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Single cell swimming dynamics of *Listeria monocytogenes* using a nanoporous microfluidic platform†

Evan Wright,[a] Suresh Neethirajan,*[a] Keith Warriner,[b] Scott Retterer[c] and Bernadeta Srijanto[c]

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Listeria monocytogenes remains a significant foodborne pathogen due to its virulence and ability to become established in food processing facilities. The pathogen is characterized by its ability to grow over a wide temperature range and withstand a broad range of stresses. The following reports on the chemotaxis and motility of the *L. monocytogenes* when exposed to relatively small concentrations of acetic acid. Using the developed nanoporous microfluidic device to precisely modulate the cellular environment, we exposed the individual *Listeria* cells to acetic acid and, in real time and with high resolution, observed how the cells reacted to the change in their surroundings. Our results showed that concentrations of acetic acid below 10 mM had very little, if any, effect on the motility. However, when exposed to 100 mM acetic acid, the cells exhibited a sharp drop in velocity and displayed a more random pattern of motion. These results indicate that at appropriate concentrations, acetic acid has the ability to disable the flagellum of the cells, thus impairing their motility. This drop in motility has numerous effects on the cell; its main effects being the obstruction of the cell's ability to properly form biofilms and a reduction in the overall infectivity of the cells. Since these characteristics are especially useful in controlling the proliferation of *L. monocytogenes*, acetic acid shows potential for application in the food industry as an active compound in designing a food packaging environment and as an antimicrobial agent.

Introduction

The bacterium *Listeria monocytogenes* (LM) is a peritrichous, opportunistic pathogen that can be found naturally in soil, water and decaying plant matter.[1] It can infect a wide variety of animal species resulting in listeriosis that can be potentially lethal especially with regards to the unborn, old, young and immunocompromised.[1] *Listeria* is one of the few pathogens that is equally adapted to growth inside and outside of the host. Outside the gastro-intestinal tract environment *Listeria* can grow under refrigerated conditions, tolerate a wide range of stresses and express motility.[1,2] The success of *Listeria* is such that the pathogen can readily become established in processing facilities and persist over years thereby presenting a continuous source of contamination. In 2008, Canadian based Maple Leaf Foods industry suffered a tragic outbreak of listeriosis that resulted in 23 deaths linked to an endemic strain of *L. monocytogenes* that became established on meat slicing machines within the processing facility.[2]

The motility and the chemotaxis of LM play a critical role in terms of movement towards nutrient sources, attachment, biofilm formation and thereby pathological processes, for host cell invasion and virulence.[3] Horizontal gene transfer between bacteria and host cells is also facilitated through chemotactic motility.[4] Flagellar motility is an important factor in both biofilm formation and infectivity of *Listeria*.[5,6] Knowledge about the transport potential of *Listeria* and its swimming behaviour in the presence and absence of chemo-attractant and chemo-repellent gradients will help in the design of antimicrobial strategies to help control and prevent outbreaks. Single cell chemotactic ability of *Listeria* also helps in developing novel diagnostics, which can identify pathogens through inclusion of attractant molecules and for investigation of early biofilm formation. Many different methods currently exist for analysing the chemotaxis of bacteria. Some of these methods include agarose plates,[7] collagen plates,[8] the Zigmond chamber,[9] the Dunn chamber[10] and capillary assays.[11] However, all of these methods possess certain flaws. These techniques rely on diffusion of chemicals

[a] BioNano Lab, School of Engineering, University of Guelph, Ontario, Guelph, N1G 2W1, Canada. E-mail: sneethir@uoguelph.ca; Tel: +1 519 824 4120
[b] Food Science, University of Guelph, Ontario, Guelph, N1G 2W1, Canada
[c] Center for Nanophase Materials Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA

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over relatively large distances leading to inaccuracies in the creation of diffusion profiles and are unable to precisely control the chemical gradient, making the quantification of chemotaxis difficult. Some of them also rely on bacterial movement as a group; individual cells cannot be viewed nor tracked. Microfluidics offers the unique ability to precisely control the cellular chemical environment and observe the subtle movements of single cells at high resolution. The potential of microfluidic devices for single cell observation of bacterial chemotaxis has begun to be realized and is becoming a more common practice.[12–18]

Understanding the chemotactic dynamics of *Listeria* in response to the change in molecular flux will aid in the development of novel food environments that prevent and control *Listeria*. The two key motility characteristics studied here are speed and randomness of the cells' movement. One of LM's main modes of movement is through the use of flagella, long, tail-like structures that spin at speeds of up to 100 Hz. This spinning motion pushes the cell forward; the rate of forward movement corresponds to the speed of the spinning motion. Since it is peritrichously flagellated, LM has multiple flagella that are able to push it in different directions. Receptors near the surface of the cell count the molecules of interest and control flagellar rotation.[19] If the current direction of movement is deemed favourable, the time of rotation is extended; the flagella powering that direction of movement spin longer than they otherwise would. This bias enables the cells to actively find regions in their environment where the cells sense preferred environment.[19] In hostile environments, due to the molecular trigger presence, the cell is constantly changing direction, while in neutral environments, it is more likely to maintain one direction. Both key factors are largely influenced by the environment of the cell, and by better understanding this influence we hope to provide valuable information about the behaviour of *Listeria* that could be applied to food industrial practices.

In this study, acetic acid was chosen as the chemoeffector because not only is it able to act as an antimicrobial agent, but it could also be easily applied in the food industry. The route of using acetic acid as a model effector includes, as a means of studying, the early events in *Listeria*'s biofilm formation. Acetic acid is safe to use; it is naturally found in many food products and provides unique advantages as both a food additive and a disinfectant. Based on various food industry related studies,[20–22] acetic acid concentrations of 1 mM, 10 mM and 100 mM were chosen for the experiments. Current research indicates that the usefulness and effect of particular concentrations can vary greatly depending on the situation and how it is applied. The selected concentrations encompass a fairly wide range and allow the tests to be simultaneously applied to some current food industry practices and used for theoretical knowledge.

The overall objective of this study is to characterize the changes in *Listeria monocytogenes*' motility in response to prescribed fluctuations of the cellular environment by changing acetic acid concentrations. The specific objectives are: (1) to utilize a nanoporous microfluidic device for creating a rapid chemical gradient using the liquid–liquid interface, and (2) to examine and quantify the effects of acetic acid on *Listeria*'s motility and to measure parameters describing its swimming behaviours such as chemotactic velocity, run direction, trajectory and run speed.

Experimental

Microfluidic platform and device characterization

The microfluidic device used in this experiment (Fig. 1) is composed of three major channels: one central channel and

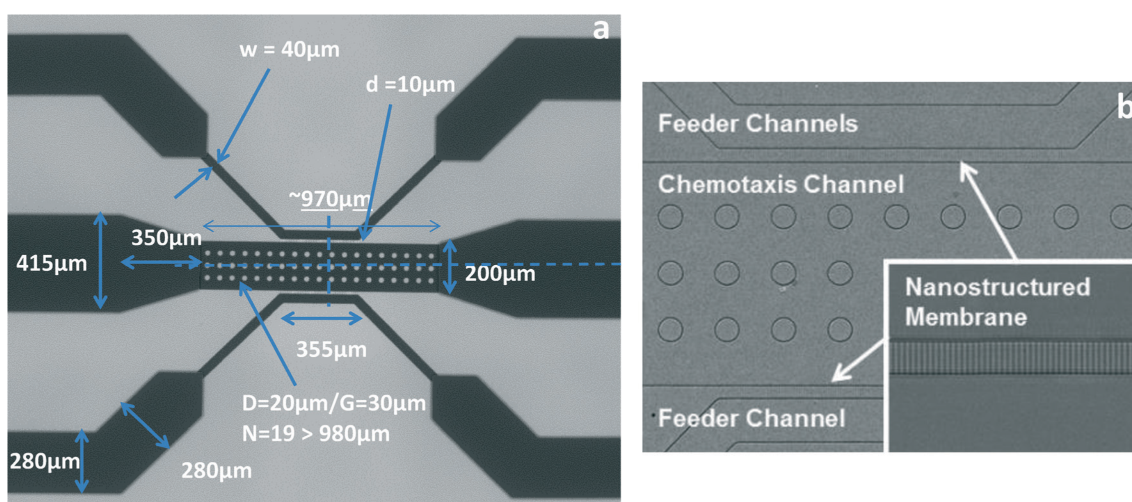

Fig. 1 (a) Scanning electron microscopy image of the fabricated microfluidic device displaying the dimensions of the diffusion channels. The nanoporous structure membranes (b) that are 800 nm in diameter connect the feeder channels with the 200 μm wide and 10 μm deep chemotaxis channel. A silicon and polymer master was formed using a combination of electron beam lithography, anisotropic silicon etching and cross linking of the polymer. This master was replicated in silicone to form the microfluidic chamber with nanostructured membranes that allow material exchange during real-time imaging of the single cell response.

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two feeder channels. At the middle of the central channel there is a viewing area with supporting pillars designed to allow images or videos to be captured in high resolution. Inlet and outlet holes are placed at the ends of every channel to allow solutions to be fed through the device. Bacteria are introduced into the central swimming channel where the environment can be manipulated by the buffer solution. The two feeder channels run alongside the viewing area and are connected by nanopores that allow the acetic acid solution to diffuse into the viewing area where its effects on the single cell bacteria can be observed. The diffusion rate of this microfluidic device was characterized using 5 μm of Fluorescein and 5 μm of Texas Red as fluorescent dyes. Dyes were injected in the feeder channels and the green and red optical filters of the microscope allow viewing of the fluorescence of a single dye at a time, making it possible to track the dyes' diffusion rate into the central swimming channel. The diffusion profiles are shown in Fig. 2 and in ESI† Video 1.

Bacterial strains and cell culturing conditions

L. monocytogenes MMB 17 was used throughout this study. The strain is a clinical isolate of *L. monocytogenes* and was a gift from the Canadian Research Institute for Food Safety (CRIFS) at the University of Guelph. The culture medium used for bacterial growth medium was tryptone soy broth (TSB) (Sigma Chemical Co., St. Louis, MO).

Cell suspensions were prepared from overnight cultures grown in 5 ml TSB at 25 °C and 100 rpm overnight. A 100 μl subculture was inoculated into 5 ml of fresh TSB which was then incubated for 4 hours and cells harvested in the exponential phase by centrifugation (SciLogex D3024, Berlin, CT) at 161 rcf or 5 minutes had the supernatant drained and then suspended in deionized water. This was repeated for two more times with the last suspension done in a motility buffer. The chemotaxis motility buffer was prepared by mixing 11.2 g of potassium phosphate dibasic, 4.8 g of potassium phosphate monobasic, 2 g of ammonium sulphate, 0.25 g of magnesium sulphate and 1 g of polyvinylpyrrolidone (Sigma Aldrich, St. Louis, MO) in 1 litre of deionized water.

Microfluidic device preparation

In the preparation of the microfluidic device, 14 g of polydimethylsiloxane or PDMS elastomer base was mixed with 1.4 g of curing agent (Dow Corning Sylgard 184, Midland, MI) and the mixture was poured over the top of the silicon chip. The solution was desiccated and then baked at 60 °C in an oven to harden the PDMS. The device was cut free of the mould using a scalpel and the inlet and outlet holes were punched (Harris Uni-Core 0.75 mm, Sigma Chemical Co., St. Louis, MO). The device and a glass slide were plasma cleaned (Harrick Plasma, Ithaca NY) for a minute. This process helped in removing debris from the surface of the PDMS and the glass as well as changing the surface functionality of both pieces and allowing them to bond together. The device and the glass slide were then placed together, creating a bond between the pieces, and then baked for 30 minutes at 60 °C to solidify the bond.

Experimental setup

When applicable, liquids were flowed into the chemotaxis device using 1 ml syringes and 0.3 mm syringe tips and tubing (Becton, Dickenson and Company, Franklin Lakes, NJ). When a constant flow was required, a syringe pump (Chemyx Fusion Touch, Stafford, TX) was used to maintain the flow rate. All solutions were flowed at a constant rate of 30 μl per hour.

The fully assembled device was first filled with 0.1% bovine serum albumin and allowed to sit for 10 minutes before it was rinsed with distilled water. The central channel

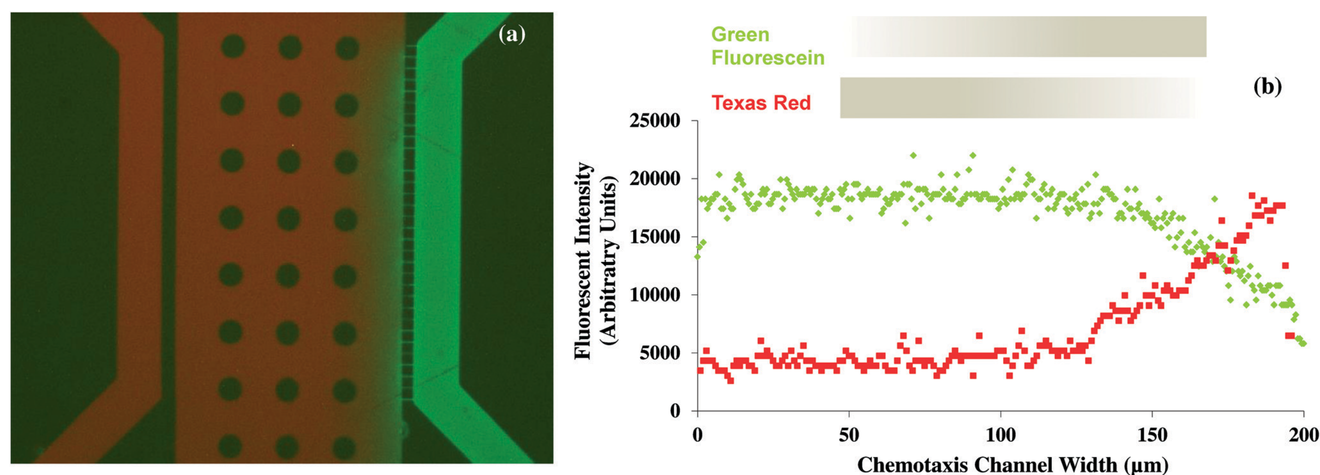

Fig. 2 Characterization of the nanoporous microfluidic device. 5 μm of Fluorescein (MW 376.3) and 5 μm of Texas Red (MW 641.5) concentration gradient generated in the chemotaxis channel at an injection flow rate of 30 μL h^{-1}. (a) Representative overlaid fluorescent image of the concentration gradient in the chemotaxis channel. (b) The pixel intensity of the concentration distribution is averaged. Concentration gradient as a fraction of the source concentration from the nanostructured membranes at the inlets of the feeder channels at a flow rate of 30 μL h^{-1}.

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and the viewing area were filled with the bacterial solution; the centre inlet and outlet were plugged with small chunks of PDMS. Syringes were filled with the relevant solution, placed in the syringe pump and hooked up to the inlet holes with tubing. The outlet holes were attached to a waste collection beaker using Teflon tubing.

To begin, a baseline of LM's motility was established. The cells were cultured and then placed in the microfluidic device so that the viewing area was filled. A motility buffer was flowed through both side channels and a video of the viewing area was recorded. The video was processed and quantitative data about the single cell motility were collected.

Acetic acid of 1 mM, 10 mM and 100 mM concentrations were then flowed through one channel instead of the motility buffer in 5 replicates. The acetic acid slowly diffuses into the viewing area where the bacteria react to the change in their environment. This reaction was recorded so that quantitative data of the bacteria's reaction to the change in cellular environment could be extracted.

As the solutions began flowing in the microfluidic device, it was placed on a micrometre stage centred on the viewing area, and the video was captured using a Nikon Eclipse Ti inverted microscope, a Nikon DS-QiMc microscope camera and a Nikon NIS Elements BR version 4.13 software (Nikon Instruments Inc., Melville, NY). Microscope hardware settings consisted of an S Plan Fluor 40× objective in the phase contrast 1 mode, with the NCB and D filters on. The video was captured at 640 × 512 resolution in a 40 ms exposure, with 2× analog gain and at 15 fps in 1 minute-long sections.

Data analysis

All video and image editing was carried out using the public domain program ImageJ (http://rsb.info.nih.gov/ij/). Once the videos were captured, they were first divided into 10 second sections and each section was processed individually. First, a projection of the entire video was used to construct an average image that was used for flat field correction, removing the background and the non-motile cells. Next, the video was converted to binary by applying a threshold using the default threshold algorithm. The cells were then tracked frame by frame using the Manual Tracking Plugin (Fabrice Cordelires, Institut Curie, Orsay France). The raw data were exported to the Chemotaxis and Migration Tool (Ibidi Software, Munich, Germany) where all the data were processed and the relevant information were extracted.

The characteristics of cellular movement analysed in this experiment included Forward Migration Index (FMI), where FMI X and FMI Y represent the efficiency of the forward migration of cells and how they relate to the direction of both axes; centre of mass which refers to the spatial average of all individual cell endpoints and indicates the movement of the entire group of cells; accumulated distance, which refers to the total length travelled by the cell; Euclidean distance, the length of the line segment connecting the starting and ending points of the cell's trajectory; velocity; and directness, a measure of the linearity of cell trajectories, which was calculated by comparing Euclidean and accumulated distances.

pH measurements

To understand the kinetics of *Listeria monocytogenes* in adapting to the acetic acid stress, additional characterization experiments were conducted to determine the change in pH over the growth time. Four samples of 40 ml broth were prepared; one sample was left as a control while the other three had acetic acid added to them to create concentrations of 1 mM, 10 mM and 100 mM. All samples were then adjusted to a pH of 5 using hydrochloric acid or sodium hydroxide when required. They were then inoculated using 100 μl of the working sample and incubated using the same conditions for 50 hours. At 5 hour intervals, a 2 ml aliquot was taken from each sample and used to record the pH and absorbance at 600 nm using a pH meter and UV-Vis spectrophotometer (Azzota Corporation, Randolph, NJ), respectively.

Results

Many different characteristics of cell motility were analysed in these microfluidic experiments. The two key factors in deciding how the cells react to changes in the environment are the cellular velocity (Fig. 3) and the directness of cellular movement (Fig. 4). The baseline (control), 1 mM and 10 mM acetic acid concentrations all displayed cellular velocity with an average of 6.5 $\mu m\ s^{-1}$, without any statistically significant difference. The directness is a comparison between the actual distance covered by the cell and the straight line distance between the starting point and the end point of the cell's movement. This means that directness is a good indication of how often the cell changed its path of travel or how random the movement was. As with the velocity, the baseline, 1 mM and 10 mM concentrations of acetic acid all displayed similar

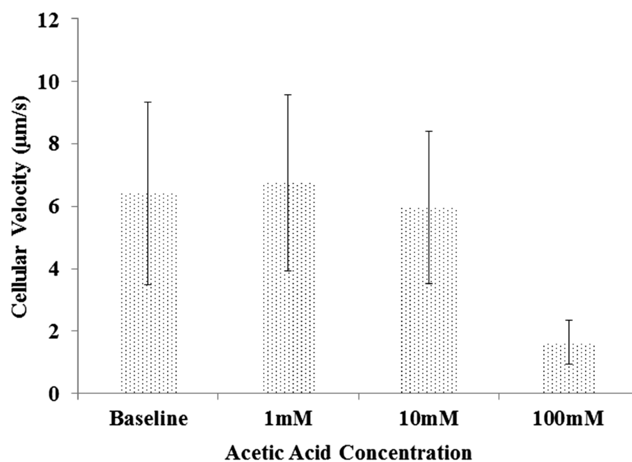

Fig. 3 A steep drop was observed in the cells' velocity when 100 mM of acetic acid was introduced to the environment, whereas no observable differences were observed between baseline and 1 mM or 10 mM acetic acid exposed cells.

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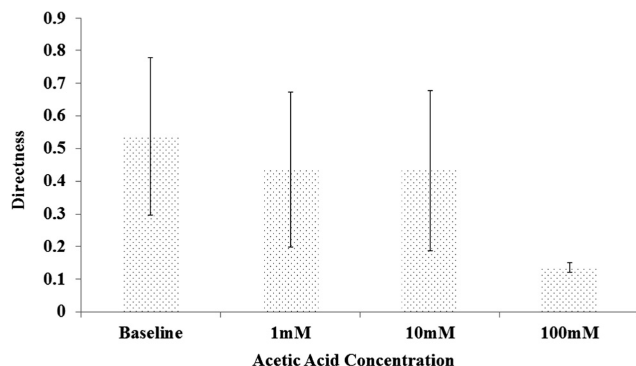

Fig. 4 The directness of the cells was greatly affected when 100 mM concentration of acetic acid was introduced to the environment of *L. monocytogenes*. No observable differences were found between baseline and 1 mM or 10 mM.

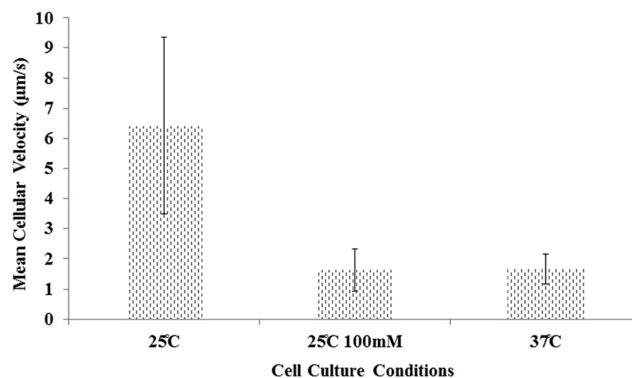

Fig. 5 *L. monocytogenes* cells cultured at 37 °C showed similar velocity to cells exposed to 100 mM of acetic acid that were cultured at 25 °C. The velocity for both of these was much lower than the baseline (control) cells cultured at 25 °C.

results of a directness of around 0.45 (no statistical difference). When the cells were exposed to the 100 mM concentration of acetic acid, their velocity reduced to 1.6 $\mu m\ s^{-1}$. Cells exposed to 1 mM and 10 mM concentrations of acetic acid showed no observable differences in reaction to the change in their environment when compared to baseline, whereas the cells exposed to the 100 mM concentration showed a drop in velocity and their movement became more random.

For the motility results to be viable the cells need to be in an environment where there is no overall flow that would bias the results in a certain direction; the viewing chamber needs to have no motion in it other than that of the cells' own self-propelled motion. By looking at the forward migration index and the centre of mass (Table 1), it can be assumed that there was no movement caused by the environment; any movement recorded was caused by the cells' own propulsion. Both the FMI and centre of mass are very low for all of the tests, indicating that the cells didn't favour any particular direction and that their movement was evenly distributed.

LM has been proven to display very different motility characteristics depending on the temperature of the environment.[6,23] To investigate the effects of temperature on motility, data were collected on cells cultured at 37 °C. The two key factors, velocity and directness, were compared to baseline at 25 °C and 100 mM acetic acid exposure tests (Fig. 5, 6). From these results, it is obvious that the cells grown at 25 °C and exposed to 100 mM of acetic acid displayed very similar motility characteristics as the cells grown at 37 °C. Both of the tests showed a much lower velocity and more random movement. LM cultured at 37 °C

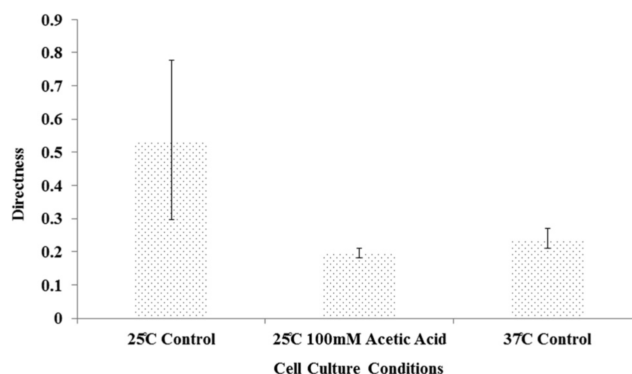

Fig. 6 *L. monocytogenes* cells cultured at 37 °C showed similar directness to cells exposed to 100 mM of acetic acid that were cultured at 25 °C. The directness for both of these was much lower than the baseline (control) cells cultured at 25 °C.

form no flagella; the genes required to form flagella are repressed at higher temperatures. This means that the cells must resort to alternative methods of motion, which in this case is actin based propulsion.[6] This provides a possible explanation for why the cells slow down when they are exposed to the 100 mM acetic acid. Acetic acid essentially depletes the ATP of the cell and hence reduces that available for motility. The similarity in their movements also suggests that the cells exposed to the 100 mM acetic acid may have had their flagella disabled and must rely on actin propulsion, causing their movement to resemble the cells that were grown without any flagella. Atomic force microscopy imaging experiments revealed that upon exposing the single cell

Table 1 Forward Migration Index (FMI) and the centre of mass (CoM) of single cell *Listeria monocytogenes* at various concentrations of acetic acid (AC) measured using the nanoporous microfluidic diffusion platform

	Baseline (25 °C)	1 mM AC	10 mM AC	100 mM AC	Baseline (37 °C)
FMI X	0.0103 ± 0.408	−0.0577 ± 0.374	0.00676 ± 0.377	0.0196 ± 0.165	0.193 ± 0.256
FMI Y	−0.0625 ± 0.427	−0.046 ± 0.324	−0.00121 ± 0.332	−0.0289 ± 0.149	−0.0901 ± 0.178
CoM X (μm)	0.214	−2.67	0.0851	0.199	0.269
CoM Y (μm)	−3.76	−2.73	−0.663	−0.281	−0.126

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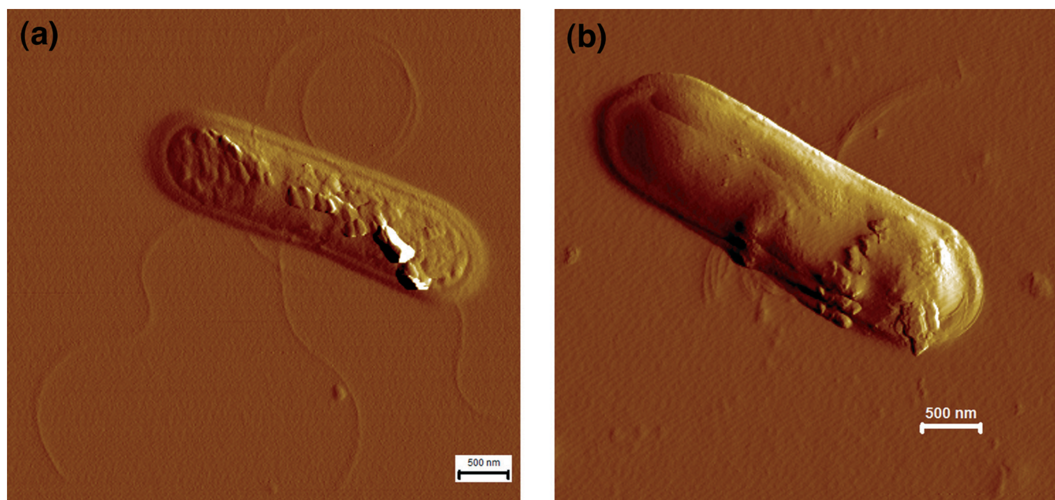

Fig. 7 Atomic force microscopy images of *L. monocytogenes* (a) control; treated with 100 mM acetic acid for 30 min (b). Flagella are clearly visible and intact in the control sample, while flagella appear detached in the acetic acid treated sample. Scale bar is 500 nm.

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Listeria monocytogenes to 100 mM acetic acid for 30 min, not only was the cell surface smoothened, it also caused changes in the microbial cell envelope (Fig. 7b), leading to the detachment of flagella. The flagellum appears intact in the control (untreated *Listeria monocytogenes*) (Fig. 7a). We were able to characterize the cell dynamics and the cell mechanics of *L. monocytogenes* during motility using a time-lapse video microscope at slower acquisition recording rates. In the presence of 10 mM acetic acid, majority of the single cell *L. monocytogenes* exhibited twirling behaviour during forward migration. After moving forward for one cell body length (2 μm) and rotating at about 180°, the cell ends up in a vertical angle position and exhibits tumbling motion (Fig. 8). This change in twirling to tumbling motion (ESI† Video 1) occurred in a time range of about 2 s. *L. monocytogenes* treated with 100 mM acetic acid did not exhibit twirling or tumbling motion (ESI† Video 2).

To further validate the results, data on pH and absorbance were collected. pH and absorbance are alternative ways to measure and track cell growth and cell density, so these aspects give a good indication of the effects that acetic acid has on the cells (Fig. 9, 10). As a normal part of their metabolism, LM naturally produces acidic by-products. Since their metabolism relies on carbohydrates, it forms and is naturally exposed to accumulations of acidic end products, including lactic acid and acetic acid.[25] These acids must then be eliminated from the cell and by doing so the pH of the environment is lowered.[24,25] Due to this effect, external pH can be an appropriate measurement of cellular growth; over time their environment should become more acidic as the cells go through their normal metabolic processes. As with the microfluidic experiments, the baseline, 1 mM acetic acid, and 10 mM acetic acid all showed very similar results, without significant effect on the growth of cells. The analysis of the cellular velocity, directness and the change in pH values confirms that 100 mM acetic acid had a significant effect on the motility and growth of the individual LM cells. There is a slight drop between the 0 and 5 hour marks, so we can assume that the cells were viable for a small amount of time at the beginning, but died soon after that.

Discussion

Acetic acid was not only chosen for its ease of application in the food industry, but also because it is biochemically effective as an antimicrobial agent. Despite the widespread use of

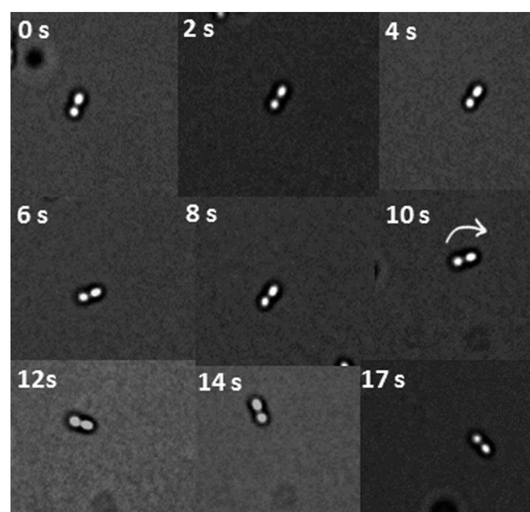

Fig. 8 Time-lapse images of *Listeria monocytogenes* LM MMB17 in 10 mM acetic acid show that the cells exhibit twirling and tumbling motility behaviour. The motility was documented using a phase-contrast video microscope with a 40× lens at 25 °C in a temperature-controlled stage. The time elapsed between each frame is indicated in seconds. Arrow indicates the direction of the movement. The final panel shows the cell position after completing the tumbling and twirling movement. The secondary constriction on the cell surface is clearly visible.

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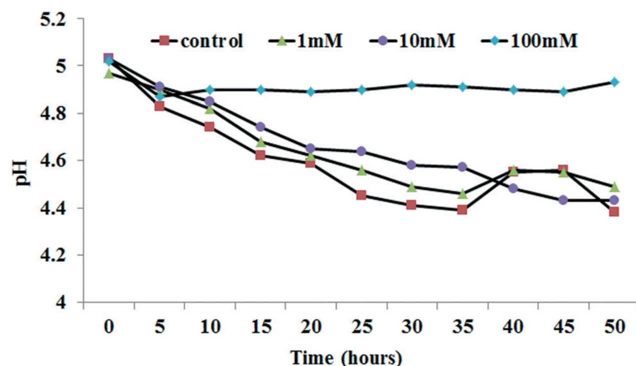

Fig. 9 Changes in pH decreased over time in the control as well as in 1 mM and 10 mM acetic acid treated *L. monocytogenes* experimental conditions. pH remained stable over time under the 100 mM condition.

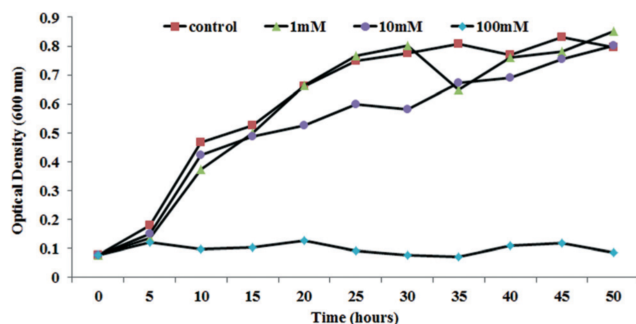

Fig. 10 Similar to pH, absorbance remained stable in cells exposed to 100 mM concentration of acetic acid but increased over time in the control and concentrations of 1 mM and 10 mM.

organic acids as antimicrobial agents, the exact mechanism of their action is not fully understood.[26,27] The accepted mechanism is that the undissociated form is able to diffuse across cellular membranes due to the uncharged form being lipid permeable. Once inside the cell, it is able to dissociate, raising the internal pH of the cell. This can have various effects on the cell such as disruption of the cell membrane, acidification of the cytoplasm, increase in osmotic pressure, stress, lowering of the intracellular ATP level, and interference with the production of various critical macromolecules. However, the most important effect caused by the lowering of the internal pH is the interference in the use of cellular energy.[25,28]

Cellular energy is affected by changes in pH in various ways. LM possesses various ways to balance the internal pH, but one of the major methods is through the use of active transport proton pumps. The enzyme complex hydrolyses ATP to generate a proton motor force and eliminate the excess protons from the cell in an attempt to bring the pH back to a stable level. By simply using up a portion of the available ATPs, the available energy of the cell is reduced.[29] At the same time, the generation of the ATP itself is directly inhibited at lower pH levels.[24] Both of these factors together mean that there is much less energy available for the cell to use for other purposes.

The bacterial flagellum is an incredibly complex mechanism measuring no more than 50 nm in diameter, built from about 20 different parts. The flagella are attached to a set of rings embedded in the cytoplasmic membrane which functions as the motor to rotate the flagella. Attached at the perimeter of the motor there are about ten torque generating units which create a motive force using an electrochemical gradient of ions across the cytoplasmic membrane.[19] The strength of this gradient has a direct effect on the speed of rotation of the flagellar motor; a decrease in the gradient results in much slower rotation of the flagellum and maintaining this gradient requires the use of active transport ATP driven proteins.[30]

We can conclude that the introduction of acetic acid into the cellular environment of LM has a large effect on the motility due to its ability to reduce available cellular energy, which in turn lowers the strength of cross membrane ion gradients and reduces the speed of flagellar rotation and cellular movement.

Another possible way to explain the decrease in motility is that the cells enter a different state where motility is not considered a high priority or is simply not needed by the cell. As part of the acid stress response, the repressor CodY is activated which controls multiple genes and has an important role in both carbon and nitrogen metabolism, flagella biosynthesis and many other cellular functions.[24] The CodY gene is considered to be responsible for inducing the change from rapid exponential growth and spread to a stationary phase.[31] When LM is exposed to an acidic environment it attempts to adapt to its new surroundings and part of this response is a decrease in motility.

This activation of the CodY regulator does come with potential problems as it also plays a large role in increasing the infectivity of LM once it is in a suitable environment. The natural environment suits the reproduction and spread of the bacterium, but once inside a host, the cells need to adapt to their new environment and change their function to be more focused on infectivity. Once inside a host, surface proteins called internalins are expressed, which induce the host cells to uptake the bacteria through endocytosis. Once inside the host, LM also produces listeriolysin, a pore forming hemolysin toxin used to escape from the vacuole. Now inside the host cytoplasm, LM multiplies rapidly and begins to express the surface protein ActA which is used to facilitate cell to cell spread. All of the virulence factors involved in these steps are regulated by the virulence activator gene PrfA, which in itself is controlled by the CodY regulator.[32] If the purpose of applying acetic acid is to reduce the spread of LM then this potential increase in infectivity due to the activation of the CodY regulator must be taken into consideration when choosing potential applications.

It is very peculiar though that no effects were noticed until the 100 mM acetic acid was applied to the cells. In other chemotaxis studies, much lower concentrations were found to have an effect on the motility of LM. It was found that there was a slight attraction towards glucose and tryptose at

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concentrations as low as 1 μM, with the most efficient and notable attraction being at concentration as low as 1 mM.[33] However, it is possible that acetic acid simply doesn't have a noticeable effect until exposure to higher concentrations. Another experiment found that the changes in genetic expression were caused by exposure to acetic acid and found that the minimum concentration to elicit a response and a change in RNA expression was 10 mM.[25] It is possible that the change caused by the 10 mM acetic acid was simply too small to be noticed in the microfluidic tests, or perhaps a strain variance simply caused less response to the acetic acid. Another possible reason for the lack of reaction at lower concentrations is the natural metabolism, which creates acidic by-products such as lactic and acetic acid,[24] LM is already adjusted to being exposed to low concentrations of acetic acid and is naturally not affected by it until exposure to higher concentrations.

The purpose of this study was to provide useful information that could be applied to the food industry in an attempt to reduce the spread and infectivity of LM. The reduction in motility that was observed achieves this purpose in multiple ways as it has been proven before that flagella are a key part of biofilm formation.[5,23] A biofilm is formed when a community of bacteria adheres to a surface, becomes non-motile and begins to excrete various substances that form a protective barrier, guarding the cells against potential hazards in the environment. This gives the cells various advantages of growth and survival and allows them to thrive in environments where it might have otherwise not been possible.[23] Biofilm-coated surfaces are particularly difficult to decontaminate, since bacteria in biofilms are more resistant to antimicrobial agents and antibiotics than their planktonic forms. Very little is known about the exact chemical mechanism behind the biofilm formation and due to this it is particularly challenging to properly design strategies to counter biofilm formation.[5] While it is agreed that flagella aid in the development of biofilms, there is conflicting evidence about how exactly they help. To address these questions, studies are underway in the Bionano Lab of the University of Guelph to investigate the biofilm formation of *Listeria monocytogenes* using microfluidic platforms.

One theory suggests that the motility itself which is caused by the flagella is critical to biofilm formation. When flagellum-minus and flagellum-paralyzed mutants were compared to a typical strain, it was noted that only the unmodified strain was able to properly form a biofilm. The suggested reasoning behind this is that the flagella supplies a force directed towards the surface that the cell is trying to attach itself to. This exerted force aids in overcoming any repulsive forces that may exist between the cells and the surface, aiding in initial attachment and the early stages of the biofilm formation.[5] Another common theory is that the flagella act as an adhesive and by sticking to a surface they help root the cells in place.[23,34] However, there are other theories that contradict both of these, stating that flagella are used purely for motility. In a more recent study it was stated that flagella do not serve as an adhesive for attachment to surfaces and are simply used as motility devices that contributed to host invasion.[6]

Regardless of the exact mechanism, it is agreed that the presence of flagella and the associated motility in some way play a vital role in infectivity.[3,6,34–36] By applying relatively low concentrations of acetic acid to the environment of LM it is possible to reduce this motility and therefore reduce the infectivity.

Conclusions

Using the developed nanoporous chemotaxis microfluidic device, we examined the influence of acetic acid on the migration of the food-borne pathogenic *Listeria monocytogenes*. The microfluidic experimental results indicated that somewhere within the 10 mM to 100 mM range there is a concentration of acetic acid that has a large effect on the flagella and flagellar motility of *Listeria*. By disabling the flagella, its ability to function as a pathogen is greatly reduced and this information could easily be applied to the food industry where outbreaks are a very serious problem that can have severe consequences. LM is a bacterium that can be difficult to control *via* conventional methods and hence development of novel antimicrobial practices is crucial for food safety and public health.

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