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Catabolic activity and biofilm formation of foodborne *Listeria monocytogenes* strains

Nowshin Shyara Sharar[1] · Lay Ching Chai[1] · Kwai Lin Thong[1] (iD)

Abstract

Listeria monocytogenes is a major foodborne pathogen causing increased morbidity worldwide. It forms resistant biofilm structures in food processing facilities after sanitization, consequently creating a public health concern. Many studies on the metabolism and transmission of *L. monocytogenes* has provided insights into its intracellular infection process, however there is limited understanding on the substrate utilization of the bacteria. Therefore, the main objective of this study was to investigate the carbon and nitrogen substrate catabolism and the biofilm forming potential of 3 Malaysian *L. monocytogenes* strains (LM41, LM92 and LM115) previously isolated from ready-to-eat foods. Biolog Phenotype Microarray (PM) system was used to study the catabolic activity of the foodborne strains in 190 carbon and 380 nitrogen sources. PM analysis showed that the carbon and nitrogen catabolic activity of *L. monocytogenes* strains were considerably limited and these strains utilised Tween 40 and Tween 80, which are commonly used for the sanitation in food and meat processing industries. Furthermore, all 3 strains showed strong biofilm forming potential in nutrient-rich and nutrient-limited media, irrespective of the serogroups. The data generated could be utilised to develop alternative measure to inhibit biofilm formation in *L. monocytogenes* in the food processing environment.

Keywords Biofilm · Foodborne pathogen · *Listeria monocytogenes* · Phenotype microarray · Ready-to-eat food

1 Introduction

Listeria monocytogenes is a facultative anaerobic, non-sporulating, Gram-positive rod shaped bacterium that is commonly found in the soil, plant material and raw and processed food products. It can survive and grow over a wide range of environmental conditions such as refrigeration temperatures (0–4 °C), low pH (2–4) and high salt concentration (10% NaCl), thus making them very hard to control (Doijad et al. 2015; Zhou et al. 2012). It is an emerging foodborne pathogen and the causative agent of human listeriosis. Infection is typically acquired through the ingestion of contaminated food products and the

common site of infection is the intestinal epithelium. *L. monocytogenes* infection has several clinical conditions, including meningitis, encephalitis, gastroenteritis, septicemia, abortions, convulsions and causes high mortality (20–30%) rates among pregnant women, neonates, elderly and immune-compromised patients (Lomonaco et al. 2015). *Listeria monocytogenes* is accountable for the majority of deaths caused by food-borne epidemics in Europe and USA (Lomonaco et al. 2015). The largest listeriosis outbreak in U.S. history occurred in 2011, due to the consumption of cantaloupe from a single farm that caused 147 illnesses, 33 deaths, and 1 miscarriage in 28 states (CDC 2012). In Malaysia, foodborne *L. monocytogenes* had been detected in raw and ready-to-eat (RTE) foods and the majority of incidences resulted from the contamination of *L. monocytogenes* in various street-side foods, salads, vegetables, raw and processed deli meats and fish products (Jamali et al. 2013; Jeyaletchumi et al. 2012; Marian et al. 2012; Ponniah et al. 2010; Wong et al. 2011). Listeriosis is not a notifiable disease in Malaysia, hence official report on foodborne listeriosis is lacking.

✉ Kwai Lin Thong
  thongkl@um.edu.my

[1] Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

Listeria monocytogenes has 4 evolutionary lineages (I, II, III and IV) containing 13 serotypes based on the subtyping of the somatic O and flagellar H antigens (Orsi et al. 2011). Lineage I consists of serotypes 1/2b, 3b, 4b, 4d and 4e (Liu 2008; Orsi et al. 2011). Lineage II contains serotypes 1/2a, 1/2c, 3a and 3c; and Lineage III includes serotypes 4a and 4c and some strains from serotype 4b (Kathariou 2002; Liu 2006, 2008). Both sporadic (serotype 1/2a, 1/2b and 4b) and outbreak cases (serotype 1/2a and 4b) of human listeriosis are mainly caused by *L. monocytogenes* strains belonging to Lineage I and II (Buchanan et al. 2017; Lomonaco et al. 2015; Orsi et al. 2011).

Listeria monocytogenes is one of the leading foodborne pathogens in both developed and developing countries and able to form resistant biofilm structures in food processing environment (Kadam et al. 2013; Lomonaco et al. 2015). Biofilms are bacterial communities which are surrounded by extracellular polymeric substances attached to biotic or abiotic surfaces (Zhou et al. 2012). *Listeria monocytogenes* biofilms have been found to show increased resistance to cleaning, disinfectants, desiccation and UV exposure, leading to enhanced persistence to processing plants (Gandhi and Chikindas 2007). Biofilm structures can disperse and contaminate the food products during processing and packaging, thus making them a food safety concern (O'Toole et al. 2000; Zhou et al. 2012). Industrially processed foods, such as cheese and meats have often been reported to be contaminated with *L. monocytogenes* (Alonso et al. 2014; Doijad et al. 2015; Hain et al. 2007). Previous studies have shown that serotype 1/2a, 1/2b and 4b are responsible for 95% of the clinical cases of listeriosis worldwide (Kadam et al. 2013; Lomonaco et al. 2015). In the current study, 3 Malaysian foodborne *L. monocytogenes* strains belonging to the pathogenic serogroups were studied for their biofilm forming ability.

Phenotype Microarray is a well-established platform to determine the cellular phenotypes of various microorganisms under different growth conditions (Bochner 2001). Studies with other bacteria have successfully determined their metabolic activity in a variety of substrates (Chelvam et al. 2015; Chong et al. 2017; Farrugia et al. 2013; Tang et al. 2010). Although there is a relatively high incidence of foodborne *L. monocytogenes* in raw and RTE foods in Malaysia (Jamali et al. 2013; Kuan et al. 2013; Marian et al. 2012; Ponniah et al. 2010; Wong et al. 2011), studies regarding the catabolic activity of *L. monocytogenes* are lacking. Furthermore, biofilm formation is a major issue in the food industry since microbial biofilms are resistant to industrial chemicals and cleaning processes (Da Silva and De Martinis 2013; Kadam et al. 2013). While previous studies have shown the formation of different biofilm structures between different serotypes of this pathogen (Doijad et al. 2015; Stepanović et al. 2000), there are no reports about the importance of substrate utilization in biofilm formation. Therefore, the present study focuses on the carbon and nitrogen substrate utilization of 3 pathogenic *L. monocytogenes* strains using Biolog Phenotype Microarray system and their biofilm forming ability in different substrates.

2 Materials and methods

2.1 Background of bacterial strains

Malaysian *L. monocytogenes* strains isolated from RTE food samples (Jamali et al. 2013) were selected for this study. The strains belong to serogroups 1/2a, 3a (LM92), 1/2c, 3c (LM41) and 4b, 4d, 4e (LM115). Among them was LM115, a multiple-drug resistant strain (Jamali et al. 2013). The strains were maintained in LB broth with 50% glycerol stock and revived in LB broth to perform the individual assays.

The Phenotype MicroArray (PM) assay included 6 × 96-well PM panels (PM1, PM2A, PM3B, PM6, PM7 and PM8), which determined the ability of 3 *L. monocytogenes* strains to show responses in 190 carbon sources and 380 nitrogen compounds (Table S1). The principle of the test relies on the reduction of tetrazolium violet dye to detect respiration (NADH formation) in various compounds (Bochner 2001).

2.2 Analysis of phenotype microarray data

PM data of plates PM1, PM2A, PM3B, PM6, PM7 and PM8 were recorded using OmniLog OL_FM_12 kinetic software (Biolog, USA) and analyzed using Microsoft Excel. The OmniLog PM Software generated a time course curve for tetrazolium color development for 48 h. For each well, the average area under growth (AUG) and the average slope of time course data were used to measure the threshold value (maximum 100 OmniLog Biolog unit, OU) for growth in the PM plates. Phenotypes were determined based on the difference in average area under growth curve (AUG). The well was considered positive if the standardized average area is equal or exceeded the threshold value. Wells with a high average area value but with a slope close to zero were considered as less significant than wells with a smaller area but increasing signal over time. During data processing, the option of A1 zero (negative control) was selected to subtract the background from each wells. Plates were analyzed in duplicates and errors were excluded from the analysis (Kalai Chelvam et al. 2014).

2.3 Validation test for PM data

To confirm the data obtained in the PM analysis, the *L. monocytogenes* strains (LM41, LM92 and LM115) were grown in minimal medium supplemented with individual carbon and nitrogen substrates. Overnight cell cultures of *L. monocytogenes* strains were harvested at 6000 rpm for 5 min, washed with $1\times$ phosphate buffered saline (PBS). Cellular turbidity was adjusted to McFarland standard No.5 which corresponds to 10^8 CFU-mL^{-1}. Serial dilutions were carried out to ensure that the final concentration of cells in each well was standardized to 10^3 CFU-mL^{-1}. An aliquot of 200 µL inoculum (M9 minimal medium) with individual carbon and nitrogen substrates were added into the wells of sterile 96-well microtiter plates and incubated at 37 °C for 24 h. After incubation, cellular turbidity was measured at O.D.600 nm wavelength using a microplate reader (Epoch, Germany). Negative control contained minimal medium and the substrates. This experiment was repeated 3 times and the results were averaged.

2.4 Biofilm assay

The biofilm assay was carried out according to the method described by Stepanović et al. (2000). The cell density of an overnight cell cultures of 3 *L. monocytogenes* strains (LM41, LM92 and LM115) were adjusted. An aliquot of 200 µL bacterial culture was added into the wells of microtiter plates and incubated at 37 °C for 24 and 48 h. After incubation, the unbound cells were removed and washed with 1x PBS. The adhered cells were heat-fixed at 80 °C for 30 min. Quantification of biofilm cells was performed by staining of adhered cells with Crystal Violet (0.5%), followed by washing and de-staining using Ethanol:Acetone (80:20%) solution. The absorption of the eluted stain was measured at O.D.590 nm wavelength.

Based the O.D.$_{590nm}$ readings and calculation of cut-off O.D. (O.D.$_c$), strains were classified as non-biofilm producer, weak, moderate and strong biofilm producer. The cut-off O.D. is defined as 3 standard deviations above the mean O.D. of negative control (Kalai Chelvam et al. 2014; Stepanović et al. 2000). Strains were:

- non-biofilm producer (0), if O.D. $\leq$ O.D.c,
- weak biofilm producer (+), if O.D.c < O.D. $\leq$ (2 × O.D.c),
- moderate biofilm producer (+ +), if (2 × O.D.c) < O.D. $\leq$ (4 × O.D.c) and,
- strong biofilm producer (+ + +), if O.D. > (4 × O.D.c).

Negative control contained sterile LB broth, and the value of negative control was deducted from the background and the experiment was performed in triplicates to ensure the reproducibility of the results.

Overnight cell cultures of 3 *L. monocytogenes* strains were harvested at 6000 rpm and washed with $1\times$ PBS. The cell density of bacterial cultures was adjusted. Aliquots of 200 µL bacterial cultures in minimal medium were added to the wells of microtiter plates and incubated at 37 °C for 24 and 48 h. The adhered cells were heat-fixed, followed by staining, washing and de-staining. The absorbance (O.D.$_{590\ nm}$) was measured. Negative control contained minimal medium only. The results were averaged and the standard deviation of negative control was calculated for the biofilm classification (Stepanović et al. 2000).

Carbon and nitrogen substrates were selected and added to M9 minimal medium to induce biofilm formation in *L. monocytogenes* strains. 20 mM solutions of each carbon and nitrogen substrates were aseptically prepared. Aliquots of 200 µL bacterial cultures were added into the wells of microtiter plates containing 20 mM carbon or nitrogen substrates and then incubated at 37 °C for 24 and 48 h. Unbound cells were removed and washed with sterile dH$_2$O. The adhered cells were heat-fixed, followed by staining, washing and destaining. Absorbance was measured at O.D.$_{590\ nm}$. Negative control contained minimal medium and the substrates. This assay was performed in triplicates and the results were averaged.

3 Results

The tested *L. monocytogenes* strains were able to catabolize different carbon and nitrogen sources. The preferred carbon substrates were: carbohydrates, polymers, nucleosides and the preferred nitrogen substrates were: amino acids, amines, nucleosides and dipeptide sources (Table 1).

Among the 190 carbon substrates tested, only 38 (20%) were catabolized by the strains. The carbon substrates were classified into carbohydrates (n = 26), nucleosides (n = 5), polymers (n = 4), amino acids (n = 2) and amide (n = 1) (Fig. 1). Out of 38 carbon substrates, only 29 (15.3%) were catabolized by all 3 strains (Fig. 2). However, alcohol, carboxylic acid, fatty acid and esters were not catabolized by any of the 3 strains.

A total of 380 nitrogen substrates were tested in PM3B, PM6, PM7 and PM8 microplates. Only 61 (16%) nitrogen substrates were catabolized by the strains. The substrates were categorized into amino acids (n = 6), amines (n = 5), nucleosides (n = 7), heterocyclic organic compounds (n = 3) and peptides (n = 10). Among them, only 7 substrates were actively metabolized by all 3 strains (Fig. 3). The substrates were: D-glucosamine, D-mannosamine, *N*-acetyl-D-glucosamine, cytidine, uridine, xanthosine and uric acid. Strain LM115 utilized only 5 peptide nitrogen

Table 1 Catabolism of carbon and nitrogen substrates by *Listeria monocytogenes* strains

|	Number of substrates tested	Number of substrates catabolized by all three strains	Strain-specific substrate catabolism			Number of substrates catabolized by at least two strains	Number of substrates not catabolized
			LM41	LM92	LM115		
Carbon substrates							
Sugar and derivatives							
Monosaccharide	20	8 (40)	1	0	1	2 (10)	10 (50)
Disaccharide	9	4 (44)	–	–	–	–	5 (56)
Oligosaccharide	10	1 (10)	–	–	–	–	9 (90)
Polysaccharide	6	1 (17)	–	–	–	–	5 (83)
Sugar alcohol	18	3 (17)	–	–	–	–	15 (83)
Amino sugar	8	2 (25)	–	–	–	–	6 (75)
Deoxy sugar	4	–	–	–	–	–	4 (100)
Aldaric acid	4	–	–	–	–	–	4 (100)
Aldonic acid	2	–	–	–	–	–	2 (100)
Uronic acid	8	–	–	–	–	–	8 (100)
Glycosides	9	2 (22)				–	7 (78)
Other	1	1 (100)	–	–	–	–	–
Polymers	11	4 (36)	–	–	–	–	7 (64)
Amide	3	–	1	0	0	–	2 (67)
Amine	5	–	–	–	–	–	5 (100)
Amino acid	30	–	2	0	0	–	25 (83)
Nucleic acid							
Nucleosides	5	4 (80)	–	–	–	1 (20)	–
Nitrogen substrates							
Amino acid	33	–	1	0	5	–	27 (82)
Other	1	–	0	0	1	–	–
Amine	17	3 (18)	0	0	1	1 (6)	12 (71)
Amide	4	–	–	–	–	–	4 (100)
Nucleic Acid	13	3 (23)	1	0	0	3 (23)	6 (46)
Heterocyclic organic compounds	10	1 (10)	0	0	0	2 (20)	7 (70)
Peptide nitrogen sources	294	–	34	1	3	2 (1)	254 (86)

sources, whereas strain LM92 utilized only 1 peptide in plate PM3B. Strain LM41 showed a higher respiration rate (10.5%) in plates PM6, PM7 and PM8, while strains LM92 and LM115 did not utilise in any of them (Figs. 1, 3).

To validate the PM analysis, 24 carbon and nitrogen substrates were selected based on the PM catabolic profile (Table 1). The validation experiment supported the PM analysis, with the exception of L-cysteine, D-xylose, riboflavin, thiamine, D-biotin, Tween 40 and L-glutamic acid. These substrates were not utilized in PM growth conditions; however they supported bacterial growth when added to the minimal medium (Table S2).

All 3 strains were able to produce strong biofilm in both LB broth and minimal medium after 24 and 48 h incubation. To induce biofilm formation in the studied strains, 23 carbon and nitrogen substrates were chosen based on the PM catabolic profile (Table 1). Out of 23 substrates tested, only 15 substrates induced biofilm formation in M9 medium. The substrates were: adenosine, cytidine, uridine, glutamic acid, rhamnose, Tween 40, riboflavin, thiamine, biotin, salicin, fructose, maltose, galactose, trehalose and xylose (Table 2). All 3 strains formed weak biofilm in threonine and glycerol. Strain LM92 formed moderate biofilm in glycine, lysine, cysteine and glucose; whereas LM41 and LM115 formed weaker biofilm. Moreover, glucose did not induce strong biofilm formation in any of the strains, instead it produced moderate to weak biofilms. All 3 strains formed strong biofilm

Fig. 1 The catabolic phenome of *Listeria monocytogenes* strains. Strengths of carbon and nitrogen utilization phenotypes of *L. monocytogenes* strains LM41, LM92 and LM115 are determined using Biolog™ Phenotype Microarray plates PM1, PM2A, PM3B, PM6, PM7 and PM8. The maximal kinetic curve height is expressed as a color-scale ranging from 0 (green) to 200 (red) area under the curve (AUC) units. Substrates are considered as 'strongly utilized' if AUC ≥ 180, 'weakly utilized' if AUC ≤ 50, and 'not utilized' if AUC < 10 (color figure online)
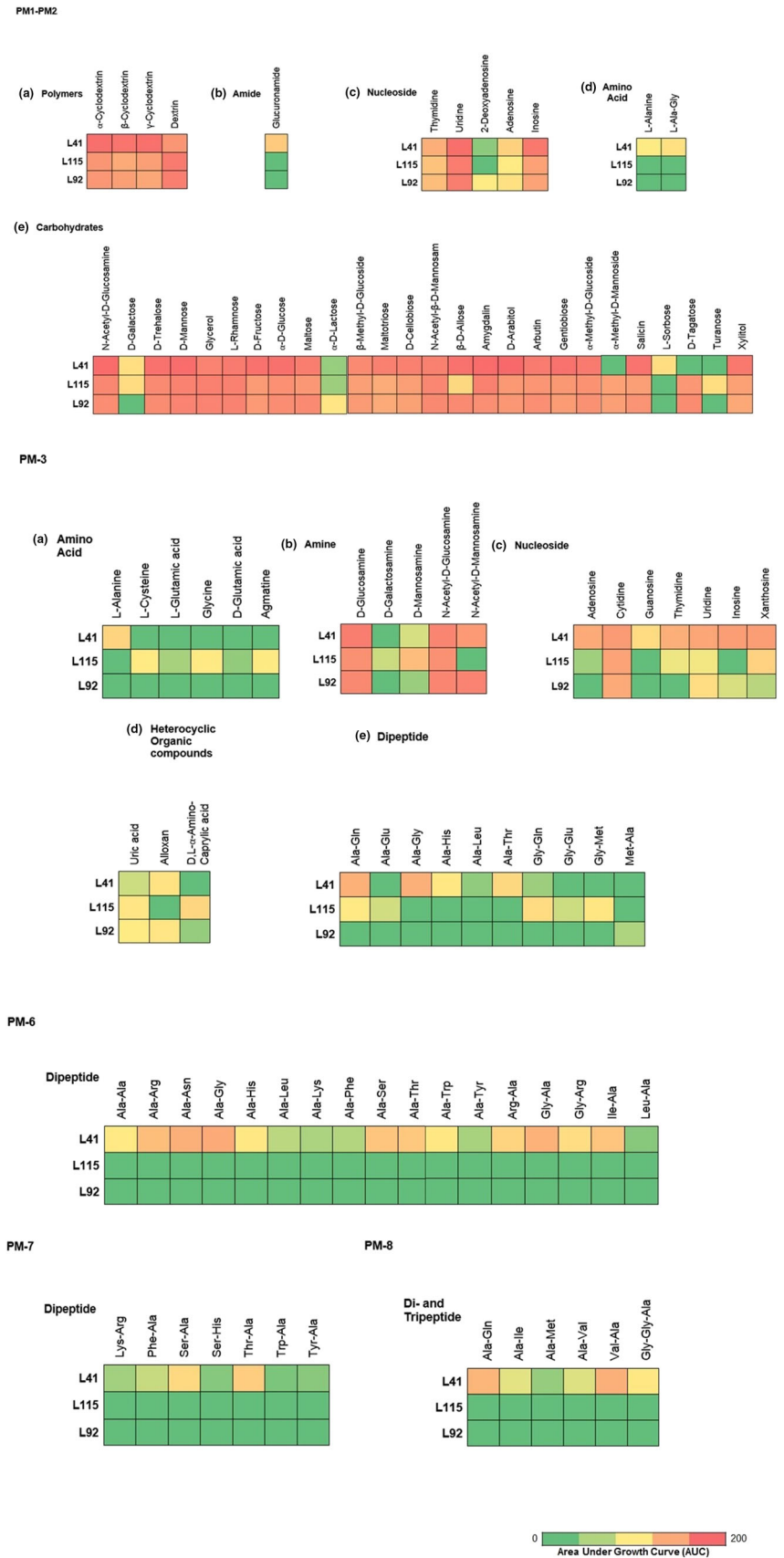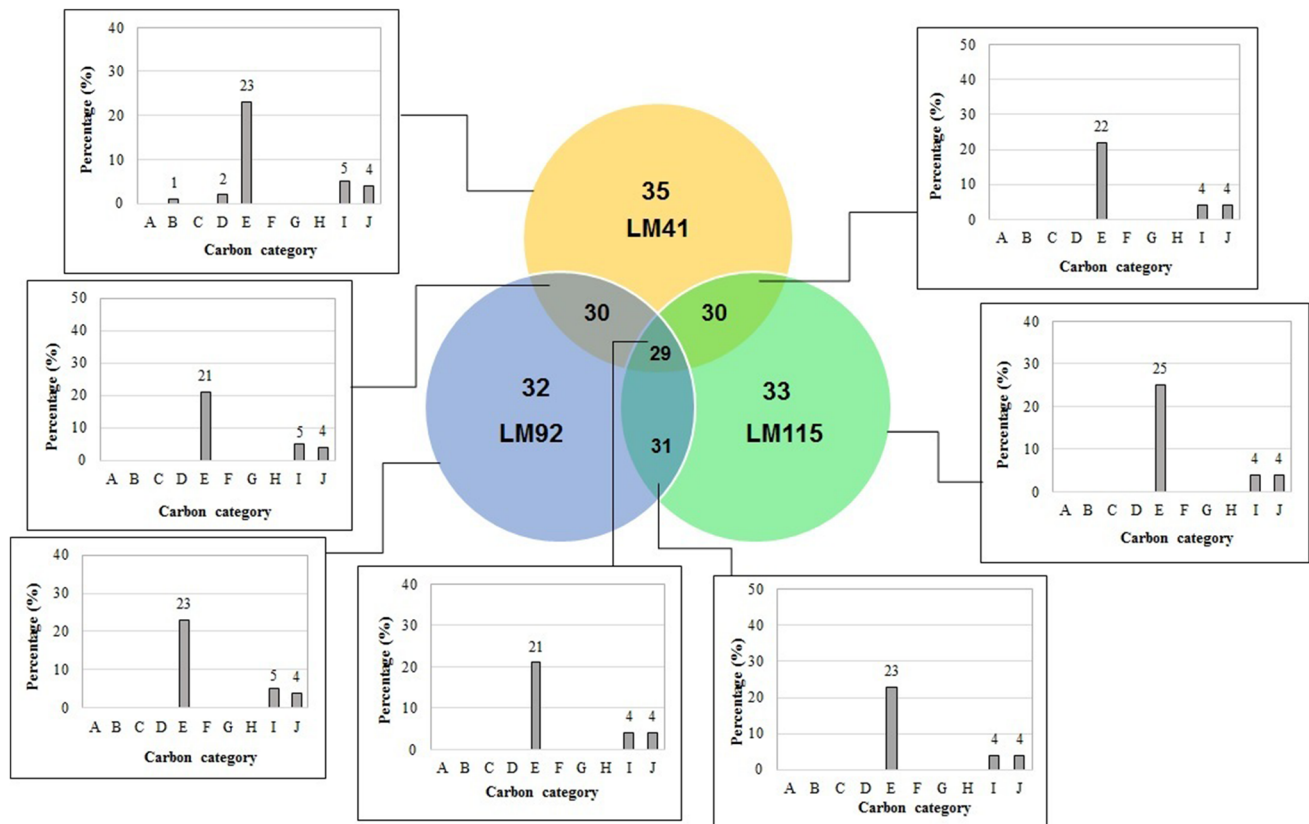
Fig. 2 Venn diagram showing the carbon catabolic activity of three *L. monocytogenes* strains. A total of 190 carbon substrates are tested. They are categorized as: A: Alcohol, B: Amide, C: Amine, D: Amino acid, E: Carbohydrate, F: Carboxylic acid, G: Ester, H: Fatty acid, I: Nucleotid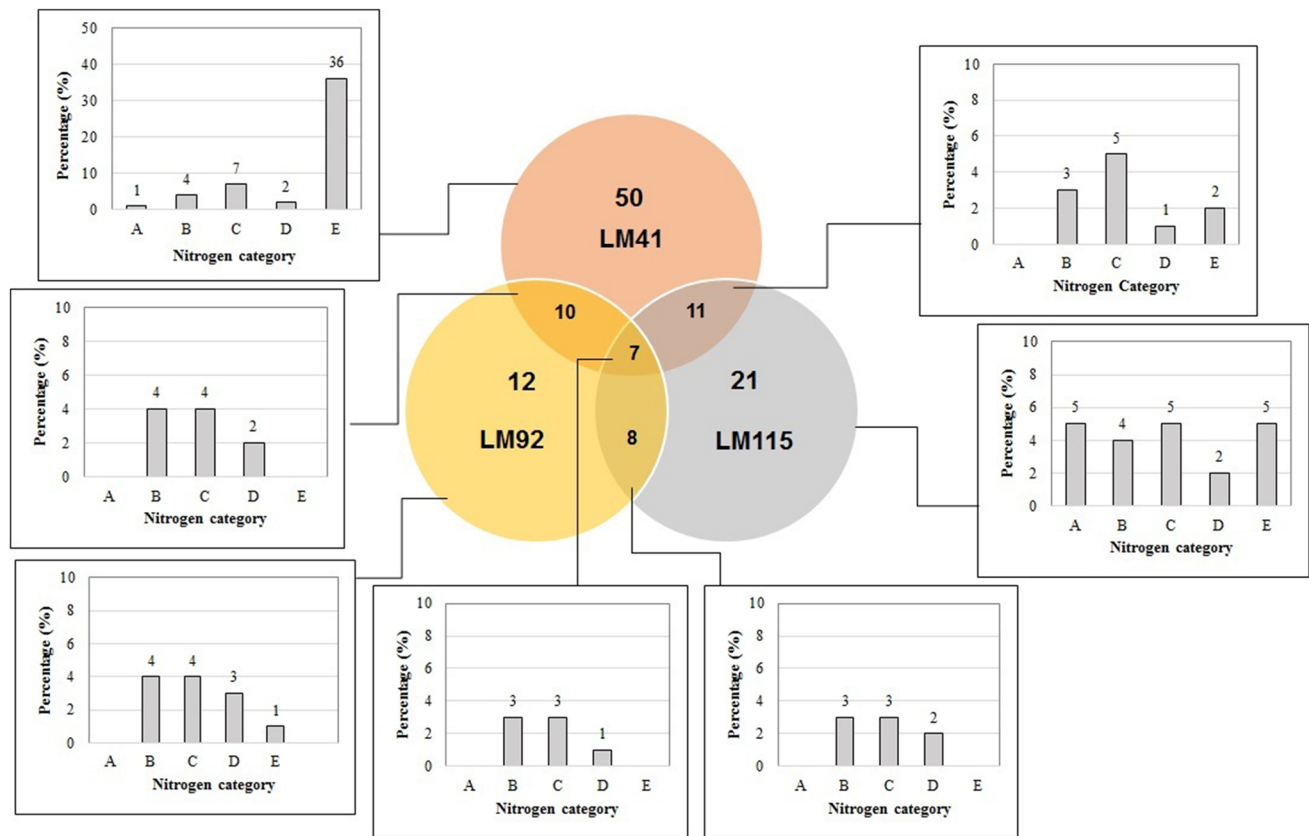e and J: Polymer. Y-axis indicates the percentage of carbon utilised. X-axis shows the carbon category for each carbon substrate tested. The Venn diagram was obtained based on average growth curve area and the numerals indicate the carbon substrates utilized by three strains, utilized by two strains and each strain individually

in minimal medium when supplemented with Tween 40 and Tween 80, although, they did not use them as sole carbon sources in PM growth conditions (Table 2). On the contrary, none of the strains formed biofilm when thymidine was added to the medium. The PM analysis indicated growth of three strains in thymidine, yet it failed to initiate biofilm formation in minimal medium.

4 Discussion

According to the PM analysis, the carbon catabolism capacity of the *L. monocytogenes* strains was extremely low, only 29–38% out of 190 carbon substrates tested (Fig. 1). Compared to other pathogenic bacteria, i.e. *Acinetobacter baumannii*, *Bacillus subtilis* and *Escherichia coli* were able to catabolize a variety of carbon and nitrogen sources (Bren et al. 2016; Farrugia et al. 2013), thus signifying that *L. monocytogenes* strains are metabolically less competent. The foodborne strains mainly utilized carbohydrates, polymers and nucleotides and the catabolism rate and choice of carbon sources were rather similar between the 3 strains (Fig. 2). Moreover, the nitrogen substrate catabolism was even lower than carbon catabolism. The strains utilized only 16% out of 380 nitrogen substrates tested, wherein 13% was utilized by strain LM41 only (Fig. 3). All 3 strains catabolized a higher number of nitrogen substrates individually and their preference for nitrogen sources were relatively extensive. For example, 7 (2%) out of 380 nitrogen sources were utilized by 3 strains, while strain LM41 utilized 10% of the dipeptide nitrogen sources and other 2 strains were unable to utilize peptides (Figs. 1, 3). The genomic analysis of two *L. monocytogenes* strains (Lim et al. 2016) showed that the strains carry a number of carbohydrate, amino acid, fatty acid and nucleotide metabolizing genes and therefore, this foodborne pathogen should be able to metabolize a wide range of carbohydrate and amino acid compounds. The strains were unable to utilize alcohol, carboxylic acid, ester and fatty acid sources and the limited carbon and nitrogen catabolic activity of the strains indicates an incomplete or restrictive metabolic pathway involved in their metabolism.

Fig. 3 Venn diagram showing the nitrogen catabolic activity of three *L. monocytogenes* strains. A total of 380 nitrogen substrates are tested. They are categorized as: A: Amino acid, B: Amine, C: Nucleoside, D: Heterocyclic Organic Compound and E: Dipeptide. Y-axis indicates the percentage of nitrogen utilised. X-axis shows the nitrogen category for each nitrogen substrate tested. The Venn diagram was obtained based on average growth curve area and the numerals indicate the nitrogen substrates utilized by three strains, utilized by two strains and each strain individually

Furthermore, *L. monocytogenes* strains showed limited growth activity in D-glucose, while an increased growth rate was observed in L-glutamic acid, fructose, maltose, galactose, salicin and plant-derived carbon sources, arbutin, D-xylose and sorbitol (Fig. 1). Conceivably, glucose is not the sole carbon source for their optimum growth. *L. monocytogenes* has a complex phosphotransferase system mediated glucose transport system, where it catabolizes fructose, mannose, cellobiose as sole carbon sources when grown in a defined minimal liquid medium and were able to utilize branched-chain amino acids (Tsai and Hodgson 2003). In this study, none of the strains were able to grow on ammonia, nitrate, nitrite and urea since they lack the genes for nitrate and nitrite reductases (Haber et al. 2017); as a result, they only catabolized organic nitrogen sources (Fig. 1). Additionally, glycine, xylose, riboflavin, thiamine, biotin and Tween 40 supported bacterial growth in the validation experiment, while they did not represent growth in PM analysis (Table S2). This could be due to the low concentration of substrates in the Biolog PM plates. Moreover, riboflavin, biotin and thiamine may act as

cofactors in minimal medium since they did not require them for growth.

Biofilm formation is a major nuisance in food manufacturing environment. Foodborne pathogens, namely *Listeria monocytogenes*, *Salmonella* spp., pathogenic *Escherichia coli*, *Bacillus cereus*, *Campylobacter* spp., *Staphylococcus aureus* have the capability to adhere and create biofilms on various surfaces in food processing industries (Giaouris and Simões 2018). Previous studies have shown that the foodborne *L. monocytogenes* strains are resistant to cleaning and sanitization and can survive after the disinfection process (Gandhi and Chikindas 2007; Mertins et al. 2007). Studies by Møretrø et al. (2017) and Dutta et al. (2013) demonstrated that *L. monocytogenes* showed increased resistance towards quaternary ammonium compounds (QAC), such as Benzalkonium chloride due to the presence of QAC resistance genes, *qacH* and *bcrABC* cassettes in their genome. Polysorbates are non-ionic surfactants often emulsified in the sanitizers to clean food and meat processing plants. Møretrø et al. (2017) demonstrated that *L. monocytogenes* can survive as biofilms in the surface water residues containing residual QAC

Table 2 The biofilm forming potential of *L. monocytogenes* strains

Media and Substrates	Substrate utilization in PM			Biofilm Classification		
	LM41	LM92	LM115	LM41	LM92	LM115
LB broth	N/A	N/A	N/A	+++	+++	+++
M9 medium only	N/A	N/A	N/A	+++	+++	+++
Adenosine	(+)	(−)	(+)	+++	+++	+++
Thymidine	(+)	(+)	(+)	−	−	−
Cytidine	(+)	(+)	(+)	+++	+++	+++
Uridine	(+)	(+)	(+)	+++	+++	+++
L-Glutamic Acid	(−)	(−)	(+)	+++	+++	+++
L-Rhamnose	(+)	(+)	(+)	+++	+++	+++
Tween 40	(−)	(−)	(−)	+++	+++	+++
Riboflavin	(−)	(−)	(−)	+++	+++	+++
Thiamine	(−)	(−)	(−)	+++	+++	+++
Biotin	(−)	(−)	(−)	+++	+++	+++
Salicin	(+)	(+)	(+)	+++	+++	+++
D-Fructose	(+)	(+)	(+)	+++	+++	+++
Maltose	(+)	(+)	(+)	+++	+++	+++
D-Galactose	(+)	(+)	(+)	+++	+++	+++
D-Trehalose	(+)	(+)	(+)	+++	+++	+++
D-Xylose	(−)	(−)	(−)	+++	+++	+++
Glycine	(−)	(−)	(+)	+	+ +	+
L-Lysine	(−)	(−)	(−)	+	+ +	+
L-Cysteine	(−)	(−)	(+)	+	+ +	+
L-Threonine	(−)	(−)	(-)	+	+	+
Glycerol	(+)	(+)	(+)	+	+	+
Tween 80	(−)	(−)	(−)	+ +	+ +	+++
D-Glucose	(+)	(+)	(+)	+	+ +	+ +

[a] (+) = positive for growth, (-) = negative for growth, N/A = not applicable

[b]Interpretation based on cut-off O.D. (O.D.c); O.D. $\leq$ O.D.c = non biofilm producer, [-]; O.D.c < O.D. $\leq$ (2 × O.D.c) = weak biofilm producer, [+]; (2 × O.D.c) < O.D. $\leq$ (4 × O.D.c) = moderate biofilm producer, [+ +]; (4 × O.D.c) < O.D. = strong biofilm producer, [+ + +] (Stepanović et al. 2000)

after disinfection, therefore conferring growth advantage to the bacteria. In this study, both Tween 40 and Tween 80 induced strong biofilm formation in the selected strains in nutrient limited conditions.

Many studies indicated that *L. monocytogenes* strains produce more biofilm in nutrient limited medium; while they have increased growth rate in nutrient-rich medium (Doijad et al. 2015; Zhou et al. 2012). In this study, all 3 strains showed enhanced growth activity and formed strong biofilms in both LB and minimal media (Table 2), suggesting that this foodborne pathogen employs an alternative mechanism to proliferate and produce biofilm in nutrient-starved conditions. This study also showed that the *L. monocytogenes* strains were able to attach and form biofilm on polystyrene microtiter plates. Polystyrene is one of the most widely used plastics, especially in commercial food packaging and the equipment used in food production facility. Several studies reported that persistent biofilm

structures are readily formed on plastic surfaces and niches that are difficult to clean (Borucki et al. 2003; Doijad et al. 2015). On the contrary, thymidine did not induce biofilm formation in any of the strains, although increased growth rate was observed when supplemented in minimal medium (Table 2). Therefore, thymidine may not be the ideal carbon source to initiate the biofilm formation in *L. monocytogenes*, since it inhibited the biofilm-forming process in a nutrient limited condition. Further experimental analysis should be taken into consideration regarding the impact of thymidine in biofilm control and prevention.

5 Conclusion

In conclusion, this study revealed that the foodborne *L. monocytogenes* strains were strong biofilm producers even in the absence of nutrients. The biofilm-forming strains

thus facilitate their survival and persistence in food processing environment which may lead to the subsequent contamination of food products. Additionally, the phenotypic analysis showed that the carbon and nitrogen catabolic activity of three strains were considerably limited. Further genomic analysis is required to investigate the biosynthetic pathways involved in their metabolism and identify the genes for the enzymes responsible for the catabolism of various substrates. Lastly, alternative approaches must be implemented to prevent biofilm formation in food processing environment to improve the food quality and ensure the welfare of human health.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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