11R-1,3,4,7-tetramethyltricyclo [5.3.1.0(4,11)] undec-2-en-8-one	0	0	6.64
		Unknown	not identified - 25	0	10.40	0
		Unknown	not identified - 29	0	0	13.3

(*) VOC detected in only one bacterial sample.

Multivariate analysis of bacterial VOCs profile

Only the detected potential bacterial VOCs underwent PCA. The score plots in Figure 3 show the VOC's variability, which was most likely due to changes in the incubation time for the *Campylobacter* strains examined. The score plots demonstrated a similar clustering pattern that is highly linked with incubation period variation (*i.e*., growth phase), implying changes in VOC patterns between the growth phases. The degree of dispersion within a cluster, on the other hand, indicated VOC reproducibility, where larger levels of within-cluster variance were seen when the number of identified VOCs varied.

For example, when *C. jejuni* 29428 (BB) was considered alone, there was a clear distinction in the VOCs collected at different incubation periods, as denoted by the exponential (12 h), early stationary (24 h), and stationary (48 h) phases, as shown in Figure 2a, where the within-cluster distance reflects the dissimilarity in measured VOCs among the replicates. Due to the time-dependent VOC emissions from the media composition, the blank media of each interval was relatively close to its respective growth phase cluster; additionally, only a few compounds (filtered putative VOC markers) were responsible to distinguish between bacterial volatiles and matrix emissions.

Furthermore, it appeared reasonable to model the relationship between VOCs and the targeted factor by blocking the other factors, *i.e*., exploring the variation pattern of all strains and culture media based on each growth phase to facilitate interpretation and further understanding of the variation in VOC patterns. The score plot depicts the relationship between the profile of *C. jejuni* strains cultivated in two different culture media and *Campylobacter* spp. over the growth phases, with each point representing a bacterial sample of a specific strain and culture media in a reduced dimension described by PC1 and PC2 (Figure 3). VOCs were identified only in *C. jejuni* ATCC 29428 (BB), *C. jejuni* ATCC 33291 (BHI), and *C. fetus* strains during the exponential phase (12 h). There was also a significant separation between them, with both main components accounting for 67.1% of the total variance (PC1 + PC2). Component 1 (26.4%) revealed that the *C. fetus* was separated from the thermotolerant species (*C. jejuni* strains and *C. lari*) after a 24-h interval. However, *C. jejuni* ATCC 33291(BB), *C. jejuni* ATCC 29428 (BHI), and *C. lari* overlapped in the centre of the plot, but *C. jejuni* ATCC 29428 (BB) and *C. jejuni* ATCC 33291 (BHI) had unique VOC profiles that separated them from the others in the PC space (Figure 4). The first two primary components revealed that most bacterial strains were fairly segregated from one another after 48 h of growth. However, *C. lari* and *C. jejuni* ATCC 29428 (BB) had stronger association with VOC profiles that overlapped.

Additionally, the profiles of volatiles emitted by the two strains of *C. jejuni* cultured in BB and BHI were distinguishable in the plots over the incubation intervals (Figure 4). At the exponential phase (12 h), *C. jejuni* 29428 (BB) and *C. jejuni* 33291(BHI) were separated from each other with PC1 (41.4%). A similar pattern was observed during 24 h, however, the samples of different strains and media (*C. jejuni* 29428-BHI and *C. jejuni* 33291-BB) overlapped in the centre, as previously mentioned. At the last interval of the stationary phase, the bacterial samples of *C. jejuni* 33291 (BHI) and *C. jejuni* 29428 (BB) were closer yet distinct, and the difference within the strains of the same medium was clearer.

Discussion

The measurement results of bacterial VOCs depended greatly on the analytical methods adopted for fingerprinting, especially the gas sampling strategy. Although the charcoal-based matrix is a sufficiently suitable adsorbent for a wide range of compounds (Matysik *et al.*, 2009), one of the major drawbacks of using the charcoal adsorbent used in the present work was the loss of volatiles, which could be caused by the solvent dilution factor, lesser affinity with polar compounds, and overload of the sorbent matrix (Matysik *et al.*, 2009; Ramirez *et al.*, 2010; Garcia-Alcega *et al.*, 2017). These might explain the detection of less putative VOC markers in this study as compared to other reported studies that used different adsorbents (Nunez-Carmona *et al.*, 2019) and techniques such as thermal desorption (Ramirez *et al.*, 2010; Reese *et al.*, 2020). However, the charcoal adsorbent was proven to perform sufficiently well to detect the most predominant bacterial volatiles that were commonly reported by other researchers, such as alcohols and ketones.

In the present work, alcohols were the most commonly detected VOCs in *Campylobacter* spp., which were produced mainly in the early stationary and stationary growth phases (Table 1). The detected

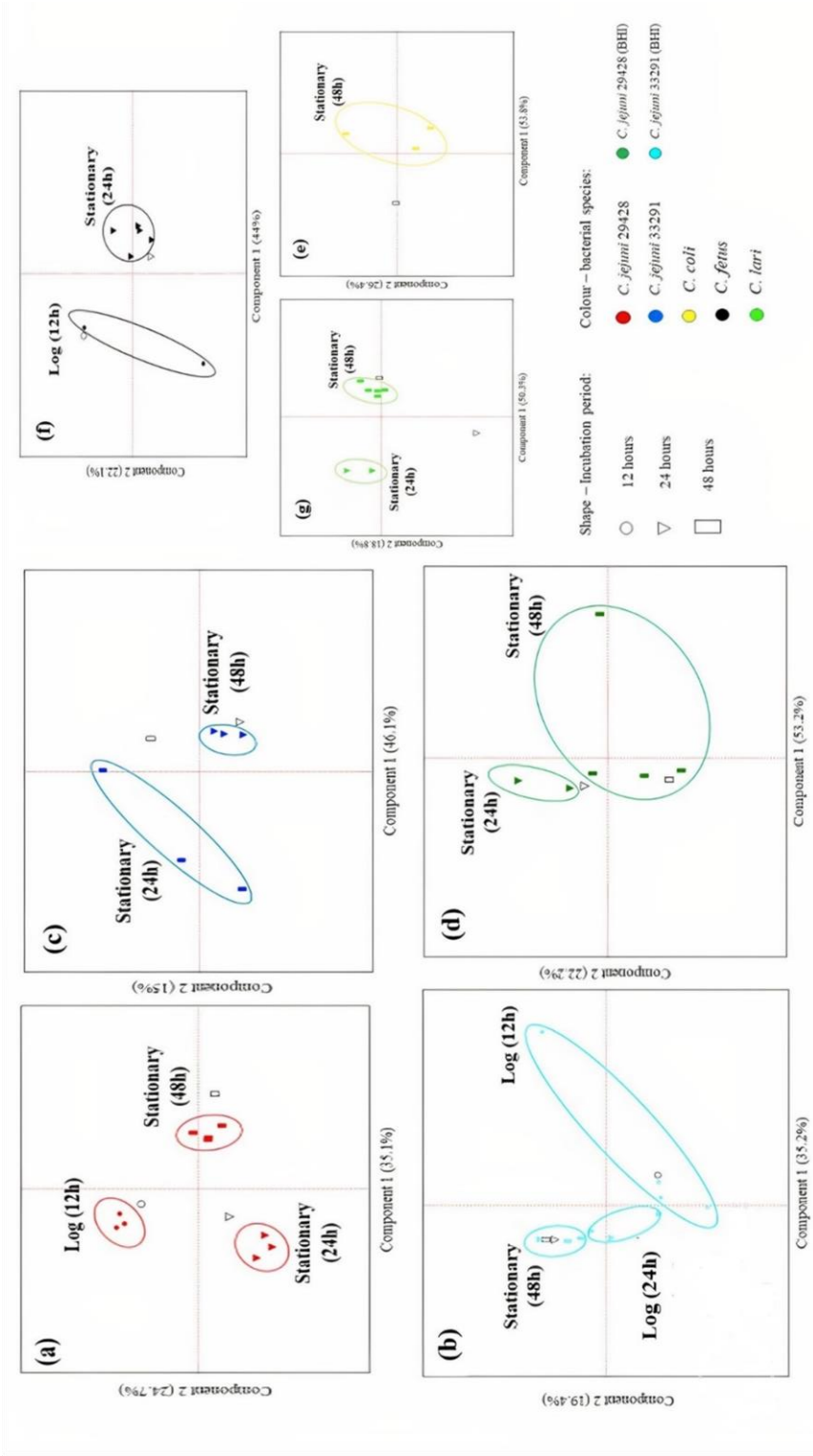

Figure 3. PCA score plots of each strain of *Campylobacter* at all incubation intervals with detected putative VOCs. **(a)** *C. jejuni* 29428 (BB); **(b)** *C. jejuni* 33291 (BHI); **(c)** C. *jejuni* 33291 (BB); **(d)** *C. jejuni* 29428 (BHI); **(e)** *C. coli*; **(f)** *C. fetus*; and **(g)** *C. lari*. Each point represents a bacterial sample with detected VOC-markers; non-coloured shape represents blank media (negative control).

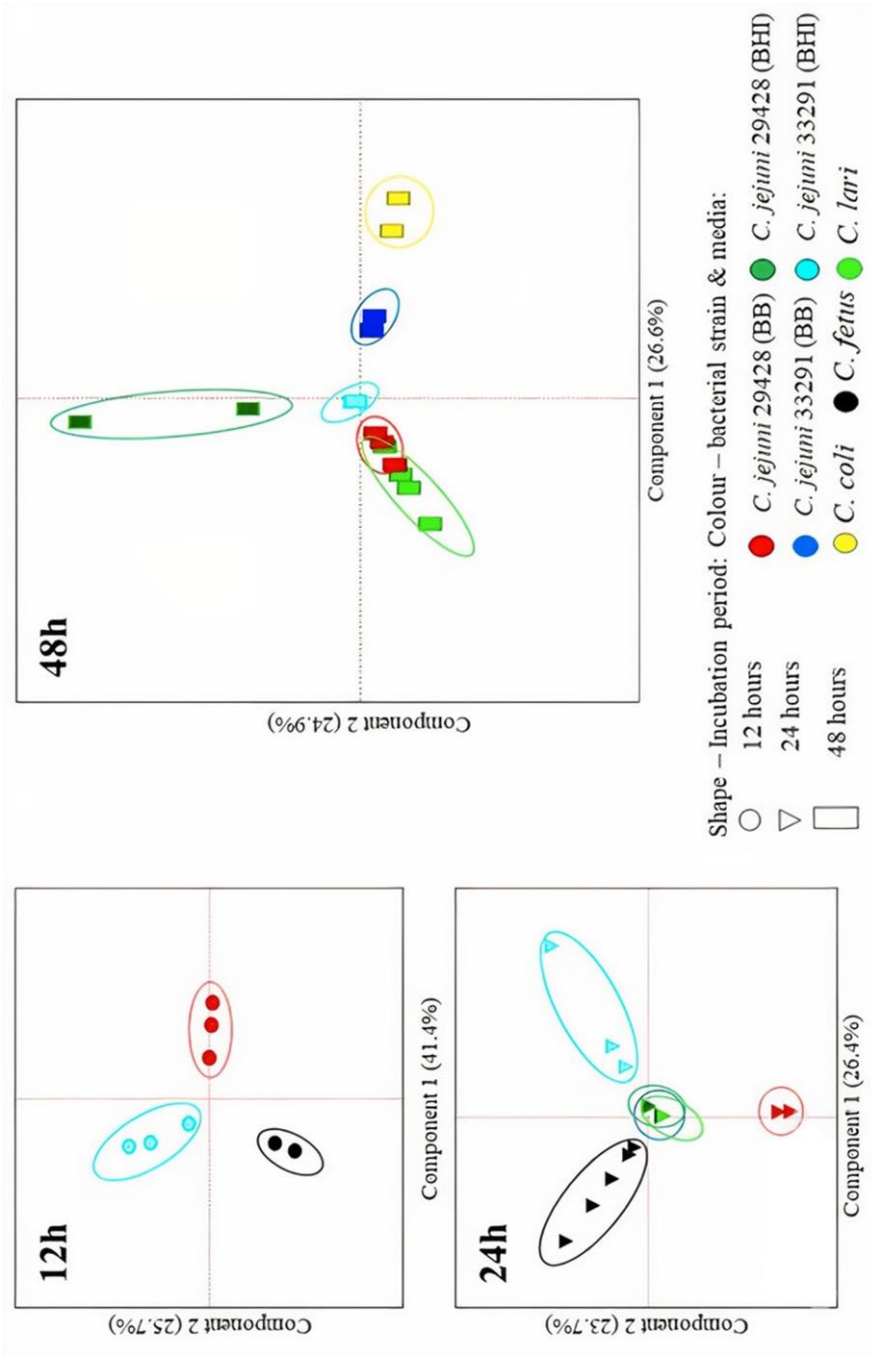

Figure 4. PCA score plots of all bacterial samples divided by each incubation period (12, 24, and 48 h). Each point represents a bacterial sample with its detected VOC markers; strains absent in one of the intervals mean that no putative VOC markers were identified in these strains in that interval.

alcohol-based VOCs include fatty alcohols 3,7,11-trimethyl-3-dodecanol and 1-heptadecanol, as well as the unsaturated alcohol 1,8-nonadien-3-ol (Figure 1). Previously, Probert *et al.* (2004) reported the presence of alcohol compounds in the stools of patients contaminated with *C. jejuni,* and Garner *et al.* (2008) identified methyl alcohol as one of the discriminant VOCs of chicken faeces contaminated with *C. jejuni* and *C. coli*. Moreover, Nunez-Carmona *et al*. (2019) reported the presence of the fatty alcohol (9E)-9-hexadecen-1-ol among other mostly saturated alcohol compounds in the bacterial samples of *C. jejuni* (ATCC 33560) cultivated in BHI broth after 20 h of incubation.

The probable pathways for bacterial synthesis of fatty alcohol and long-chain alcohols are through the hydrogenation of methyl esters of fatty acids (Kelm and Wickett, 2017), as well as by the β- or α-oxidation of fatty acid by-products (Audrain *et al.*, 2015). For instance, the production of fatty alcohols in *E. coli* begins with a thioesterase-mediated conversion of a fatty acyl-ACP (acyl carrier protein) to a free fatty acid. The free fatty acid is then converted to fatty acyl-CoA by a fatty acyl-CoA synthase. The resulting fatty acyl-CoA can subsequently be metabolised *via* the β-oxidation route or reduced to its corresponding fatty alcohol through the generation of its corresponding fatty aldehyde in an NADPH-dependent fatty acyl-CoA reductase-catalysed process (Fillet and Adrio, 2016).

While the roles of 3,7,11-trimethyl-3-dodecanol in bacteria are yet to be explored, Hu *et al.* (2023) recently reported 3,7,11-trimethyl-3-dodecanol as a discriminant VOC of Wuchang rice, influencing its flavour and producing a milder odour. On the other hand, 1,8-nonadien-3-ol has already been reported to be emitted by *Pseudomonas putida* as an antimicrobial volatile against plant pathogens (Sheoran *et al.*, 2015), and 1-heptadecanol was found to be one of the antifungal volatiles emitted by *Pseudomonas* spp., and isolated from canola and soybean (Fernando *et al.*, 2005). The roles of 1,8-nonadien-3-ol and 1-heptadecanol in *Pseudomonas* spp. may indicate that these metabolites have similar roles in *Campylobacter* spp. in inhibiting the growth of other bacteria and fungi. Also, according to Schulz-Bohm *et al.* (2017), bacterial VOCs are not only known for their negative effects on competitors; they also increase bacterial virulence factors and promote the growth of distant nutrient-limited bacteria. However, more studies are necessary to better understand the specific roles of these alcohol compounds in *Campylobacter* sp.

The two most predominantly isolated volatile ketones emitted by *Campylobacter* spp. in the present work were isophorone and 1s,4R,7R,11R-1,3,4,7-tetramethyltricyclo [5.3.1.0(4,11)] undec-2-en-8-one (Figure 1 and Table 1). The ketones were found to be emitted by the thermophilic *Campylobacter* (*C. jejuni*, *C. coli*, and *C. lari*) in both Bolton and Brain Heart Infusion broths (Table 1).

Although these specific ketones were not previously associated with *Campylobacter* spp., other researchers have already linked ketone compounds to the VOC profile of *Campylobacter* (Probert *et al.*, 2004; Garner *et al.*, 2008). Nunez-Carmona *et al.* (2019) reported that 2-undecanone was present in *C. jejuni*-inoculated BHI broth samples but not in the negative control (non-inoculated media).

Isophorone is an unsaturated cyclic ketone (Kim *et al.*, 2019), and its probable metabolic pathway is through the oxidation of large cyclic alkanes and cyclic alcohols, for instance, by the oxidation of cyclododecane into cyclododecanone that has already been observed in *Rhodococcus ruber* (Cheng *et al.*, 2002), and through a bienzymatic cascade for the oxyfunctionalisation of cycloalkanes to cyclic ketones (Wu *et al*., 2023). This volatile can be naturally found in cranberries (Kim *et al.*, 2019), honey (Karabagias, 2018), and beetle pheromone (Vidal *et al.*, 2019). Also, it has already been reported by Schulz and Dickschat (2007) as a volatile emitted by the cyanobacteria *Oscillatoria perornata*. However, the specific metabolic pathways and roles of isophorone in *Campylobacter* spp. have yet to be studied.

On the other hand, 1s,4R,7R,11R-1,3,4,7-tetramethyltricyclo [5.3.1.0(4,11)] undec-2-en-8-one is a terpenoid of the sesquiterpene family with a molecule of oxygen as the ketone functional group. According to Rudolf *et al*. (2021), even though bacterial terpenoids are rare, nearly all bacteria can produce the C5 building blocks required for terpenoid biosynthesis. Gabrielsen *et al*. (2004) had previously reported that *C. jejuni* exclusively uses the non-mevalonate pathway, starting from pyruvate and D-glyceraldehyde 3-phosphate *via* 1-deoxy-d-xylulose 5-phosphate to produce terpenoids. Sesquiterpenoids are derived from farnesyl diphosphate (FPP), which is also synthesised through the non-mevalonate

pathway and later converted into several farnesene isomers through the enzyme terpene synthase (TS) (Rudolf *et al*., 2021).

1s,4R,7R,11R-1,3,4,7-tetramethyltricyclo [5.3.1.0(4,11)] undec-2-en-8-one has been reported as one of the volatiles of lignin isolated from oil palm's empty fruit (Hidayati *et al.*, 2020). Bacterial sesquiterpene ketones have an important antibiotic role as a bacterial secondary metabolite (Zhao *et al.*, 2008), for instance, as an antibiotic agent against *Bacillus subtilis* (Rudolf *et al*., 2021). However, more studies are necessary to understand whether 1s,4R,7R,11R-1,3,4,7-tetramethyltricyclo [5.3.1.0(4,11)] undec-2-en-8-one has a similar role as an antibiotic agent against competitors in *Campylobacter* spp.

The VOC profiling of other clinically important *Campylobacter* spp., such as *C. coli*, *C. fetus* subsp. *fetus*, and *C. lari* is a pioneering step towards the characterisation of the volatiles of these pathogens. Isophorone and 3,5-bis(1,1-dimethylethyl)-phenol were discriminant volatiles emitted by *C. coli*. Meanwhile, 3,7,11-trimethyl-3-dodecanol and 1s,4R,7R,11R-1,3,4,7-tetramethyltricyclo [5.3.1.0(4,11)] undec-2-en-8-one was identified as biomarkers of *C. lari*. However, the profile of *C. fetus* consisted of only non-identified compounds, and despite their unconfirmed identity, these compounds were important to demonstrate the distinction of *C. fetus* among other species. Hence, future research should be able to widen the identification of the volatiles of these foodborne bacteria such as by employing more sensitive VOC-analytical methods.

The VOC marker profiles of all four bacterial strains assessed in the present work exhibited variations. Specifically, *C. jejuni* subsp. *jejuni*, *C. lari*, and *C. fetus* subsp. *fetus* showed significant variations at each stage of their growth. This was further illustrated through the application of PCA (Figure 3), which revealed distinct clustering of each bacterial strain across the investigated growth phases at three intervals. For instance, 3,7,11-trimethyl-3-dodecanol and 1-heptadecanol were only detected within 24 and 48 h. This implies that these VOCs may be related to the secondary metabolism of the bacteria, which is triggered by environmental conditions (*i.e*., competition) and usually appears within the late log and stationary phases (Wang *et al.*, 2016).

Based on PCA (Figure 4), distinct groupings of VOC markers were observed for *C. jejuni* between the two studied strains of the bacterium (*C. jejuni* ATCC 29428 and *C. jejuni* ATCC 33291). These findings corroborated other studies on the specificity of VOC markers and its potential application for rapid bacterial identification (Chen *et al.*, 2017; Milanowski *et al*., 2019; Reese *et al.*, 2020).

It is noteworthy to highlight that the PCA score plots (Figure 4) show a clear distinction between the strains of *C. jejuni* compared to different culture media at each interval (BB and BHI). The composition of the media was likely the primary factor that influenced the production of distinctive volatiles within the same bacterial strain (Wang *et al.*, 2016). For instance, the emission of 1,8-nonadien-3-ol and 3,7,11-trimethyl-3-dodecanol by *C. jejuni* ATCC 29428 cultivated in BB (Table 1) depended on the availability of fatty acids (Audrain *et al.*, 2015), which were more abundant in BB due to the supplementation of lysed horse blood.

Conclusion

The study on the variation in the VOCs emitted by the various clinically important *Campylobacter* spp., including *C. jejuni* subsp. *jejuni*, *in vitro*, *via* active sampling with an activated charcoal sorbent, revealed the culture- and growth phase-associated volatile profiles of thermophilic and non-thermophilic *Campylobacter* spp. The findings provided insight into the potential roles of bacterial VOCs in their growth, and survival in various niches in the environment and their host. The findings may also provide a baseline for the development of a VOC-based detection method for the fastidious *Campylobacter* spp. Nevertheless, in-depth studies are required to identify the unknown VOCs detected in the present work. Also, it will be meaningful to investigate other influencing factors, such as the coculturing of *Campylobacter* spp. with other bacterial populations, to fully understand the mechanisms and role of bacterial VOCs.

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References

Audrain, B., Farag, M. A., Ryu, C. and Ghigo, J. 2015. Role of bacterial volatile compounds in bacterial biology. FEMS Microbiology Reviews 39(2): 222-233.

Battersby, T., Walsh, D., Whyte, P. and Bolton, D. J. 2016. *Campylobacter* growth rates in four different matrices: Broiler caecal material, live birds, Bolton broth, and Brain heart infusion broth. Infection Ecology and Epidemiology 6: 31217.

Bessa, M. C., Haas, S., Ramos, R. C., Rocha, R., Voss-Rech, D., Rebelatto, R., ... and Vaz, C. S. L. 2020. Survey of *Campylobacter* in foods implicated in foodborne diseases in Southern Brazil. Revista do Instituto de Medicina Tropical de São Paulo 62: e90.

Brondz, I. and Olsen, I. 1991. Multivariate analyses of cellular fatty acids in bacteroides, *Prevotella*, *Porphyromonas*, *Wolinella*, and *Campylobacter* spp. Journal of Clinical Microbiology 29(1): 183-189.

Buss, J. E., Cresse, M., Doyle, S., Buchan, B. W., Craft, D. W. and Young, S. 2019. *Campylobacter* culture fails to correctly detect *Campylobacter* in 30% of positive patient stool specimens compared to non-cultural methods. European Journal of Clinical Microbiology and Infectious Diseases 38(6): 1087-1093.

Chai, L. C., Robin, T., Ragavan, U. M., Gunsalam, J. W., Bakar, F. A., Ghazali, F. M., … and Kumar, M. P. 2007. Thermophilic *Campylobacter* spp. in salad vegetables in Malaysia. International Journal of Food Microbiology 117: 106-111.

Chen, J., Tang, J., Shi, H., Tang, C. and Zhang, S. 2017. Characteristics of volatile organic compounds produced from five pathogenic bacteria by headspace-solid phase micro-extraction/gas chromatography-mass spectrometry. Journal of Basic Microbiology 57(3): 228-237.

Cheng, Q., Thomas, S. and Rouviere, P. 2002. Biological conversion of cyclic alkanes and cyclic alcohols into dicarboxylic acids: Biochemical and molecular basis. Applied Microbiology and Biotechnology 58: 704-711.

Chlebicz, A. and Slizewska, K. 2018. *Campylobacter*iosis, salmonellosis, yersiniosis, and listeriosis as zoonotic foodborne diseases: A review. International Journal Environmental Research Public Health 15(5): 863.

Fang, S., Liu, S., Song, J., Huang, Q. and Xiang, Z. 2021. Recognition of pathogens in food matrixes based on the untargeted *in vivo* microbial metabolite profiling *via* a novel SPME/GC × GC-QTOFMS approach. Food Research International 142: 110213.

Fernando, W. G. D., Ramarathnam, R., Krishnamoorthy, A. S. and Savchuk, S. C. 2005. Identification and use of potential bacterial organic antifungal volatiles in biocontrol. Soil Biology and Biochemistry 37: 955-964.

Ferone, M., Gowen, A., Fanning, S. and Scanell, A. G. M. 2020. Microbial detection and identification methods: Bench top assays to omics approaches. Comprehensive Reviews in Food Science and Food Safety 19(6): 3106-3129.

Fillet, S. and Adrio, J. L. 2016. Microbial production of fatty alcohols. World Journal of Microbiology and Biotechnology 32(9): 152.

Franchina, F. A., Purcaro, G., Burklund, A., Beccaria, M. and Hill, J.E. 2019. Evaluation of different adsorbent materials for the untargeted and targeted bacterial VOC analysis using GC×GC-MS. Analytica Chimica Acta 1066: 146-153.

Gabrielsen, M., Rohdich, F., Eisenreich, W., Gräwert, T., Hecht, S., Bacher, A. and Hunter, W. N. 2004. Biosynthesis of isoprenoids: A bifunctional IspDF enzyme from *Campylobacter jejuni*. European Journal of Biochemistry 271(14): 3028-3035.

Garcia-Alcega, S., Nasir, Z. A., Ferguson, R., Whitby, C., Dumbrel, A. J., Colbeck, I., … and Coulon, F. 2017. Fingerprinting outdoor air environment using microbial volatile organic compounds (MVOCs) - A review. Trends in Analytical Chemistry 86: 75-83.

Garner, C. E., Smith, S., Elviss, N. C., Humphrey, T. J., White, P. W., Ratcliffe, N. M. and Probert, C. S. 2008. Identification of *Campylobacter*

infection in chickens from volatile faecal emissions. Biomarkers 13(4): 413-421.

Gundogdu, O. and Wren, B. W. 2020. Microbe profile: *Campylobacter jejuni* - Survival instincts. Microbiology 166(3): 230-232.

Hidayati, S., Satyajaya, W. and Fudholi, A. 2020. Lignin isolation from black liquor from oil palm empty fruit bunch using acid. Journal of Materials Research and Technology 9(5): 11382-11391.

Hu, S., Ren, H., Song, Y., Liu, F., Qian, L., Zuo, F. and Meng, L. 2023. Analysis of volatile compounds by GCMS reveals their rice cultivars. Scientific Reports 13(1): 7973.

Igwaran, A. and Okoh, A. I. 2019. Human Campylobacteriosis: A public health concern of global importance. Heliyon 5(11): e02814.

Jahiddin, F. S. A. and Low, K. H. 2020. Evaluation of terpene variability in the volatile oils from *Zingiber officinale* using chemometrics. Current Analytical Chemistry 16(6): 695-702.

Kaakoush, N. O., Castaño-Rodríguez, N., Mitchell, H. M. and Man, S. M. 2015. Global epidemiology of *Campylobacter* infection. Clinical Microbiology Reviews 28(3): 687-720.

Karabagias, I. K. 2018. A targeted metabolomic procedure for the identification of isophorone related compounds in honey. Journal of Plant Biochemistry and Physiology 6: 1.

Kelm, G. R., and Wickett, R. R. 2017. The role of fatty acids in cosmetic technology. In Ahmad, M. U. (eds). Fatty Acids, p. 385-404. United States: AOCS Press.

Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., … and Bolton, E. E. 2019. PubChem 2019 update: Improved access to chemical data. Nucleic Acids Research 47(D1): D1102–D1109.

Koo, I., Kim, S. and Zhang, S. 2013. Comparative analysis of mass spectral matching-based compound identification in gas chromatography-mass spectrometry. Journal of Chromatography A 1298: 132-138.

Kos, R., Brinkman, P., Neerincx, A. H., Paff, T., Gerritsen, M. J., Lammers, A., … and Maitland-van der Zee, A. H. 2022. Targeted exhaled breath analysis for detection of *Pseudomonas aeruginosa* in cystic fibrosis patients. Journal of Cystic Fibrosis 21(1): e28-e34.

Matysik, S., Herbarth, O. and Mueller, A. 2009. Determination of microbial volatile organic compounds (MVOCs) by passive sampling onto charcoal sorbents. Chemosphere 76(1): 114-119.

Mikulic, M., Humski, A., Njari, B., Ostovic, M., Duvnjak, S. and Cvetnic, Z. 2016. Prevalence of thermotolerant *Campylobacter* spp. in chicken meat in Croatia and multilocus sequence typing of a small subset of *Campylobacter jejuni* and *Campylobacter coli* isolates. Food Technology and Biotechnology 54(4): 475-481.

Milanowski, M., Zloch, M., Ratiu, I. A., Pomastowski, P., Ligor, T., De Martinis, B. S. and Buszewski, B. 2019. Profiling of VOCs released from different salivary bacteria treated with non-lethal concentrations of silver nitrate. Analytical Biochemistry 578: 36-44.

Mortada, M., Cosby, D. E., Shanmugasundaram, R. and Selvaraj, 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.

Nunez-Carmona, E., Abbatangelo, M. and Sberveglieri, V. 2019. Innovative sensor approach to follow *Campylobacter jejuni* development. Biosensors: 9(1): 8.

Probert, C., Jones, P. and Raticliffe, N. 2004. A novel method for rapidly diagnosing the causes of diarrhea. Gut 53(1): 58-61.

Ramirez, N., Cuadras, A., Rovira, E., Borrull, F. and Marce, R. M. 2010. Comparative study of solvent extraction and thermal desorption methods for determining a wide range of volatile organic compounds in ambient air. Talanta 82(2): 719-727.

Reese, K. L., Rasley, A., Avila, J. R., Jones, A. D. and Frank, M. 2020. Metabolic profiling of volatile organic compounds (VOCs) emitted by the pathogens *Francisella tularensis* and *Bacillus anthracis* in liquid culture. Scientific Reports 10: 9333.

Ricke, S. C., Feye, K. M., Chaney, W. E., Shi, Z., Pavlidis, H. and Yang, Y. 2019. Developments in rapid detection methods for the detection of foodborne *Campylobacter* in the United States. Frontiers in Microbiology 9: 3280.

Rudolf, J. D., Alsup, T. A., Xu, B. and Li, Z. 2021. Bacterial terpenome. Natural Product Reports 38(5): 905-980.

Schulz, S. and Dickschat, J. S. 2007. Bacterial volatiles: The smell of small organisms. Natural Product Reports 24(4): 814-842.

Schulz-Bohm, K., Martín-Sánchez, L. and Garbeva, P. 2017. Microbial volatiles: Small molecules with an important role in intra-and inter-kingdom interactions. Frontiers in Microbiology 8: 2484.

Sheoran, N., Valiya Nadakkakath, A., Munjal, V., Kundu, A., Subaharan, K., Venugopal, V., … and Kumar, A. 2015. Genetic analysis of plant endophytic *Pseudomonas putida* BP25 and chemo-profiling of its antimicrobial volatile organic compounds. Microbiological Research 173: 66-78.

Smart, A., de Lacy Costello, B., White, P., Avison, M., Batty, C., Turner, C., … and Ratcliffe, N. 2019. Sniffing out resistance - Rapid identification of urinary tract infection-causing bacteria and their antibiotic susceptibility using volatile metabolite profiles. Journal of Pharmaceutical and Biomedical Analysis 167: 59-65.

Tang, Y., Jiang, Q., Tang, H., Wang, Z., Yin, Y., Ren, F., ... and Huang, J. 2020. Characterization and prevalence of *Campylobacter* spp. from broiler chicken rearing period to the slaughtering process in eastern China. Frontiers in Veterinary Science 7: 227.

United States Environmental and Protection Agency (USEPA). 2013. Most probable number (MPN) calculator version 2.0. Retrieved on June, 27, 2021 from USEPA website: https://mostprobablenumbercalculator.epa.gov/index.html

Van Den Dool, H. and Kratz, P. D. 1963. A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography. Journal of Chromatography 11: 463-471.

Vidal, D. M., Moreira, M. A. B., Coracini, M. D. A. and Zarbin, P. H. G. 2019. Isophorone derivatives as a new structural motif of aggregation pheromones in Curculionidae. Scientific Reports 9: 776.

Wagenaar, J. A., van Bergen, M. A., Blaser, M. J., Tauxe, R. V., Newell, D. G. and van Putten, J. P. 2014. *Campylobacter fetus* infections in humans: Exposure and disease. Clinical Infectious Diseases 58(11): 1579-1586.

Wang, Y., Li, Y., Yang, J., Ruan, J. and Sun, C. 2016. Microbial volatile organic compounds and their application in microorganism identification in foodstuff. Trends in Analytical Chemistry 78: 1-16.

World Health Organization (WHO). 2013. The global view of Campylobacteriosis: Report of an expert consultation. United States: WHO.

Wright, J. A., Grant, A. J., Hurd, D., Harrison, M., Guccione, E. J., Kelly, D. J. and Maskell, D. J. 2009. Metabolite and transcriptome analysis of *Campylobacter jejuni in vitro* growth reveals a stationary-phase physiological switch. Microbiology 155(1): 80-94.

Wu, B., Wang, X., Yang, B., Ma, Y., Hollmann, F. and Wang, Y. 2023. Biosynthesis of cyclic ketones by a H_2O_2 self-sufficient cascade reaction. Molecular Catalysis 547: 113396.

Zhao, B., Lin, X., Lei, L., Lamb, D. C., Kelly, S. L., Waterman, M. R. and Cane, D. E. 2008. Biosynthesis of the sesquiterpene antibiotic albaflavenone in *Streptomyces coelicolor* A3(2). The Journal of Biological Chemistry 283(13): 8183-8189.