



Estimating the antimicrobial effect of grape seed extract on *L. monocytogenes* Δ sigB on xanthan gum gels

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ABSTRACT

The substitution of chemical preservatives with natural antimicrobials has emerged as an important topic of interest for both researchers and the food industry. The utilisation of grape seed extract (GSE) has the potential to serve as an effective natural antimicrobial agent, while also offering the advantage of being a sustainable antimicrobial strategy, since GSE is a by-product of the fruit industry. The aim of this study was to quantitatively investigate the antimicrobial efficacy of GSE (1 % w/v) that was added in xanthan gum (XG)-based viscoelastic models of various XG concentrations (3, 5, 7 % w/v XG) against the wild-type (WT) strain and isogenic Δ sigB mutant of the foodborne pathogen *L. monocytogenes*. The gene regulator SigB is responsible for the general stress response of *L. monocytogenes* and its adaptation to environmental stresses. The GSE treatment effectively inactivated both strains (microbial inactivation ≥ 3 log CFU/ml) in all viscoelastic models regardless of the model firmness. However, the mutant strain Δ sigB was more sensitive to GSE treatment evidenced by the reduced viable population count and the increased percentage of sublethal injury in comparison to the WT. Lastly, at 7 % w/v XG (GSE-free) concentration, which was the highest gelling agent concentration used in this study, the mutant formed smaller colonies on the model surface as compared to the WT, suggesting the impact of SigB on the microbial growth/colony formation, especially on stiffer surfaces. The results of our study shed light on the impact of matrix surface structure on the response of *L. monocytogenes* and its Δ sigB mutant to the waste product GSE. Therefore, this study contributes to the development of enhanced and sustainable antimicrobial control strategies.

1. Introduction

Grapes are one of the world's most prized crops, producing 25 million tonnes annually (USDA Foreign Agricultural Service, 2021). Approximately 20 % of the total weight of grapes is estimated to be grape by-products, presenting a substantial disposal challenge. A by-product of the wine and juice industries is known as grape seed extract, or GSE for short (Chedea & Pop, 2019; Costa et al., 2022; Kar-nopp et al., 2017; Shrikhande, 2000). GSE has been found to possess strong antimicrobial and antioxidant activity. Therefore, the utilisation of natural antimicrobials, such as GSE, offer a promising sustainable alternative in substituting chemical preservatives in the food industry

(Ahn et al., 2004; Delgado Adámez et al., 2012; Farhadi et al., 2016; Rhodes et al., 2006; Sheng et al., 2016). Numerous mechanisms have been suggested for the antibacterial activity of GSE such as the penetration of polyphenol to the bacterial cell wall, the inactivation of extracellular enzymes and the formation of complexes with metal ions, which deplete the bacterial environment of these ions (Begg, 2019; Corrales et al., 2009; Silván et al., 2013). To date, limited research exists on the antimicrobial efficacy of GSE in terms of microbial dynamics (kinetics). More specifically, most studies to date utilize qualitative methods, such as the agar/disk diffusion tests and the minimum inhibitory concentration determination, to assess microbial inhibitory effects, while in cases where quantitative methods are employed, microbial

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concentration is typically measured only once post-treatment (Kao et al., 2010; Klančnik et al., 2010; Sheng et al., 2016; Silván et al., 2013). Furthermore, there are very limited studies investigating the role of different genes including *sigB*, i.e., a gene responsible for the general stress response of *L. monocytogenes* and its adaptation to environmental stress factors, on the sensitivity/tolerance to natural antimicrobials which would provide deeper insights into the mechanism of microbial inactivation and/or response to antimicrobials (Begley et al., 2006; Kitsiou et al., 2023a; Palmer et al., 2009). We recently evaluated the microbial population dynamics of *L. monocytogenes* wild-type (WT) and Δ *sigB* in liquid nutrient medium (TSBYE) supplemented with 1 % w/v GSE and observed increased susceptibility of Δ *sigB* to GSE suggesting for the first time that gene(s) of the SigB regulon significantly contribute to the bacterial response to GSE (Kitsiou et al., 2023a).

Generally, the main existing body of GSE research has primarily examined their antimicrobial properties in liquid substrates and/or specific foods (fresh produce, meat and fish products) (Ahn et al., 2004, 2007; Sagdic et al., 2011; Sivaroban et al., 2007; Zhao et al., 2020). However, the high complexity and batch-to-batch variation of food products limits the broader application of such findings to other food products (Baka et al., 2016; Bisha et al., 2010; Costello et al., 2021a). Prior research on the antimicrobial efficacy of GSE has demonstrated significant microbial inhibition on fresh produce like tomatoes, but its effectiveness decreased in more complex foods such as minced beef and turkey sausages, which have higher protein and fat content (Ahn et al., 2007; Bisha et al., 2010; Sivaroban et al., 2007). Comparable findings were noted in our recent study, where the microbial population dynamics of *L. monocytogenes* WT were monitored on the surface viscoelastic models of various biochemical complexities in which GSE was incorporated. More specifically, our study indicated that the antimicrobial activity of GSE against *L. monocytogenes* WT was not influenced by the increasing xanthan gum (XG) concentration in the monophasic models. However, the level of inactivation of *L. monocytogenes* WT was diminished when GSE was added in the more complex polyphasic models (Kitsiou et al., 2023b).

The microbiological response to any treatment can indeed be influenced by the biochemical composition, physicochemical properties, and structural attributes of the surrounding system/environment (Costello et al., 2019, 2021a; El Kadri et al., 2021; Garcia-Gonzalez et al., 2009; Smet et al., 2017; Vandekinderen et al., 2009; Verheyen et al., 2019, 2020). Therefore, testing the antimicrobial efficacy of novel treatments only in controlled liquid media could result in different microbial dynamics, as compared to solid or solid like systems. More specifically, cells in liquid nutrient broths grow planktonically, whereas in solid environments, they are immobilised and grow in colonies, aggregates, or biofilms. Additionally, in solid and solid (like) systems, gradients of oxygen or of the stress factor (e.g. the natural antimicrobial concentration) can take place, along with the accumulation of metabolic by-products such as acids. This accumulation can result in enhanced bacterial tolerance to the treatment through cross-protection mechanisms (Costello et al., 2018, 2019, 2021a, 2021b; Baka et al., 2017; Skandamis & Jeanson, 2015; Smet et al., 2017; Velliou et al., 2013; Wang et al., 2017). Various studies have reported that microbial inactivation and/or growth can be significantly different between solid and liquid systems. Microbial adaptation to environmental stresses and cross-protection, is a major challenge for food safety, especially with milder processing techniques that may allow for increased microbial resistance and survival, potentially resulting in unsafe food products (Antwi et al., 2008; Aryani et al., 2016; Baka et al., 2016; Boons et al., 2013; El Kadri et al., 2021; Karina et al., 2011; Mertens et al., 2009; Piyasena et al., 2003; Pol et al., 2001; Wang et al., 2017).

Recent outbreaks of *L. monocytogenes* like the one in February 2024 linked to queso fresco and cotija cheese resulting in wide-spread recalling of several products including dressings and sauces highlights the critical need for improved understanding of its survival and adaptation in solid like viscoelastic environments (similar to those that the

microorganism can experience in sauces and dressings; FDA, 2024). In *L. monocytogenes*, SigB is known to enhance the bacterium's ability to survive environmental stress, showing increased activity when exposed to heat, acidic, osmotic conditions (Boura et al., 2016; Cheng et al., 2015; O'Byrne & Karatzas, 2008; Raengpradub et al., 2008). Most existing studies examining the microbial growth and inactivation of mutant strains use liquid substrates, which is valuable for understanding fundamental physiological responses, stress adaptation mechanisms, and genetic regulation under controlled conditions (Han et al., 2016; Perni et al., 2007; Smith et al., 2003; Van Der Veen & Abee, 2010). However, these findings may not fully capture microbial growth and survival in more complex environments, such as solid or semi-like substrates, where factors like spatial organization, diffusion limitations and therefore environmental stress play a crucial role. To the authors best knowledge there are no studies to date studying the role of SigB, the main stress gene regulator of Gram-positive bacteria on the surface of viscoelastic model systems of controlled composition. Studying the *L. monocytogenes* Δ *sigB* mutant in comparison to the WT on the surface of viscoelastic models with or without the addition of GSE can provide valuable information on the microbial environmental behaviour and response to antimicrobial treatments. In general, the utilisation of viscoelastic *in vitro* models offers improved reproducibility due to their ability to regulate the composition and complexity, in contrast to real food products that exhibit variability between batches and possess product-specific characteristics. In addition, these models enable a more accurate understanding of the spatial microbial organization and growth and the response to different treatment approaches in comparison to liquid *in vitro* systems.

The aim of this work was to quantitatively explore, for the first time, the microbial response of *L. monocytogenes* WT and the role of its main stress gene regulator, SigB on the surface of monophasic XG-based models incorporated with 1 % w/v GSE and varying concentrations of gelling agent. XG was selected for its widespread use in food processing due to its viscoelastic properties, which can mimic the texture of specific food matrices and influence microbial behaviour (Costello et al., 2018, 2021a; Katzbauer, 1998; Lopes et al., 2015; Pirsá & Hafezi, 2023; Purk et al., 2023). XG is commonly utilised in the food industry in plethora of products such as salad dressings, sauces, gravies, dairy products, sweets, dairy alternatives and low-calorie food products (Henden et al., 2024; Katzbauer, 1998). Additionally, XG has an excellent gelling ability, requiring low concentrations, and stability under a wide range of pH levels and temperatures which allows us to study how the firmness of the model can affect the antimicrobial effect of GSE (Pirsá & Hafezi, 2023; Velliou et al., 2013). This work contributes towards the design of novel waste-based, sustainable antimicrobial strategies.

2. Materials and methods

2.1. Inoculum preparation

Stock cultures of *L. monocytogenes* 10403S WT and Δ *sigB* were preserved at -80°C in Tryptone Soy Broth (TSB, Oxoid Ltd, UK) with 15 % v/v glycerol. The inoculum was prepared as previously described (Velliou et al., 2011a, 2011b, 2011c, 2012). Briefly, after the culture was thawed, a loopful was inoculated into 20 ml TSB enriched with 0.6 % w/v of Yeast Extract (TSBYE, Oxoid Ltd, UK). The culture was incubated at 37°C for 9.5 h using a shaking incubator at 175 rpm. Subsequently, 20 μl were placed into 20 ml of liquid nutrient broth (TSBYE) and incubated for an additional 15 h (early stationary phase).

2.2. Grape seed extracts (GSE)

Commercially available grape seed extract (GSE, Bulk, UK) was utilised in this study. The concentration of polyphenols (oligomeric proanthocyanidins) in the GSE powder was at least 95 %. For both experiments the powder was firstly dissolved in TSBYE and the tested GSE

concentration was 1 % w/v in both tested substrates (liquid and solid systems). The selection of GSE concentration was based on our previous studies, conducted in TSBYE (liquid) or in viscoelastic models (Kitsiou et al., 2024; Kitsiou, Purk, Ioannou, et al., 2023).

2.3. Preparation of monophasic food model systems

The procedure by which the monophasic XG models were prepared has been outlined in our previous publications (Kitsiou et al., 2023b; Purk et al., 2023; Velliou et al., 2013).

Briefly, 3 %, 5 % and/or 7 % (w/v) Xanthan Gum (XG; Xantural® 75; CP Kelco, UK) was incorporated into TSBYE. The XG concentrations were chosen to represent a wide range of viscosities, and to allow for comparisons with our previous studies (Costello et al., 2018, 2021a; Kitsiou et al., 2023b; Velliou et al., 2013). Additionally, changes in moisture level were considered to be minimal across the XG concentrations under study and within the optimal growth range for *L. monocytogenes*, therefore the moisture content is expected to have a minimal effect on the bacterial kinetics (Purk et al., 2023). The mixtures were subjected to mechanical stirring (2000 rpm) for a minimum duration of 5 min, ensuring complete homogeneity (Omni Mixer Homogenizer, Omni International Inc., USA). To assess GSE's antimicrobial activity, the powder was dissolved in TSBYE to achieve 1 % (w/v) final concentration (see section 2.2). Thereafter, viscoelastic models were formed by mechanically stirring in XG to achieve the previously mentioned final concentrations. The viscoelastic models were then transferred in falcon tubes and autoclaved. In order to observe the microbial dynamics of *L. monocytogenes* 10403S WT and Δ sigB when treated with GSE, the monophasic XG models were transferred in 24-well plates to ensure the consistency of their size (approx. 2 cm² surface area).

2.4. Microbial dynamics in the presence of GSE on monophasic XG models

To investigate the antimicrobial efficacy of GSE against the *L. monocytogenes* 10403S WT and Δ sigB, bacterial cells were inoculated in 1 % TSBYE-GSE solution or spread on the surface of the viscoelastic models. Additionally, cells were added in TSBYE or on the viscoelastic model systems without GSE (controls). The initial bacterial concentration was approximately 10⁵ CFU/ml. Following inoculation, the samples were incubated at 37 °C. The bacterial survival was systematically enumerated at intervals of 0, 2, 4, 8, 12, and 24 h post-treatment during a 24-h period using the spread plate method in non-selective agar i.e., Tryptone Soy Agar enriched with 0.6 % of Yeast Extract (TSAYE, Oxoid Ltd, UK). Additionally, the samples were also plated onto selective media for *L. monocytogenes* i.e., Polymyxin Acriflavin Lithium-chloride Ceftazidime Esculin Mannitol (PALCAM, Oxoid Ltd, UK) in order to quantify the number of sub-lethally damaged cells. The sublethal injury (%) was calculated based on the following equation (Busch & Donnelly, 1992):

$$\% \text{ Injured cells} = \left[1 - \frac{\text{Count on selective agar}}{\text{Count on non - selective agar}} \left(\frac{\text{CFU}}{\text{ml}} \right) \right] \times 100 \quad (1)$$

2.5. Rheological characterization

The monophasic XG models were characterised rheologically, determining the storage modulus G' (Pa) and the loss modulus G'' (Pa). The analysis was performed as previously outlined (Costello et al., 2018; Kitsiou et al., 2023b). The viscoelastic behaviour was evaluated at 37 °C by conducting dynamic oscillatory measurements. This temperature was chosen as the antimicrobial effect of GSE incorporated in the viscoelastic models occurred at that temperature, which is the optimal growth temperature for *L. monocytogenes*.

The G' and G'' were quantified as functions of angular frequency ω

ranging from 1 to 100 (rad/s) utilising a HR-2 Discovery Series Hybrid Rheometer (DHR, TA instruments, USA) with a maximum strain of 2 % and a circulating water bath for temperature regulation (TA instruments, USA). The analysis was performed utilising a cone and plate geometry (50 mm diameter, 2° angle).

2.6. Imaging of colonies

To obtain images of *L. monocytogenes* 10403S WT and Δ sigB colonies on the surface of the monophasic model systems without the GSE, the same procedure described in section 2.4 was followed. Briefly, the cells were spread on the surface of the viscoelastic models at final concentration 10⁵ CFU/ml and incubated at 37 °C. After stationary phase was reached, the 24-well plate was transferred to the Cytation 5 Imaging reader (BioTek, Winooski, VT, USA) in order to capture images of the colonies formed on the surface of the monophasic models. The images were processed using the Gen5 software (Biotek). A minimum of three images were captured for each sample, three samples were analysed for each experiment, and a minimum of two independent experiments were conducted.

2.7. Statistical analysis

The statistical analysis was carried out using GraphPad Prim and Microsoft Excel. All measurements and conditions under investigation were subjected to a minimum of two independent experiments, each comprising three replicate samples. A *t*-test was applied to evaluate statistical significance ($p < 0.05$) when comparing two mean values, while a two-way ANOVA followed by Tukey's HSD post hoc analysis was utilised to determine statistically significant ($p < 0.05$) differences among independent experimental groups in the case of multiple comparisons. When the viable cell count was below the detection limit (<10 CFU/ml) in the non-selective and selective media the number of viable and percentage sub-lethally damaged cells were determined to be 1 log CFU/ml and/or 100 %, respectively.

3. Results and discussion

As previously stated, the objective of this study was to examine the microbial dynamics of *L. monocytogenes* WT and Δ sigB, as affected by 1 % w/v grape seed extract (GSE), incorporated on the surface of viscoelastic xanthan gum-based models with varying xanthan gum concentration (3, 5, 7 % w/v XG).

To the authors' best knowledge this is the first study exploring the microbial dynamics of *L. monocytogenes* WT and the role of the main stress gene regulator SigB in the presence of GSE on solid-like models with robustly controlled structural and biochemical composition.

Fig. 1 shows the microbial dynamics of *L. monocytogenes* WT and Δ sigB in liquid nutrient medium (TSBYE; Kitsiou et al., 2023a) and on the surface of the viscoelastic model systems under study. The antimicrobial effect of GSE against *L. monocytogenes* WT and Δ sigB was not affected by the increasing XG concentration in the viscoelastic model systems. Additionally, the mutant strain Δ sigB was more sensitive to the GSE treatment when compared to the WT strain. More specifically, the microbial inactivation of *L. monocytogenes* WT by GSE for all tested viscoelastic model systems was on average 1.6, and 2.9 log CFU/ml after 8 and 24 h, respectively. Additionally, Δ sigB was inactivated by an average of 2.0 and 3.3 log reduction of CFU/ml after 8 and 24 h, respectively (Fig. 1). To investigate further the increased susceptibility of Δ sigB to the GSE treatment, the level of sublethal injury of both strains after treatment was tested (as described in section 2.4). The percentage of sub-lethally injured cells for both strains increased with increasing treatment time (Fig. 2, $p < 0.05$). For instance, the percentage of sub-lethally injured cells of *L. monocytogenes* WT was on average 36, 44, 48 and 54 % after 4, 8, 12 and 24 h, respectively. However, after 4 h, the average of sublethal injury of Δ sigB was 55 % and by 8 h, all cells had

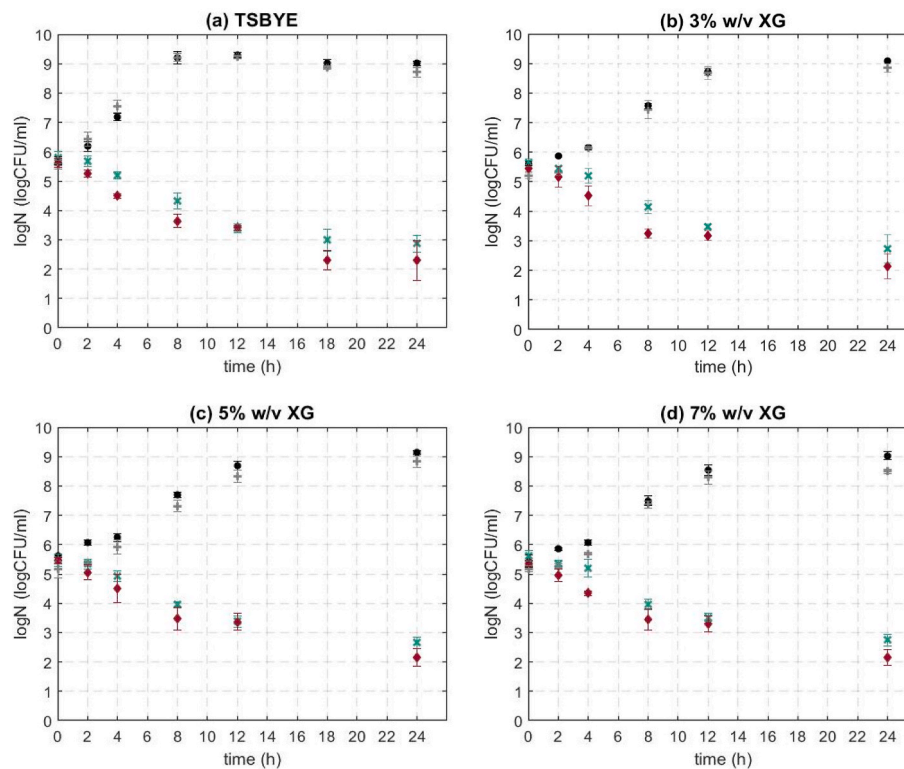


Fig. 1. Microbial population dynamics of *L. monocytogenes* 10403S WT and $\Delta sigB$ in (a) liquid TSBYE (Kitsiou et al., 2023a) and on the surfaces of monophasic models of TSBYE incorporated with (b) 3 % w/v XG, (c) 5 % w/v XG, (d) 7 % w/v XG. In all plots (●) control (without GSE) WT (w/o GSE) (+), control (without GSE) $\Delta sigB$ (w/o GSE) (x), treated sample WT with 1 % w/v GSE, and (♦) treated sample $\Delta sigB$ with 1 % w/v GSE are shown. Each time point represents the average of two independent experiments with three technical replicates per experiment. Error bars show standard deviation.

reached sublethal injury state (below detection limit for our method), confirming the high sensitivity of the mutant strain to GSE. It is also worth mentioning that the level of sublethal injury was similar across all viscoelastic model systems, despite the increased gel firmness (higher G' and G'') associated with higher XG concentrations (Fig. 3, Table 1) (Choppe et al., 2010; Costello et al., 2018; Kitsiou et al., 2023b; Velliou et al., 2013).

The observed sensitivity of the mutant strain in the viscoelastic gels is consistent with our previous observations in liquid nutrient broth (Kitsiou et al., 2023a; 2024), as well as with other limited studies in liquid systems (Begley et al., 2006; Gomes Neto et al., 2015; Palmer et al., 2009), showing that structure affects the mutant sensitivity similarly to a liquid broth. The increased susceptibility of $\Delta sigB$ to natural antimicrobials could be attributed to the role of SigB in regulating mechanisms protecting the cell membrane, including the charge or lipid composition of the latter, in response to the stressor. Furthermore, SigB-regulated proteins aid in the efflux of the antimicrobial compounds out of the cell. Both stress adaption mechanisms play a role in overcoming the imposed stress and promote the recovery of sublethally injured cells (Begley et al., 2006; Gomes Neto et al., 2015; Guerreiro et al., 2020; Kitsiou et al., 2023a).

In terms of the properties of the gels, an increase in rigidity of the viscoelastic model systems can also lead to diffusional limitations of antimicrobials, resulting in diminished antimicrobial activity (Aspridou et al., 2014; Costello et al., 2018, 2019; Makariti et al., 2021; Skandamis & Jeanson, 2015; Velliou et al., 2013). However, in our viscoelastic models, no change in the antimicrobial effect of GSE was observed with varying XG concentrations, up to 7 % w/v. Our results show changes in firmness did not affect the response of the mutant to GSE. These results align with the previous published work of our group (conducted for the WT strain; Costello et al., 2018; Kitsiou et al., 2023a, Kitsiou, Purk, Ioannou, et al., 2023). In a prior study, 1 % w/v GSE was added in three

monophasic XG models with lower xanthan gum (XG) concentration (1.5, 2.5 and 5 % w/v) than the current study as well as in structurally complex biphasic systems, containing XG and Whey Protein Isolate (WPI; 5 % w/v XG, 10 % w/v WPI) and triphasic systems, containing XG, WPI and fat (5 % w/v XG, 10 % w/v WPI, 10 % v/v fat). We reported that the antimicrobial activity of GSE against *L. monocytogenes* WT (initial microbial load 10^5 CFU/ml) was not influenced by the increasing XG concentration in the monophasic models i.e., the level of inactivation was similar when tested in TBSYE and monophasic models. However, the level of inactivation of *L. monocytogenes* WT was diminished when GSE was added in the more complex model system i.e., biphasic and triphasic model systems. Lastly, Costello et al., (2019) examined the antimicrobial activity of nisin against *L. innocua* in liquid and on monophasic models (3 & 5 % w/v XG). The results of this investigation showed that the microbial dynamics of *L. innocua* treated with nisin followed the same trend in liquid and both viscoelastic model systems despite the increased firmness of the XG models (Costello et al., 2019). Our current study suggests that a further increase in the stiffness of the viscoelastic models, using up to 7 % w/v XG concentration, did not affect the antimicrobial activity of the GSE. Therefore, the biochemical composition and structural complexity of the viscoelastic model might have a higher impact than the gel firmness on reducing the level of inactivation under GSE treatment both for the WT and mutant strain.

In terms of growth on the surface of the monophasic XG models (in absence of GSE), we observed that, as the rigidity/firmness of the viscoelastic model increased, a small disturbance in the growth, of $\Delta sigB$ occurred mainly at the final (stationary phase) cell concentration, i.e., lower cell concentration in stationary phase when grown on the surface of viscoelastic model containing 7 % w/v XG in comparison to those with 3 % w/v XG. More specifically, after 24 h, a difference in cell concentration of 0.5 log CFU/ml was noted for $\Delta sigB$ compared to the WT on the surface of the viscoelastic model containing 7 % w/v XG

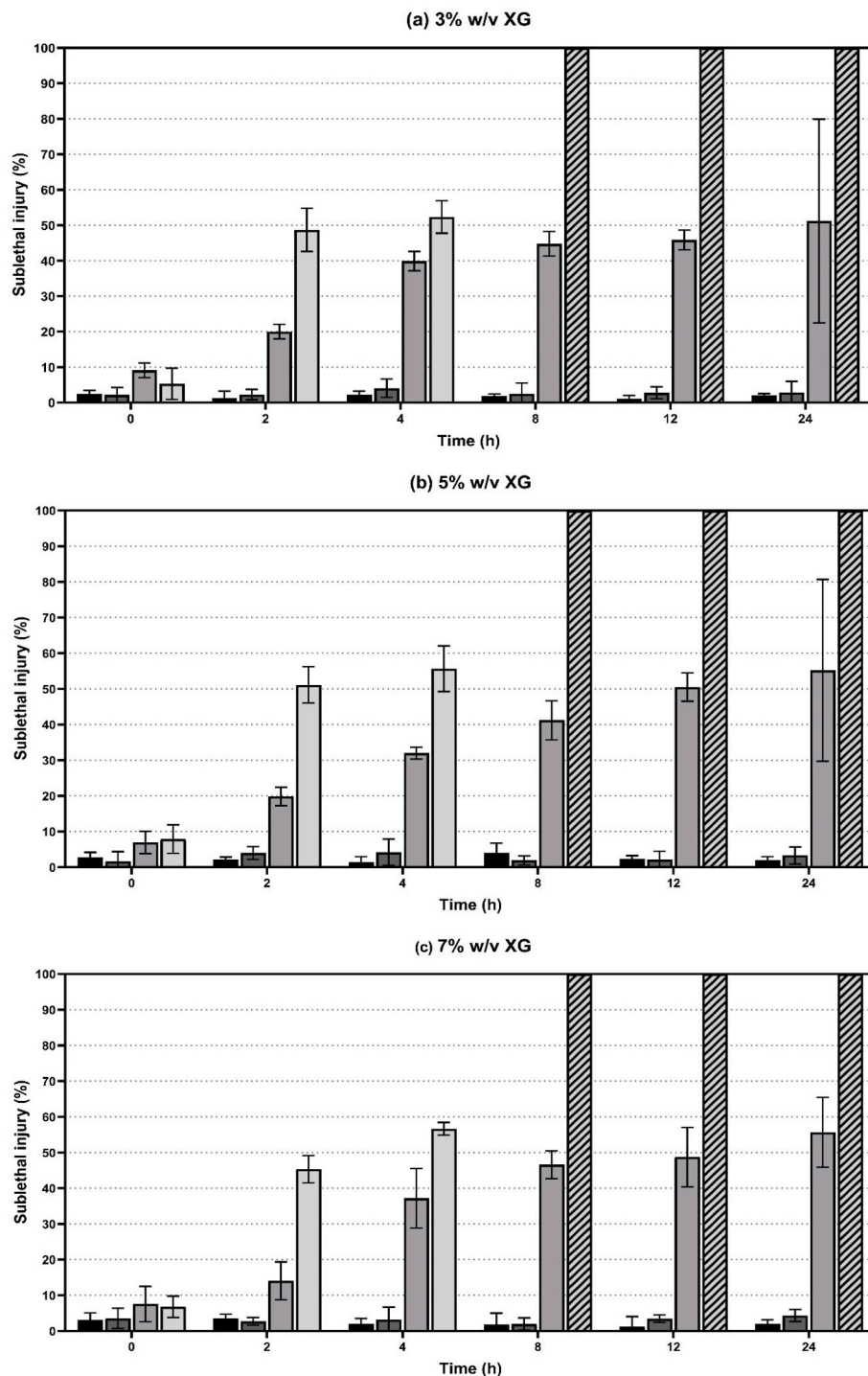


Fig. 2. Quantification of the sublethally injured population (%) of *L. monocytogenes* 10403S WT and $\Delta sigB$ on the surface of (a) 3 % w/v XG, (b) 5 % w/v XG, (c) 7 % w/v XG models. In all plots (■), control WT (without GSE) (■), control $\Delta sigB$ (without GSE) (■), treated sample WT with 1 % w/v GSE, and (▨) treated sample $\Delta sigB$ with 1 % w/v GSE are shown. In cases where the viable cell count in the selective media was below detection limit (<10 CFU/ml) the number of sublethal damaged cells was set to 100 % (bar with stripes). Each bar represents the average of two independent experiments with three technical replicates per experiment. Error bars show standard deviation.

(Fig. 1d). Moreover, images of the colonies formed on the surface of the monophasic XG models showed that the colonies of the WT strain were larger and more spread out on the surface of the gels, as the firmness of the viscoelastic model increased, as expected since this phenomenon has been reported previously by us and others (Fig. 4a–c) (Be'er et al., 2009; Costello et al., 2018; Kitsiou et al., 2023b). The same trend was observed for $\Delta sigB$, when the XG concentration increased from 3 to 5 % w/v

(Fig. 4d and e). However, interestingly, no significant changes in the colony size were detected when the XG concentration was further increased to 7 % w/v, in contrast to the WT and to the expected trend from the literature (Fig. 4f; Be'er et al., 2009; Costello et al., 2018; Kitsiou et al., 2023b). As previously mentioned, colony growth may result in diffusion limitations of oxygen and nutrients, as well as accumulation of metabolic compounds that can reduce the pH levels in and

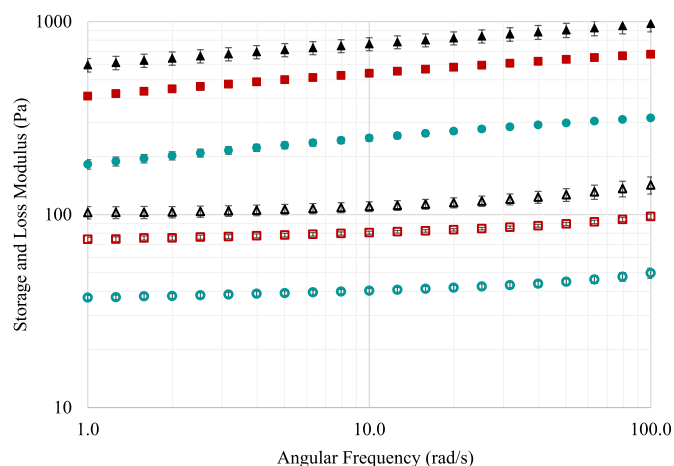


Fig. 3. Rheological characterization of the viscoelastic models incorporated with 1 % w/v GSE. Storage modulus G' and the loss modulus G'' as a function of the angular frequency at 37 °C: G' (○) 3 % XG (□), 5 % XG (△), 7 % XG; G'' (●): 3 % XG (■), 5 % XG (▲), 7 % XG.

around the colonies. This can result in a decrease in microbial growth rate due to self-induced acid stress and starvation. Additionally, the localised reduction of pH can activate the general environmental stress response of *L. monocytogenes* enhancing cross-protection against different environmental stresses. Lastly, the increase of the viscoelastic model's rigidity may enhance the previously-mentioned effect of colony

growth i.e., increased diffusional limitations resulting in cells that are more stressed and the formation of smaller colonies (Aspridou et al., 2014; Costello et al., 2018, 2019; Makariti et al., 2021; Skandamis & Jeanson, 2015; Velliou et al., 2013). Therefore, the observed disturbance in growth of $\Delta sigB$ can be attributed to the absence of the stress regulator SigB which is responsible for the expression of >40 environmental stress related genes including acid stress and starvation, which are stresses that can occur in colony growth especially in viscoelastic models with higher XG that are more rigid (Abee, 1999; Herbert & Foster, 2001; Liu et al., 2019; NicAogáin & O'Byrne, 2016; O'Byrne & Karatzas, 2008). Additionally, some studies have shown that SigB enhances biofilm formation especially under stress, thus its absence could result in the formation of smaller cell aggregates (Lee et al., 2014; Van Der Veen & Abee, 2010).

Overall, the results of the current study expand our understanding of the stress adaptation mechanisms of *L. monocytogenes* in the presence of GSE, in viscoelastic models. Additionally, our data offer new insights into the microbial dynamics of the $\Delta sigB$ mutant strain which exhibits lower cell concentrations in stationary phase in solid like environments. Hence, our findings can be used to create innovative and sustainable antimicrobial control strategies by utilising food waste. Future work should focus on understanding how, next to structure, additional factors such as (i) biochemical complexity (ii) acidity (fluctuations in pH levels of the gels) and (iii) changes in moisture content could potentially affect the microbial population dynamics, as all those factors, further to structure, can differ in different food products.

Table 1

Rheological parameters for the monophasic XG models with 1 % w/v GSE. Letters indicate statistical significance ($p < 0.05$), different letters are significantly different. Means with the same letter are not significantly different.

Monophasic model	Temperature	G'			G''			$\tan\delta$		
3 % XG	37 °C	249.6	±	42.2 ^a	41.2	±	3.71 ^a	0.17	±	0.02 ^a
5 % XG	37 °C	541.2	±	82.0 ^b	82.2	±	6.76 ^b	0.15	±	0.01 ^b
7 % XG	37 °C	772.4	±	125.4 ^c	114.0	±	13.47 ^c	0.15	±	0.01 ^b

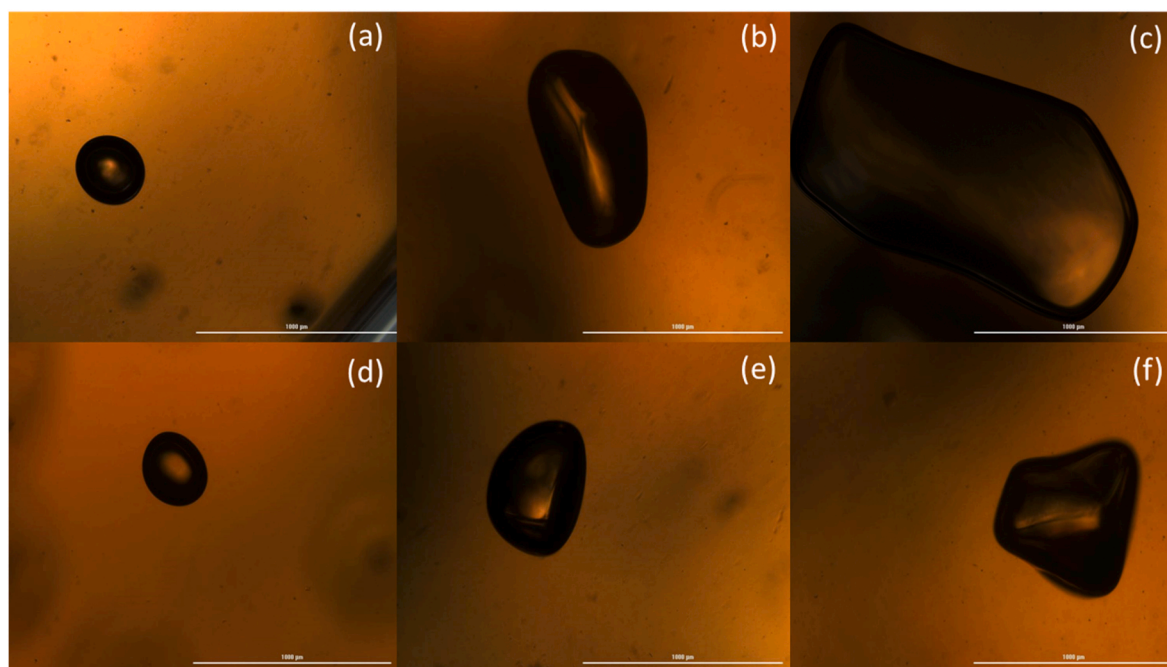


Fig. 4. Representative images of *L. monocytogenes* WT colonies on the surface of (a) 3 % w/v XG (b) 5 w/v % XG (c) and 7 % w/v XG and *L. monocytogenes* $\Delta sigB$ colonies on (d) 3 % w/v XG (e) 5 w/v % XG (f) and 7 % w/v XG systems at 37 °C.

4. Conclusion

In this study, the microbial dynamics of the pathogen *L. monocytogenes* wild type (WT) and mutant ΔsigB (which is associated with the general environmental stress response of the pathogen) were quantitatively investigated, under grape seed extract (GSE, 1 % w/v) treatment. The cells were spread on the surface of monophasic models of controlled xanthan gum concentrations (3, 5, 7 % w/v XG). In all viscoelastic models under study, the GSE effectively inactivated both strains, regardless of the increase in the firmness of the monophasic models. However, the ΔsigB exhibited increased susceptibility to the GSE treatment, indicated by lower viable population counts and a higher percentage of sublethal injury, in comparison to the WT. Additionally, a disturbance in the growth of the mutant strain was observed as the XG concentration in the viscoelastic models increased to 7 % w/v resulting in the formation of smaller colonies when compared to the WT.

Our findings provide insight into the efficacy and mechanism of inactivation of GSE against one of the most lethal foodborne pathogens and its' general environmental stress response mutant. These data can be used to develop novel antimicrobial control strategies including approaches of targeting the sensitivity of the mutant strain. Additionally, the observed antimicrobial activity of GSE in viscoelastic models highlights the possibility of utilising waste by-products from the food industry as efficient and ecologically friendly antimicrobial agents. This not only helps reduce food waste but also could improve food safety measures by replacing chemical preservatives and antibiotics.

CRedit authorship contribution statement

Melina Kitsiou: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Jorge Gutierrez-Merino:** Writing – review & editing, Supervision, Resources. **Oleksiy V. Klymenko:** Writing – review & editing, Supervision, Formal analysis. **Kimon Andreas Karatzas:** Writing – review & editing, Supervision, Resources, Investigation, Formal analysis. **Eirini Velliou:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests nor personal relationships that could have appeared to influence the work reported in this article.

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Data availability

Data will be made available on request.

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