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Posted Date: 31 May 2024

doi: 10.20944/preprints202405.2144.v1

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 doi:10.20944/preprints202405.2144.v1

Article

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Abstract: *Listeria monocytogenes* is an emerging pathogen responsible for serious foodborne disease, listeriosis. Commensal gut microbiota is the first line of defense against pathogen internalization. Gut microbiome can be modified by prebiotic substrates, which are among others frequently added to food products and dietary supplement. Prebiotics should selectively support the growth of beneficial microbes and thus improve host health. Nevertheless, little is known about their effect on growth of L. monocytogenes. The aim of this study was to evaluate the growth ability of four L. monocytogenes strains, representing the most common serotypes, on prebiotic oligosaccharides: beta(1,3)-D-glucan, inulin, fructooligosaccharides, galactooligosaccharides, lactulose, raffinose, stachyose, 2´-fucosyllactose and mixture of human milk oligosaccharides as a sole carbon source. The results showed that only beta(1,3)-D-glucan was metabolized by L. monocytogenes. Therefore, the health-safety of beta(1,3)-D-glucan in respect to listeriosis development should be furthermore studied.

Keywords: *Listeria monocytogenes*; prebiotics; oligosaccharides; beta(1,3)-D-glucan; pathogen growth

1. Introduction

Listeria monocytogenes is ubiquitous gram-positive, facultative anaerobic, non-spore forming bacterium. This organism is opportunistic intracellular pathogen responsible for foodborne disease, listeriosis. Despite the fact, that the disease is relatively rare, it is characterized by severe symptoms and high mortality rate (it accounts for 20-30 % of the deaths of infected individuals worldwide). Ingestion of highly contaminated food (aprox. 10^9 cells) is in most cases associated with mild to severe febrile gastroenteritis. By contrast, in the case of high risks individuals such as children, elderly and immunocompromised people the disease is characterized by sepsis, meningitidis and meningoencephalitides [1–3]. Pregnant women are also highly endangered group because *L. monocytogens* traverse fetoplacental barrier and cause infection of fetus, resulting in fetal death, premature birth, or complications to pregnancy [4]. The infectious dose for these groups of people is as low as 100 to 1000 cells [1–3]. In general, listeriosis is the fifth most reported zoonosis in the EU. Despite strict hygiene precaution and regular food control, annually, around 2,000 people become infected in the EU. Around 50 % of cases are hospitalized and 10 % percent of those infected die, which is much more compared to other foodborne and zoonotic diseases [5]. *L. monocytogenes* can be subclassified in 13 known serotypes, from which 1/2a, 1/2b, 1/2c and 4b are the most common serotypes in food and are related with 95% of all listeriosis outbreaks. However, serotype 4b accounts for 50% of cases [3,6,7].

This disease can be treated by various antibiotics such as benzylpenicillin, ampicillin, erythromycin, meropenem, moxifloxacin, linezolid and trimethoprim-sulfamethoxazole (EUCAST, 2023). Nevertheless, not all individuals can be treated with antibiotics, especially pregnant women. In general, the best form of protection is prevention. One of the most important approaches of

 doi:10.20944/preprints202405.2144.v1

prevention is to avoid the consumption of food where these bacteria may be present, e.g., raw meat, soft ripening cheeses, uncooked fish products etc. [8]. However, due to *L. monocytogenes* ubiquitous dispersal, the risk of ingestion and disease outbreak cannot be fully excluded even in foods such as fresh vegetable or fruits [9,10].

Today, it is already known that the main barrier to the invasion of *L. monocytogenes* into the host's body is commensal bacteria, which are normally found in the digestive tract. Due to the spread of antibiotic resistant pathogenic strains, there are efforts to support the viability and metabolic activity of commensal microbiota in the digestive tract of the host. This effort can be supported by the administration of prebiotics in host nutrition, which can nourish the health-benefit bacteria. These bacteria can than occupy the adhesion sites on the intestinal mucosa, produce antimicrobial substances and thus prevent the colonization of digestive tract with pathogens and/or inhibit their growth [11–13].

Prebiotics are defined as substrates that are selectively utilized by host microorganisms conferring a health benefit. The most commonly used prebiotics in the food industry or in the pharmaceutical sector are mainly non-digestive nonstarch polysaccharides and oligosaccharides [14]. The most popular prebiotics are inulin, beta-glucans, fructooligosaccharides (FOS), galactooligosaccharides (GOS), xylooligosaccharides (XOS), isomaltooligosaccharides (IMO), raffinose series oligosaccharides (RSO) or lactulose. For infants, the best prebiotics are human milk oligosaccharides (HMO), which are found in breast milk [15,16]. Despite that prebiotics should only be metabolized by health-beneficial bacteria, it was found that even undesirable bacteria can grow on these substrates [17,18].

Because *L. monocytogenes* is able to live in different environments such as soil or vegetation as a saprophyte or in the cytosol of mammalian cells as an intracellular pathogen, it can use different substrates as sources of the main elements for its growth. Nutrient requirements are strain-specific and may vary depending on the strain origin [19]. This organism requires amino acids (leucin, isoleucine, valine, histidine, arginine, glutamine, and methionine) and organically bound sulphur for its growth, but it can also metabolize peptides or complex proteins [19,20]. In the absence of these substances in the environment, bacteria can use hexose-phosphate or glycerol as alternative energy sources. Bacteria use this mechanism especially in the role of an intracellular pathogen, when there is lack of amino acids in the cytosol [19]. The growth is improved by various saccharides especially by glucose. *L. monocytogenes* can ferment differently mono- and oligosaccharides, e.g., fructose, lactose, mannose, trehalose, cellobiose, L-rhamnose and maltose [21–24]. It is known that *L. monocytogenes* can also utilize some polysaccharides such as maltodextrin [22] and it has a functional chitinolytic system and an active lytic polysaccharide monooxygenase, the mechanisms associated with the ability to cleave chitin and cellulose [25,26].

To the best of our knowledge, there are no studies focused on the ability of *L. monocytogenes* to metabolize prebiotics. Therefore, the aim of this work was to investigate the growth properties of the most common serotypes of *L. monocytogenes* in media containing different prebiotics as the sole source of energy and to evaluate the safety of prebiotics oligosaccharides used in foodstuff and dietary supplements with respect to the possible support of listeria growth.

2. Materials and Methods

2.1. Isolation, Identification, and Characterization of Strains

Listeria strains were isolated from foodstuffs purchased in the market network of the Czech Republic and from a smear from the work surface in the food processing plants according to ISO 11290-1:2017 standard. Subsequently, presumptive *L. monocytogenes* colonies were taken, aseptically transferred into the Brain-heart infusion broth (Oxoid, UK) and cultivated at 37 °C for 24 hours. Freshly grown isolates were checked for purity using a phase-contrast microscope (Nikon Eclipse E200LED MV RS, Japan) and identified by MALDI-TOF mass spectrometry (Bruker Daltonik GmbH, Germany) as described in Salmonová et al. [27] to the genus level. *Listeria* spp. were than identify by sequencing of 16S rDNA. Briefly, genomic DNA was isolated from fresh overnight cultures using the

 doi:10.20944/preprints202405.2144.v1

PrepMan Ultra kit (Applied Biosystems, USA) according to the manufacturer's instructions. Amplification of 16S rRNA gene was performed using fD1 [5' AGAGTTTGATCCTGGCTCAG 3'] and rP2 [5' ACGGCTACCTTGTTACGACTT 3'] primers [28] under the conditions described in Salmonová et al., [27]. The amplified PCR products were verified by electrophoresis on a 1% agarose gel, purified with E.Z.N.A.® Cycle Pure kit (Omega Bio-tek, USA) and sequenced by Sanger method (GATC Biotech, Germany). Sequences data were analyzed using the ClustalX package in BioEdit [29,30], and compared with sequences available in the GenBank nucleotide database in NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and EZ Biocloud (https://www.ezbiocloud.net). The sequences were deposited in GenBank via the BankIt program at the NCBI website (https://www.ncbi.nlm.nih.gov/WebSub/?tool=genbank).

For confirmed *L. monocytogenes*, serotype was determined by slide agglutination method using commercial antisera according to the manufacturer's instructions (Merck Group, Germany). Furthermore, the pathogenic potential was studied (as follows). First the hemolytic activity was detected. Cultures were spread on the Columbia blood agar (Oxoid, UK) supplemented with 5% sheep blood (v/v) and incubated for 24 h at 37 °C. Next, the presence of internalin B was detected by the PCR method using primers lmo2821-F [5' TGTAACCCCGCTTACACAGTT 3'] and lmo2821-R [5' TTACGGCTGGATTGTCTGTG 3'] [31].

Four hemolytic and internalin B positive strains of different origin and serotype were selected for substrate preference testing. Strain LM1 (serotype 4b, Access. No. OR725603) was isolated from salami, LM11 (serotype 1/2b, Access. No. OR725605) from a smear from the work surface of the food industry, LM56 (serotype 1/2a, Access. No. OR725613) from raw beef meat and LM79 (serotype 1/2c, Access. No. OR725613) from vegie minced meat.

2.2. Tested Prebiotics and Cultivation Media Composition

The growth characteristics were determined *in vitro* in basal medium containing 5 g/L of tryptone (Oxoid, UK) and 9 g/L NaCl, which was prepared anaerobically by Hungate technique [32]. For testing, the most commonly used prebiotics for human and livestock nutrition were selected in form of food supplements: beta(1,3)-D-glucan (purity of 100% yeast beta-glucans of which > 86.6% beta(1,3)-D-glucan, Brainway Inc., USA), inulin (purity > 90%, Frutafit, Hages, SK), fructooligosaccharides (purity of 95%, Nutri-Extract, CZ), galactooligosaccharides (purity of 95%, Nutri-Extract, CZ) and 2´-fucosyllactose (purity > 94%, RAW´s, CZ). The less common prebiotics: lactulose (purity > 95%, Sigma-Aldich, CZ), raffinose (purity > 99%, Sigma-Aldich, CZ) and stachyose (purity > 98%, Sigma-Aldich, CZ) were also tested. All supplements were used as received without further purification. Finally, ability of listeria strains to grow on mixture of human milk oligosaccharides isolated from human milk according Rockova et al. [33] was studied. Concentrated stock solutions of each substrate (20 g/L) were filter-sterilized using syringe PES filters with a 0.22 µm pore size membrane (Rotilabo®, Germany). Vials with 9 mL of basal medium were supplemented with solution of each substrate to obtain a final concentration of 2 g/L. Due to the partial solubility of beta(1,3)-D-glucan in water and its heat resistance [34,35], this prebiotic was added directly to the basal medium and sterilized by autoclaving at 121 °C for 15 min. An amount of residual or released glucose after autoclaving was verified spectrophotometrically using Glucose Test (Supelco, Canada) and measured with Reflectoquant® RQflex 10 (Sigma-Aldich, Germany). The resulting amount of glucose in medium with beta(1,3)-D-glucan after autoclaving was as low as 2 mg/L.

As a positive control, glucose at the concentration of 2 g/L was used as a sole carbohydrate source. Due to the incomplete purity and residual content of undefined monosaccharides in tested substrates, control with 0,1 g/L of glucose was also included (residual control). The medium without any carbohydrate source was tested as a negative control (basal medium). The control media were sterilized by autoclaving.

2.3 Testing of Bacterial Growth

Fresh overnight cultures grown under aerobic condition at 37 °C in Brain-heart infusion broth (Oxoid, UK), were checked for purity and washed twice with sterile saline. Media were inoculated

 doi:10.20944/preprints202405.2144.v1

by bacteria suspension to final density of 3 x 10^6 CFU/mL and cultivated anaerobically at 37 °C for 24 h. All experiments were prepared in triplicates. Bacterial growth was determined spectrophotometrically at 620 nm using spectrometer UV/VIS SPEKOL 1300 (Analytik Jena GmbH, Germany), and calculated as a difference in optical density (OD) at the time of inoculation and after 24 hours of cultivation. Since the medium with beta(1,3)-D-glucan showed high density (OD=0,4), bacterial counts were evaluated by the agar plate count technique [36] using BHI agar, after 24 h cultivation under aerobic conditions at 37 °C. The growth of *L. monocytogenes* strains for beta(1,3)-D-glucan was analyzed by subtracting initial values of log of colony-forming units (CFU) per 1 mL from the final values.

The pH values of cultivation media were measured using HI98100 pH tester Checker® Plus (Hanna instruments, USA) at the time of inoculation and after 24 hours of cultivation.

Growth curves were constructed for strains showing growth (significant changes) on tested carbohydrates. Optical density (CFU/mL for beta(1,3)-D-glucan) as described above were measured in one-hour intervals during bacterial growth.

The specific growth rates were calculated for individual samples according to Rada et al. (2008) [17] using formula:

$$\mu = (\ln x - \ln x0)/(t - t0)$$

where x and x0 were optical densities or CFU/mL measured within the exponential growth phase at time t and t0, respectively.

2.4 Statistical Analyses

All data were evaluated by Multiple range comparison with Multiple range test in program Statgraphics Centurion XV Version 15.02.06 (Statgraphics technologies, Inc., USA).

3. Results

3.1. Utilization of Water-Soluble Prebiotics

The results of listeria strains growth on prebiotics soluble in water are summarized in Table 1. The growth of all tested strains in positive control (2 g/L glucose) was significantly ($P < 0.05$) higher than on the tested carbohydrates. Slightly increased bacterial densities, which were significantly ($P < 0.05$) higher than in the negative control (no saccharides), were observed on inulin, fructooligosaccharides, galactooligosaccharides and in the residual control (0,1 g/L glucose). Other prebiotics were not utilised, and the optical density values of all strains were similar to the negative control. The strain specific utilization was found for inulin, galactooligosaccharides, stachyose, 2′-fucosyllactose and mixture of human milk oligosaccharides. *Listeria* growth in the negative and residual control was also strain specific.

Table 1. *Listeria monocytogenes* growth on selected prebiotic saccharides soluble in water.

Strain code	**LM1**	**LM11**	**LM56**	**LM79**	**Average ± SD**
Serotype	**4b**	**1/2b**	**1/2a**	**1/2c**	**-**
Positive control (2 g/L glucose)	0.156 ± 0.036^{Aa}	0.140 ± 0.027^{Aa}	0.127 ± 0.032^{Aa}	0.130 ± 0.030^{Aa}	0.138 ± 0.032^{A}
Residual control (0,1 g/L glucose)	0.028 ± 0.002^{Ba}	0.029 ± 0.001^{BCa}	0.028 ± 0.001^{BCDab}	0.026 ± 0.001^{BCb}	0.028 ± 0.002^{BC}
Negative control (no saccharides)	0.016 ± 0.015^{Ba}	-0.006 ± 0.026^{Db}	0.008 ± 0.008^{Deab}	0.005 ± 0.005^{Dab}	0.006 ± 0.017^{EF}

 doi:10.20944/preprints202405.2144.v1

Inulin	0.027 ± 0.006^{Bb}	±	0.028 ± 0.004^{BCb}	±	0.047 ± 0.013^{Ba}	±	0.043 ± 0.005^{Ba}	±	0.036 ± 0.012^{B}	
Fructooligosaccharides	0.026 ± 0.031^{Ba}	±	0.029 ± 0.008^{Bca}	±	0.034 ± 0.011^{Bca}	±	0.016 ± 0.005^{Cda}	±	0.027 ± 0.016^{BCD}	±
Galactooligosaccharides	0.008 ± 0.018^{Bb}	±	0.046 ± 0.006^{Ba}	±	0.018 ± 0.002^{CDEb}	±	0.011 ± 0.007^{CDb}	±	0.021 ± 0.018^{BCD}	±
Lactulose	0.014 ± 0.040^{Ba}	±	0.010 ± 0.014^{Cda}	±	-0.001 ± 0.005^{Ea}	±	-0.005 ± 0.001^{Da}	±	-0.001 ± 0.010^{F}	
Raffinose	0.004 ± 0.006^{Ba}	±	0.006 ± 0,002^{Cda}	±	0.004 ± 0.006^{DEa}	±	-0.003 ± 0.007^{Da}	±	0.003 ± 0.006^{EF}	
Stachyose	0.032 ± 0.005^{Ba}	±	0.011 ± 0.009^{CDbc}	±	0.015 ± 0.005^{CDEb}	±	0.003 ± 0.005^{CDc}	±	0.015 ± 0.012^{CDE}	±
2′-fucosyllactose	0.019 ± 0.002^{Ba}	±	0.013 ± 0.003^{Cdab}	±	0.015 ± 0.004^{CDEab}	±	0.011 ± 0.006^{CDb}	±	0.014 ± 0.005^{CDE}	±
Mixture of HMOs	0.028 ± 0.014^{Ba}	±	0.010 ± 0.007^{Cdab}	±	0.016 ± 0.007^{CDEa}	±	-0.006 ± 0.010^{Db}	±	0.012 ± 0.015^{DEF}	±

Data are expressed as differences in OD at the time of inoculation and after 24 hours of cultivation measured at 620 nm. All data for individual strains are average from triplicates ± SD. OD – optical density. SD – standard deviation. HMOs – Human milk oligosaccharides. ^{ABCDEF}data with different superscripts differ ($P < 0.05$) in column. ^{abc}data with different superscripts differ ($P < 0.05$) in line.

3.2. Utilization of beta(1,3)-D-glucan

L. monocytogenes growth on beta(1,3)-D-glucan are summarized in Table 2. Bacterial cell counts in media containing beta(1,3)-D-glucan were in line with counts determined in positive control. The growth in negative control was significantly ($P < 0.05$) lower than on bought above-mentioned media.

Bacterial growth was accompanied by a decrease in the pH of the culture medium. The initial pH value was 6.9 in all batches. In positive control and beta(1,3)-D-glucan batches, the pH decreased to 5.7. In the inulin experiments, pH decreased to 6.3, and in the residual control, fructooligosaccharides and galactooligosaccharides to pH 6.6. Values of pH did not change in media with saccharides, where bacterial growth was not detected. This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

Table 2. *Listeria monocytogenes* growth on beta(1,3)-D-glucan.

Strain code	**LM1**	**LM11**	**LM56**	**LM79**	**Average ± SD**
Serotype	**4b**	**1/2b**	**1/2a**	**1/2c**	**-**
Positive control (2 g/L glucose)	1.56 ± 0.72^{Aa}	1.2 ± 0.04^{Aba}	0.95 ± 0.01^{Aa}	1.24 ± 0.15^{Aa}	1.24 ± 0.39^{A}
Negative control (no saccharides)	0.33 ± 0.06^{Ba}	0.49 ± 0.12^{Ba}	0.16 ± 0.02^{Ba}	0.24 ± 0.11^{Ba}	0.31 ± 0.15^{B}
Beta(1-3)-D-glucan	1.05 ± 0.09^{Aba}	1.43 ± 0.67^{Aa}	0.96 ± 0.07^{Aa}	1.48 ± 0.72^{Aa}	1.23 ± 0.48^{A}

Data are expressed as differences in log CFU/mL at the time of inoculation and after 24 hours of cultivation. All data for individual strains are average from triplicates ± SD. CFU – colony forming units. SD – standard deviation. ^{AB}data with different superscripts differ ($P < 0.05$) in column. ^{abc}data with different superscripts differ ($P < 0.05$) in line.

3.3. Growth CURVES and rates

 doi:10.20944/preprints202405.2144.v1

Growth curves were constructed and specific growth rates were calculated for positive control, residual control, beta(1,3)-D-glucan, inulin, fructooligosaccharides and galactooligosaccharides. Growth curves during the cultivation of listeria on water-soluble prebiotics are shown in Figure 1. The specific growth rates are summarized in Table 3. Although the same specific growth rates of *L. monocytogenes* on prebiotics and controls was found, the maximal optical density value was significantly higher in positive control than in prebiotic case. The growth curves show that for bacteria cultured on medium with residual control, inulin, fructooligosaccharides and galactooligosaccharides, the exponential phase last around 3 hours and growth was terminated at OD values between 0.028 and 0.047. Longer exponential phase (8 hours) was observed during the cultivation in a positive control, when the growth terminated at OD 0.128. As in the case of bacterial growth ability, the strain-specific growth rates were found for residual control, inulin, galactooligosaccharides and at this case also for fructooligosaccharides.

Table 3. *Listeria monocytogenes* specific growth rates on prebiotic saccharides soluble in water.

Strain code	**LM1**	**LM11**	**LM56**	**LM79**	**Average ± SD**
Serotype	**4b**	**1/2b**	**1/2a**	**1/2c**	**-**
Positive control (2 g/L glucose)	0.42 ± 0.21^{Aa}	0.63 ± 0.04^{Aa}	0.49 ± 0.02^{Ba}	0.64 ± 0.10^{Aa}	0.55 ± 0.14^{A}
Residual control (0,1 g/L glucose)	0.48 ± 0.07^{Aab}	0.32 ± 0.03^{Bb}	$0.40 \pm 0.08B^{ab}$	0.55 ± 0.20^{ABCa}	0.44 ± 0.14^{A}
Inulin	0.35 ± 0.02^{ABc}	$0.63 \pm 0.12A^{ab}$	0.68 ± 0.03^{Aa}	0.51 ± 0.04^{ABb}	0.54 ± 0.11^{A}
Fructooligosaccharides	0.38 ± 0.10^{ABb}	0.67 ± 0.12^{Aa}	0.70 ± 0.09^{Aa}	0.47 ± 0.09^{BCb}	0.55 ± 0.20^{A}
Galactooligosaccharides	0.19 ± 0.05^{Bb}	0.66 ± 0.03^{Aa}	0.73 ± 0.18^{Aa}	0.28 ± 0.13^{Cab}	0.46 ± 0.19^{A}

All data for individual strains average from triplicates ± SD. SD - standard deviation. ^{ABCD}data with different superscripts differ ($P < 0.05$) in column. ^{abc}data with different superscripts differ ($P < 0.05$) in line. .

The growth curves of bacteria cultivated in media with positive control and beta(1,3)-D-glucan are presented in Figure 2, and the calculated specific growth rates are shown in Table 4. No statistically significant difference ($P < 0.05$) was found for the specific growth rates obtain for these two media, although the growth curve shapes were different with a maximal count of 3.50×10^8 CFU/mL after 9 hours of cultivation in positive control and 2.8×10^8 CFU/mL after 8 hours of cultivation in media with beta(1,3)-D-glucan.

Table 4. *Listeria monocytogenes* specific growth rates on beta(1,3)-D-glucan.

Strain code	**LM1**	**LM11**	**LM56**	**LM79**	**Average ± SD**
Serotype	**4b**	**1/2b**	**1/2a**	**1/2c**	**-**
Positive control (2 g/L glucose)	0.53 ± 0.01	0.53 ± 0.01	0.52 ± 0.01	0.47 ± 0.16	0.51 ± 0.07
Beta(1,3)-D-glucan	0.53 ± 0.01	0.54 ± 0.01	0.53 ± 0.01	0.52 ± 0.01	0.53 ± 0.01

All data are average from triplicates ± SD. SD - standard deviation. No different superscripts differ ($P < 0.05$) was found.

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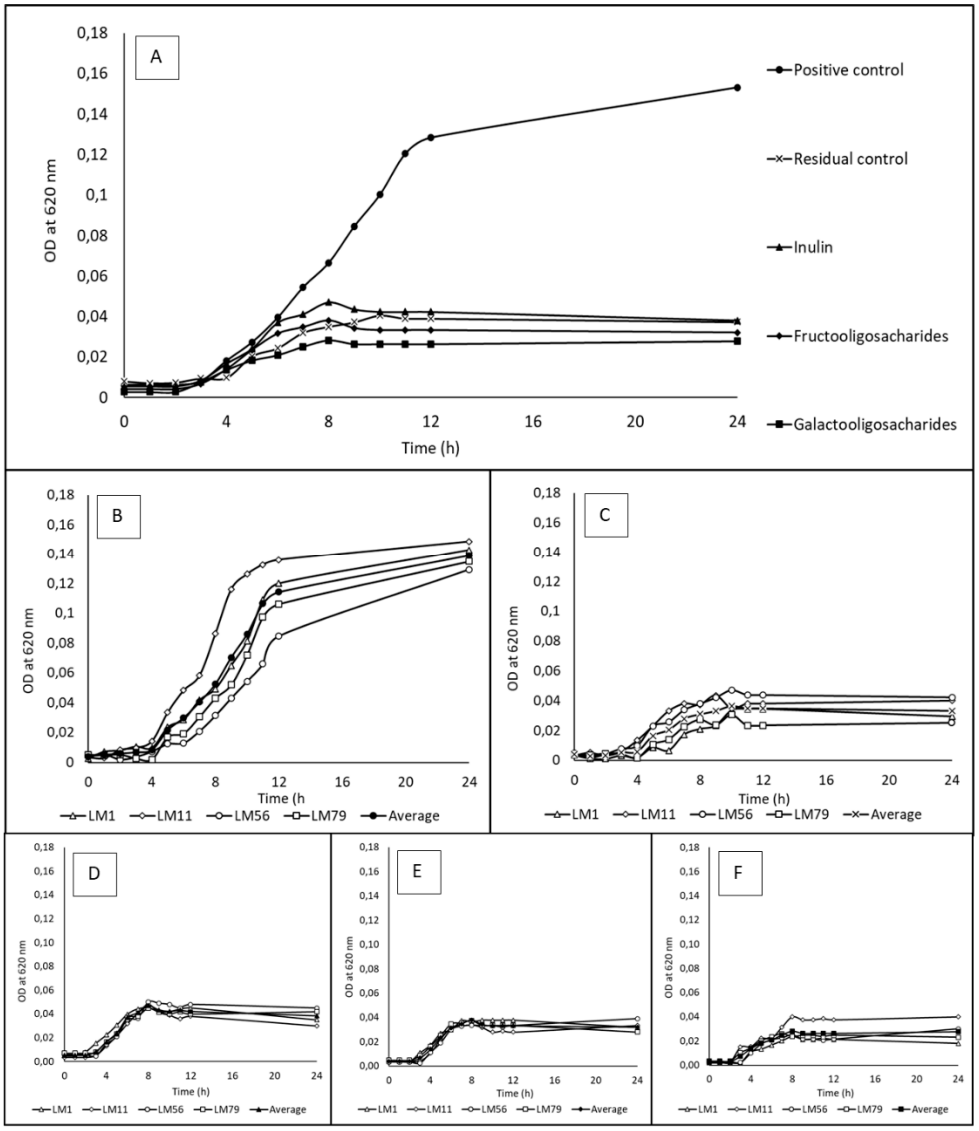

Figure 1. The growth curves of *Listeria monocytogenes* strains cultivated on prebiotics (2 g/L) soluble in water. All data for individual strains are average from triplicates. Positive control = 2 g/L glucose, Residual control = 0,1 g/L glucose. OD = optical density. A: Data show average of 4 strains – LM1 (serotype 4b), LM11 (serotype 1/2b), LM 56 (serotype 1/2a), LM 79 (serotype 1/2c); B: The growth of individual strains in positive control (2 g/L glucose); C: The growth of individual strains in residual control (0,1 g/L glucose). D: The growth of individual strains on inulin (2 g/L); E: The growth of individual strains on fructooligosaccharides (2 g/L); F: The growth of individual strains on galactooligosaccharides (2 g/L).

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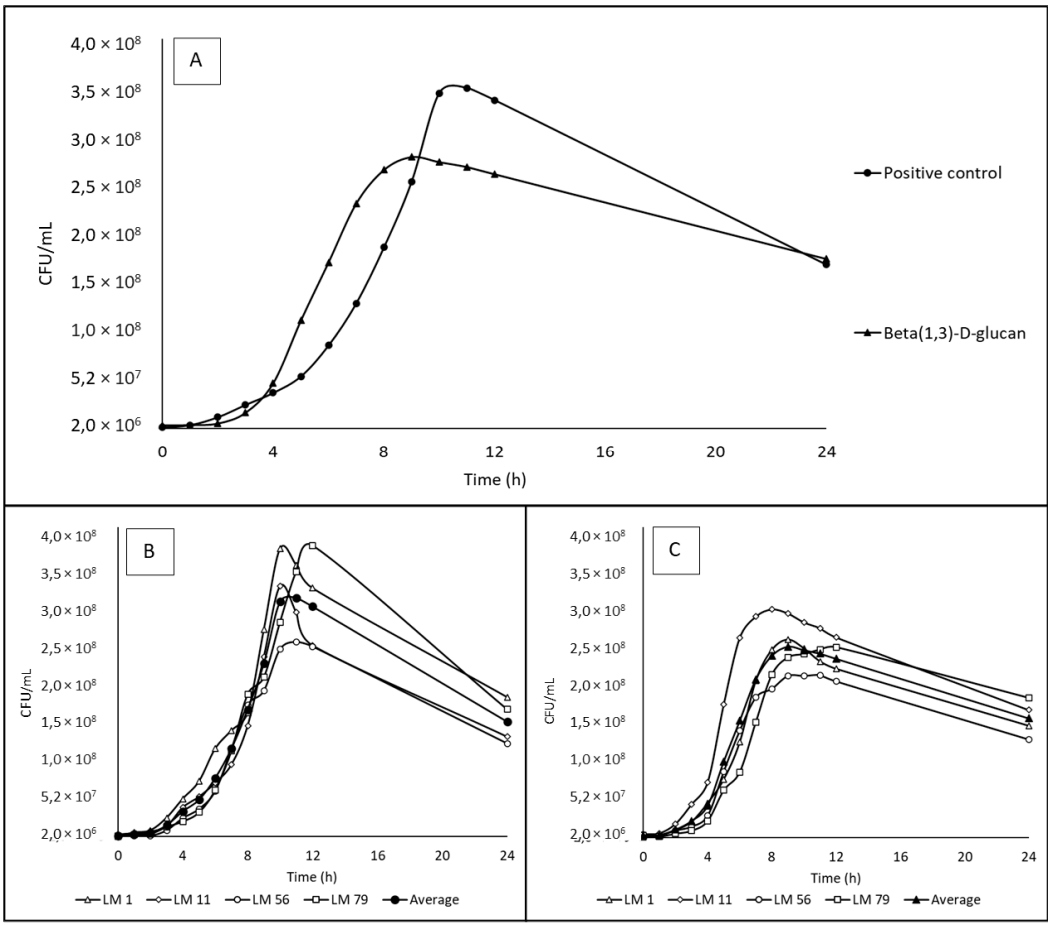

Figure 2. The growth curves of *Listeria monocytogenes* strains on beta(1,3)-D-glucan and positive control. All data for individual strains are average from triplicates. CFU – colony forming units. A: Data show mean of 4 strains – LM1 (serotype 4b), LM11 (serotype 1/2b), LM 56 (serotype 1/2a), LM 79 (serotype 1/2c); B: The growth of individual strains in positive control (2 g/L glucose); C: The growth of individual strains on beta(1,3)-D-glucan (2 g/L).

4. Discussion

The consumption of prebiotics saccharides should selectively stimulate the growth of health-promoting (probiotic) bacteria, suppress occurrence of pathogenic bacteria and conferring benefit(s) upon host health [14,18]. However, it has been noted that some prebiotics can be utilized by undesirable bacteria as well [17,18]. *Listeria monocytogenes* as a foodborne pathogen, can also occur in the digestive tract, where it can use various nutrients for its growth, including several specific saccharides [19,20]. The ability to utilise different saccharides by bacteria depends on the bacteria's genetic predisposition [19,37,38]. To the best of our knowledge, there are no studies focused on the ability of *L. monocytogenes* to metabolize prebiotics. Therefore, the aim of this study was to evaluate the growth of the most common listeria serotypes on several prebiotics' supplements with a purity of 90-100 %, depending on the product.

The results showed that lactulose, raffinose, stachyose, 2′-fucosyllactose and mixture of human milk oligosaccharides were unable to promote *L. monocytogenes* growth and even residual substrates presented in tested supplements did not affect the results. A slight increase in cell numbers was observed for supplements containing inulin, fructooligosaccharides and galactooligosaccharides. Inulin and fructooligosaccharides are prebiotics composed of linear chains of fructose units, linked by β-(2,1) glycosidic bonds, terminated by a non-reducing sucrose end. The difference between these two prebiotics is in the length of chain, whereas fructooligosaccharides comprise of 3 – 7 fructose units, inulin is mainly built form 10 – 60 units [12,39]. L. monocytogenes is capable to utilize fructose

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[24], but it seems that it is not able to cleave the β-(1,2) glycosidic bond that connects individual fructose units. Galactooligosaccharides are composed of 2 – 8 galactose units linked together with β-(1,6) glycosidic bonds and one molecule of terminal glucose connected by β-(1,3) or β-(1,4) glycosidic bond [40]. This prebiotic saccharide does not promote growth of *L. monocytogenes*, because galactose is not among substrates that provide a source of carbon for listeria growth [23]. The bacterial growth and specific growth rate on these prebiotic supplements were comparable to the growth in residue control. Therefore, we assume that the growth of *L. monocytogenes* strains was probably caused by the presence of residual monosaccharides in the prebiotic supplements, which constituted 5 % of fructooligosaccharides and galactooligosaccharides supplements and ≤ 10 % of inulin supplement. Higher growth on inulin than on fructooligosaccharides and galactooligosaccharides could be affected by higher content of monosaccharides. Strain-specific growth of listeria on certain substrates are known [19,38], which was also shown in our study. Although a statistically significant strain specificity growth was observed, no comparable growth of *L. monocytogenes* strains as in the positive control was found.

Beta(1,3)-D-glucan was the only prebiotic supplement, on which the comparable growth of *L. monocytogenes* strains as on the positive control was found. Beta(1,3)-D-glucan is a prebiotic polysaccharide belonging to the group of beta-glucans composed only from monomers of glucose that are linked by β-(1,3) glycosidic bonds [41,42]. They are generally considered effective and widely applied prebiotics, which are used for their ability to protect the body against various pathogens, cancer, high LDL cholesterol level and their antioxidant effects, which overall supports the host's health [43]. Beta-glucans are major structural components of plant and fungi and include the major biopolymer cellulose. The structure of cellulose is similar to beta(1,3)-D-glucan, but glucose units are linked by β-(1,4) glycosidic bonds [44]. It has been demonstrated that *L. monocytogenes* is able to utilize cellulose [25,26]. Therefore, it could be assumed that *L. monocytogenes* is capable to cleave beta-glucans as well. Beta-glucans used in this study were of S. cerevisiae origin, and in addition to β-(1,3)-glucans also contain less than 15% of β-(1,6)-glucans [43]. Since the results confirmed this presumption, it could be expected, that *L. monocytogenes* can clave both β-(1,3) and β-(1,6) bonds, and metabolize released glucose, which is the main source of carbon in listeria metabolism. Regarding the growth curve, there were observed some differences between the course of *L. monocytogenes* growth on beta(1,3)-D-glucan and the positive control. Slightly lower cell counts on beta(1,3)-D-glucan could be easily explained by the fact that part of the energy, that could have been used for reproduction, was spent on the substrate cleavage. It could be also result of gradual or incomplete cleavage. The decrease in the number of viable cells on beta(1,3)-D-glucan was slower compared to the positive control, probably due to the gradual release of glucose from the beta-glucan chain.

There are various natural sources of beta-glucans, e.g., yeast, barley, bacteria, seaweed, fungi or dahlia tuber. They differ not only in origin, but also in glycosidic bond position as well as in their other characteristic such as solubility in water or alkalis. Except the β-(1,3)- also β-(1,4)- or β-(1,6)-glucans are found in supplements [45–47]. The differences between the growths of *Bifidobacterium* sp. depending on the source of beta-glucans were demonstrated [47]. Thus, we can hypothesize that the *L. monocytogenes* ability to metabolize these prebiotics also depends on the source.

Regardless of whether the *L. monocytogenes* is able to utilize these prebiotics or not, effects against pathogens, such as supporting the individual's immunity, growth of beneficial microbes and reduced adherence ability of pathogens in general, have been reported for these substances [48–52]. However, the results are sometimes ambiguous or contradictory. For example, Ebersbach et al. [53] found that inulin, which *L. monocytogenes* is not able to use as source of carbon, decreased the resistance of the host body against the *L. monocytogenes*. On the contrary, despite that *L. monocytogenes* strains are able to utilize beta(1,3)-D-glucan, it has been found that these prebiotics stimulate immune system in mice or guinea pig and effectively inhibit pathogens proliferation, reduce adhesion to the digestive tract wall and suppress *L. monocytogenes* infection [54–56]. In addition beta(1,3)-D-glucan are metabolized by commensal and probiotic intestinal bacteria which leads to competition for substrate. Moreover, short-chain fatty acids (SCFA), which are formed through microbial metabolite cross-feeding, suppress listeria growth and support the body's disease defense. The health-promoting bacteria also

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produce various substances that inhibit *L. monocytogenes*, such as bacteriocins [57–64]. The fact that *L. monocytogenes* is able to utilize beta(1,3)-D-glucan is therefore not alarming. Compared to the growth of previously studied *Lactobacillus*, *Bifidobacterium* and commensal *Clostridium* strains in the presence of selected prebiotics, the growth of *L. monocytogenes* was lower [17,47,65]. This is most likely affected by the medium composition. The media used for probiotics/commensals testing are composed for fastidious bacteria and contained complex nutritional mixture except the energy source. Some of these nutrients can *L. monocytogenes* utilize for its growth even without the presence of saccharides, therefore nutrient-poor medium is required for this organism [17,47,65].

Finally, the question how else prebiotics can affect metabolism and behavior of this pathogen because different saccharides have been found to affect the pathogenic potential [66–69], biofilm formation potential [69] and resistance to antimicrobial substances [21], heat or acids [69], arises. To our best knowledge, there are no studies focusing on these topics for prebiotics used in our study.

Authors should discuss the results and how they can be interpreted from the perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted.

5. Conclusions

Our study attempted to evaluates the growth ability of listeria on different prebiotics. The results showed that inulin, fructooligosaccharides, galactooligosaccharides, lactulose, raffinose, stachyose, 2′-fucosyllactose and mixture of human milk oligosaccharides are not used by *L. monocytogenes* as a nutrient and source of energy. Therefore, these prebiotics can be considered as health-safe with respect to the possible support of listeria growth. Beta(1,3)-D-glucan was the only prebiotic utilized by *L. monocytogenes*, thus this substrate does not meet the general definition of prebiotics. Even though the results showed that beta(1,3)-D-glucan support the growth of *L. monocytogenes*, available studies highlight suppressive effects on listeriosis, probably due to the function complexity. Therefore, the health-safety of beta(1,3)-D-glucan with respect to listeriosis development should be furthermore studied.

Author Contributions: "Conceptualization, Kodešová, T. and Šubrtová Salmonová, H..; methodology, Kodešová, T.; Šubrtová Salmonová, H. and Musilová Š.; validation, Kodešová, T.; formal analysis, Kodešová, T.; investigation, Kodešová, T.; Mašlejová, A., Horváthová, K.; resources, Vlková, E.; writing— original draft, Kodešová, T.; writing— review & editing, Šubrtová Salmonová, H., Vlková, E.; visualization, Kodešová, T.; supervision, Šubrtová Salmonová. H.; funding acquisition, Vlková, E. All authors have read and agreed to the published version of the manuscript."

Funding: This work was supported by the NutRisk Centre, Ministry of Education, Youth and Sports of the Czech Republic [CZ.02.1.01/0.0/0.0/16_019/0000845, 2019-2023]; and METROFOOD-CZ research infrastructure project [MEYS Grant No: LM2023064] including access to its facilities.

Data Availability Statement: The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Acknowledgments:

Conflicts of Interest: The authors declare that they have no conflict of interest.

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