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Short communication

Effect of lemongrass essential oil on *Listeria monocytogenes* gene expression

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ABSTRACT

The aim of the present study was to assess the transcriptomic response of *L. monocytogenes* isolates on the exposure to lemongrass essential oil. Overnight cultures of six strains previously isolated from a strawberry sample were spread over the surface of BHI agar and exposed to 5 and 10 μL of lemongrass essential oil applied on a Whatman paper placed on the lid. Three of them survived in the former case and one in the latter; in all cases biomass was collected and RNA was isolated. The expression of the key virulence genes *prfA*, *sigB*, *plcA*, *plcB*, *hly*, *inlA*, *inlB*, *inlC*, *inlJ*, *lmo2470* and *lmo2672*, as well as *accA*, *acpP* and *fapR* involved in fatty acid biosynthesis/metabolism and *murE* and *pbpB* involved in peptidoglycan biosynthesis was assessed by RT-qPCR. Transcription of *murE*, *pbpB*, *accA*, *fapR*, *prfA*, *sigB*, *inlA* and *lmo2672* was not affected. On the contrary, downregulation of *hly* and *inlJ* was observed for all strains. Moreover, upregulation of *acpP* and downregulation of *plcA*, *plcB*, *inlB*, *inlC* and *lmo2470* were observed according to the strain. The increased amount of lemongrass essential oil affected significantly the expression of *acpP*, *hly* and *inlJ*.

1. Introduction

The significance of *L. monocytogenes* as a food contaminant has been adequately highlighted (Allen et al., 2016; Hadjilouka, Paramithiotis, & Drosinos, 2014a). Among the intervention strategies that have been applied to control the growth of this ubiquitous pathogen, use of essential oils has drawn specific interest over the last decades (Perricone, Arace, Corbo, Sinigaglia, & Bevilacqua, 2015). Lemongrass essential oil is mostly composed of monoterpene compounds with citral, a natural mixture of geranial and neral, being the main component (Verma, Verma, Chauhan, & Bisht, 2015). The antimicrobial activity of lemongrass essential oil has been studied against a diverse range of microorganisms (Mishra & Dubey, 1994; Baratta et al., 1998; Hammer, Carson, & Riley, 1999; Cimanga et al., 2002; Daferera, Ziogas, & Polissiou, 2003; Pereira, Sumita, Furlan, Jorge, & Ueno, 2004; Naik, Fomda, Jaykumar, & Bhat, 2010; Korenblum et al., 2013; Balakrishnan, Paramasivam, & Arulkumar, 2014) both *in vitro* and in a variety of food matrices (Arrebola, Sivakumar, Bacigalupo, & Korsten, 2010; Azarakhsh, Osman, Ghazali, Tan, & Adzahan, 2014; De Oliveira et al., 2013; Raybaudi-Massilis, Mosqueda-Melgar, & Martin-Belloso, 2006). As far as the antilisterial effect was concerned, *in vitro* effectiveness was not always accompanied by a respective *in situ*, most probably due to the effect of the food components and storage conditions (Barbosa et al., 2009; Hadjilouka, Polychronopoulou, Paramithiotis, Tzamalis, & Drosinos, 2015a).

The transcriptomic responses of *L. monocytogenes* to a variety of environmental stimuli such as heat (Ripio, Vazquez-Boland, Vega, Nair, & Berche, 1998; Hanawa et al., 1999; Hanawa, Yamanishi, Murayama, Yamamoto, & Kamiya, 2002; Gaillot, Pellegrini, Bregenholt, Nair, & Berche, 2000; Van der Veen, Hain, Wouters, Hossain, de Vos, Abee et al., 2007), cold (Phan-Thanh & Gormon, 1995; Nelson et al., 2004; Schmid et al., 2009; Durack, Ross, & Bowman, 2013), acid (Cotter, Gahan, & Hill, 2000, 2001; Milecka, Samluk, Wasiak, & Krawczyk-Balska, 2015) and osmotic shock (Sleator, Gahan, Abee, & Hill, 1999; Sleator, Gahan, & Hill, 2001a; b; Duche, Tremoulet, Glaser, & Labadie, 2002; Brondsted,

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Kallipolitis, Ingmer, & Knochel, 2003; Sleator & Hill, 2005) as well as the presence of antimicrobials (Dutta, Elhanafi, & Kathariou, 2013; Elhanafi, Dutta, & Kathariou, 2010; Van der Veen & Abee, 2010; Laursen, Bahl, Licht, Gram, & Knudsen, 2015; Liu, Basu, Miller, & McMullen, 2014; Pleitner, Trinetta, Morgan, Linton, & Oliver, 2014; Romanova, Wolffs, Brovko, & Griffiths, 2006) *in vitro* and in an actual food matrix (Liu & Ream, 2008; Olesen, Thorsen, & Jespersen, 2010; Bae, Crowley, & Wang, 2011; Alessandria, Rantsiou, Dolci, Zeppa, & Cocolin, 2013; Mataragas et al., 2015; Hadjilouka et al., 2016) have been extensively studied improving our understanding of the pathogen's physiology (Hadjilouka, Paramithiotis, & Drosinos, 2015b). Genes associated with virulence potential as well as ones that contribute to the activation of response mechanisms have been in the epicenter of these studies. However, the transcriptomic response resulting from the exposure to an essential oil has not yet been studied.

The aim of the present study was to assess the expression of the transcriptional regulators *prfA* and *sigB*, the key virulence genes *hly*, *plcA*, *plcB*, *inlA*, *inlB*, *inlC*, *inlJ*, *lmo2672* and *lmo2470*, as well as genes that are involved in fatty acid (*acpP*, *accA*, *fapR*) and peptidoglycan (*murE*, *pbpB*) biosynthesis upon exposure to lemongrass essential oil vapors.

2. Materials and methods

2.1. Bacterial strains and culture conditions

L. monocytogenes strains LQC 15257, 15258, 15259, 15260, 15261 and 15262, belonging to serotype 4b, previously isolated from a strawberry sample (Hadjilouka, Andritsos, Paramithiotis, Mataragas, & Drosinos, 2014b) were used throughout this study. Long-term storage took place at −20 °C in nutrient broth supplemented with 50% glycerol. Before use, strain was grown twice in Brain Heart Infusion broth (Biolife, Milan, Italy) at 37 °C for 24 h.

2.2. Analysis of essential oil

Lemongrass (*Cymbopogon citratus*) essential oil was purchased

Table 1
Primer sequences and respective amplicon sizes used for the gene expression assay.

Genes		Sequence	Concentration (μM)	Amplicon size (bp)	PCR efficiency
Reference genes					
IGS	IGS_f[a]	GGCCTATAGCTCAGCTGGTTA	1.2	135	2.03
	IGS_r[a]	GCTGAGCTAAGGCCCCGTAAA	1.2		
rpob	Rpob_f[b]	CCGCGATGCGAAAACAAT	0.9	69	2.04
	Rpob_r[b]	CCWACAGAGATACGGTTATCRAATGC	0.9		
16S	16S_f[c]	GATGCATAGCCGACCTGAGA	0.9	114	2.05
	16S_r[d]	CTCCGTCAGACTTTCGTCCA	0.9		
Virulence-associated					
prfA	PrfA_f	CTATTTGCGGTCAACTTTTAATCCT	0.9	100	2.09
	PrfA_r	CCTAACTCCTGCATTGTTAAATTATCC	0.9		
hly	Hly_f[a]	TACATTAGTGGAAAGATGG	1.2	153	1.98
	Hly_r[a]	ACATTCAAGCTATTATTTACA	1.2		
plcA	PlcA_f[a]	CTAGAAGCAGGAATACGGTACA	1.2	115	1.94
	plcA_r[a]	ATTGAGTAATCGTTTCTAAT	1.2		
plcB	PlcB_f[b]	CAGGCTACCACTGTGCATATGAA	0.9	72	2.00
	PlcB_r[b]	CCATGTCTTCYGTTGCTTGATAATTG	0.9		
sigB	SigB_f[a]	CCAAGAAAATGGCGATCAAGAC	1.2	166	2.13
	SigB_r[a]	CGTTGCATCATATCTTCTAATAGCT	1.2		
inlA	InlA_f[b]	AATGCTCAGGCAGCTACAMTTACA	0.9	114	2.12
	InlA_r[b]	CGTGTCTGTTACRTTCGTTTTTCC	0.9		
inlB	inlB_f[b]	AAGCAMGATTTCATGGGAGAGT	0.9	78	2.04
	inlB_r[b]	TTACCGTTCCATCAACATCATAACTT	0.9		
inlC	InlC_f[d]	ACTGGTCAGAAATGTGTGAATGA	0.9	80	2.06
	InlC_r[d]	CCATCTGGGTCTTTGACAGT	0.9		
inlJ	InlJ_f[d]	TGCGTAAATGCTCACATCCAAG	0.9	81	2.03
	InlJ_r[d]	TTGCCCTTCAGCATCCAAGT	0.9		
Lmo2672	Lmo2672_f[e]	CGGCACACTTGGATTCTCAT	0.9	90	2.10
	Lmo2672_r[d]	AAACACATGGGACTTGCACC	0.9		
Lmo2470	Lmo2470_f[e]	TGATTCCATGCAATTACTAGAACG	0.9	86	2.10
	Lmo2470_r[d]	ACTCCGTTAGTTTAGCCCCA	0.9		
Fatty acid biosynthesis					
murE	murE_f[f]	GCCACAACCAACAACGACAA	0.9	85	1.97
	murE_r[f]	TCATACTCCAGACGGCTTGC	0.9		
accA	accA_f[f]	GCGGTCAAAGTGAAGCCATT	0.9	94	1.99
	accA_r[f]	CCACTTCCACCTTCACCGAT	0.9		
acpP	acpP_f[f]	TGAAGACGAGTTCGGAGTTGA	0.9	91	2.05
	acpP_r[f]	TGCGTTCGCCTCTATGTACT	0.9		
Peptidoglycan biosynthesis					
pbpB	pbpB_f[f]	AACGCATCGTCTTTCGACCA	0.9	95	1.93
	pbpB_r[f]	AACGCCGAAATCATGCAAGG	0.9		
fapR	fapR_f[f]	CGCCGTCATCCCAAATGAAA	0.9	88	2.02
	fapR_r[f]	TGCGATGATGCGTTCTCCTT	0.9		

Thermocycling conditions: initial denaturation at 95 °C for 20 sec and then 40x (95 °C for 10 sec, 60 °C for 30 sec, 72 °C for 30 sec). Melting curve analysis: 95 °C for 15 sec then 60 °C for 1 min and raise to 95 °C at 0.3 °C/sec.

[a] Rantsiou, Mataragas, Alessandria & Cocolin, 2012a.
[b] Olesen, Vogensen & Jespersen, 2009.
[c] Van der Veen and Abee (2010).
[d] Hadjilouka, Molfeta, Panagiotopoulou, Paramithiotis, Mataragas & Drosinos, 2014c.
[e] Liu, Ainsworth, Austin & Lawrence, 2003.
[f] This study.