A review of minimal and defined media for growth of *Listeria monocytogenes*

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Abstract

Listeria monocytogenes is known to be an important foodborne pathogen and is the causative agent of one of the deadliest foodborne illnesses. The organism has a wide range of environmental conditions under which it will survive and grow, and often contaminates processing plants and retail environments in which ready-to-eat foods are manufactured, prepared and served. Although *L. monocytogenes* is not a fastidious organism and can grow in a variety of rich media, defined and minimal media are necessary to elucidate the minimal environmental nutrients that are required for the survival and growth of this organism. In addition, many of the virulence factors required for *L. monocytogenes* to invade and multiply in mammalian host cells are not produced in rich media and thus induction of these factors is best studied in a minimal medium. This review covers the historical development of minimal media for *Listeria* spp. and explores the various factors required for survival and growth of this organism. To the best of our knowledge, this is the first time that these media have been compared side by side. In order to better compare studies using different chemical substrates such as a different carbon source, all concentrations of components in each medium have been converted to molar concentrations.

Keywords: *Listeria*, defined media, minimal media, growth, physiology, nutrition

1. Introduction

The genus *Listeria* consists of seventeen species: *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, and *L. grayi*, *L. marthii*, *L. rocourtiae*, *L. fleischmannii*, *L. weihenstephanensis*, *L. floridensis*, *L. aquatica*, *L. cornellensis*, *L. riparia*, *L. grandensis*, *L. booriae*, and *L. newyorkensis*, all of which are widespread in nature and can often be found in soil, decaying plants, sewage, silage, dust, and water (Sauders & Wiedmann, 2007; Weller, Andrus, Wiedemann, & den Bakker, 2015). Because of this extensive distribution, *L. monocytogenes* and other *Listeria* spp. regularly contaminate the receiving, handling, and processing areas of food manufacturing establishments. Urban environments have also been identified as reservoirs including sidewalks, door handles, signs, and grab rails (Sauders et al., 2012). While *L. seeligeri* and *L. welshimeri* are mostly associated with natural environments, *L. innocua* and *L. monocytogenes* are often identified in urban environments (Sauders et al., 2012). Only two of the species, *L. monocytogenes* and *L. ivanovii*, are considered pathogenic for animals, with *L. monocytogenes* being predominantly associated with human illnesses such as meningitis, encephalitis, and sepsis (Painter & Slutsker, 2007; Scallan et al., 2011).

How and why certain strains and species of *Listeria* persist in specific environments or prefer one type of host over another is a complex question. It has been shown that *L. innocua* is often able to outgrow *L. monocytogenes* when they are cultured together under some enrichment conditions and that certain *L. monocytogenes* strains will outcompete other strains (Bruhn, Vogel, & Gram, 2005; MacDonald & Sutherland,

1994). It has also been shown that rich/complex media or easily convertible carbohydrates suppress transcription of some of the virulence genes of *L. monocytogenes* (Gray, Freitag, & Boor, 2006). While *Listeria* does require a number of essential nutrients, which will be described later in this review, it is not overly fastidious or difficult to cultivate, and can often be regularly cultured in common rich media such as tryptic soy broth (TSB) or brain heart infusion (BHI). Yeast extract is added to certain media at 0.6 %, although it is not obligatory (Lavieri et al., 2014). Before a selective medium became available, a cold enrichment was employed when isolating *L. monocytogenes* from food, animal specimens, or environmental samples to take advantage of the ability of *Listeria* to grow under refrigerated conditions (Donnelly & Nyachuba, 2007). While outside the scope of this review, several reviews have been published on media for the isolation and identification of *Listeria* spp. (Beumer & Hazeleger, 2003; Curtis & Lee, 1995; Jantzen et al., 2006). In the current review a general background on media definitions and growth measurements typically used will be provided followed by an in-depth examination of minimal media development and uses for *L. monocytogenes* growth studies.

2. Growth media – general concepts

Complex media are frequently used to cultivate microorganisms due to their ease of preparation and their ability to recover isolates from a wide range of ecosystems. Complex media contain extracts of plant or animal tissue, such as peptone or tryptone, as well as a sugar (often glucose) to serve as an energy source; the exact composition of complex media is not fully defined (Lim, 2003). The variability of media constituents

and the difficulty of quantifying these components makes a defined medium (DM) the choice when there is a need to control certain nutrients or to elucidate metabolic mechanisms (Greasham & Herber, 1997). A defined medium is a medium suitable for *in vitro* growth of an organism that is made with chemically defined ingredients that are carefully measured into distilled water; the exact composition of these media are known (Lim, 2003). Defined media contain a simple sugar as a carbon and energy source, an inorganic source of nitrogen as well as mineral salts and any required growth factors (Lim, 2003). In contrast, a minimal medium (MM) provides only the exact essential nutrients (including any growth factors) needed for a particular organism to grow (Todar, 2012). Uses of DM are outlined in **Table 1**. Unless specifically referring to a MM, DM will be used as the general term in this review article.

Because *Listeria* spp. are able to grow in such a wide range of environmental conditions, focusing on one or two factors requires the use of DM to eliminate other factors. In the history of media development for *L. monocytogenes*, MM or DM were initially developed to elucidate the minimal environmental nutrients that *L. monocytogenes* required. As the basic vitamins, carbon, and nitrogen sources were discovered, MM and DM came to be used more for determining the various metabolic pathways of the organism. Often, a new and improved MM was developed for *L. monocytogenes* because it was found that previous media failed to provide all the necessary nutrients for the growth of the particular strains being studied. In this way, MM and DM have been useful for differentiating between the **metabolisms** of the various strains.

At this time, no single MM or DM is exclusively used for the study of *Listeria spp.* growth, physiology, or pathogenicity. Whether this is because of a need for more research on *Listeria* metabolism or because of the varying metabolic capabilities of *Listeria spp.* and strains remains in question. In an attempt to address this, the current review explores the historical development of widely varying MM for *Listeria spp.* and argues that the search for a universal listerial MM may indeed be futile while the prospects for a universal DM are hopeful.

To the best of our knowledge, this is the first time that the DM for *L. monocytogenes* have been presented for comparison. The tables were designed to provide a comparative understanding of the variability of DM used for *Listeria* research, which is not easily seen when depending solely on the individual publications alone, and to provide a starting point for the development of a universal *Listeria* DM. To this end, all concentrations of components in each medium taken from the respective publications have been converted to molar concentrations. This facilitates more ready comparison between studies which used differing hydrates of salts, different carbon sources, or nitrogen sources and allows the comparison of total molecular concentrations.

3. Microbial growth definitions and methods of quantification

Before comparing studies employing variations of MM and DM, it is important to discuss how bacterial growth responses are collected and analyzed to draw conclusions. The following describes some of the fundamental definitions for growth and growth measurements. The various papers discussed in this review use a variety of definitions

of what constitutes growth of the organism. For example, some of the investigators emphasized vigorous growth as the criterion rather than just growth; without a quantitative definition of what constitutes vigorous growth direct medium comparisons become difficult. However, even when growth is quantified in some manner, there still remain a variety of laboratory approaches to measuring growth. Bacterial growth can be measured in terms of increase in cell mass or increase in numbers of cells (Pirt, 1975; Jay, 2000; Todar, 2012).

Measuring optical density (OD) of a suspension of bacteria and comparing that to a standard curve is a common method of measuring bacterial growth; this method is quick and inexpensive but cannot differentiate between live and dead cells and is not as sensitive as direct plate counts (Kavanagh, 1963; Todar, 2012). Differences also exist in measurements depending on the spectrophotometer used and on whether it is a tube, cuvette, or microplate reader-based format (Hall, Acar, Nandipati & Barlow, 2014). In addition, the OD of bacteria grown in DM does not always correlate well with direct plate counts and many researchers have found it extremely difficult to even construct a standard curve in DM (Horáková, Greifová, Seemannová, Gondová, & Wyatt, 2004). In addition, many stress factors and culture conditions affect the relationship between viable counts and OD values in *L. monocytogenes* (Francois et al., 2005). The direct plate count is more sensitive than OD measurements although it can still be influenced by culture conditions (medium, atmosphere, agitation and other factors) and due to clumps and chains of cells a colony does not necessarily represent a single cell (Jay, 2000; Todar, 2012).

Another complication in growth based studies of DM is that growth rates can vary depending on the population density of the organism and how nutrients are provided in the culture system. Batch cultures typically involve initial nutrient levels which are depleted as the organism grows and utilizes the nutrients with growth halting when the first limiting nutrient concentration can no longer support growth (Pirt, 1975). Consequently, in batch cultures, bacterial cells in many different growth and physiological states (lag, log, death) may be present, but in a continuous culture all cells are in the log phase and growth rate is dependent on the rate at which fresh medium is added (Todar, 2012). A "continuous culture" is defined as a culture to which is added a continuous stream of fresh medium and removal of spent medium and cells for the purpose of maintaining the culture at some quantifiable steady-state level of growth (James, 1961; Monod, 1949; Pirt, 1975). Contois (1959) studied *Aerobacter aerogenes* in continuous culture in a chemically defined medium and determined that specific growth rate was a function of population density as well as the concentration of the limiting nutrient. Jones, Shama, Andrew, Roberts and Jones (1995) and Kelly and Patchett (1996) all observed increasing growth rates of *L. monocytogenes* and *L. innocua* with increased dilution rates in continuous cultures. Jones et al. (1995) using the medium of Trivett and Meyer (1971), observed that the critical dilution rate (D_c) for *L. monocytogenes* NCTC 7973 was 0.22 h^{-1} ; this was also the maximum specific growth rate ($\mu_{\max}$) at a steady state. The $D_c/\mu_{\max}$ of *L. innocua* NCTC 11289 was between 0.14 and 0.2 h^{-1} as determined by Kelly and Patchett (1996). Unfortunately the two studies used different DM and so the $\mu_{\max}$ of the two species cannot be compared.

4. Background on minimal and defined media used for *Listeria spp*

Due to the wide and varied uses of various DMs, it would be impossible for a single review to present all studies which have done so. Thus a select sampling of DM and MM will be drawn from the literature to illustrate key concepts. Listed in **Table 2** are the numerous and varied DM and MM used to study *L. monocytogenes*; Modified Welshimer's Broth (MWB) is the most widely used for the majority of reported research investigations. However, further evaluation of the literature reveals large discrepancies and variations among studies with regards to the media ingredients, quantification of growth, strain choice, and methods used. This contradictory state of affairs makes it difficult at times to directly compare the usefulness of the various media. For example, many studies use different species of *Listeria* or even different strains of *L. monocytogenes*, making it impossible to conclude whether the differing results are due to the change in media or to the species or strains having differing metabolic and growth characteristics. Consequently, new media continue to be developed because a particular strain did not grow well in previously published MM, indicating that there may be significant strain to strain variations in growth needs or significant laboratory-to-laboratory variation.

While not the first to study growth requirements for *L. monocytogenes*, Friedman and Roessler (1961) were the first to attempt to define a complete MM for the growth of this organism. Previous researchers had only published results of metabolic requirements and growth characteristics without defining a specific medium (Cury, Portellada, & Hutner, 1954; Hutner, 1942, 1944; Miller & Silverman, 1959; Porter & Pelczar, 1941). In addition to delivering a specified medium for five strains, Friedman and Roessler (1961) reported a detailed account of their trials. Of historical interest, unlike methods currently

206 employed today in which an aliquot of liquid growth or a streak from a slant is used as
207 inoculum, Friedman & Roessler (1961) grew the strains on slants for seven hours and
208 subsequently removed the bacterial growth from the slant by washing with sterile
209 deionized water, before resuspending to a standard OD reading of 0.08 at 655 nm. To
210 measure growth, they took OD measurements at 660 nm between 16 and 24 hours to
211 document the rate of growth (Friedman & Roessler, 1961). They were unable to achieve
212 growth under shaking conditions, but obtained dense growth when the culture remained
213 stationary, although they did not identify why this was the case. Two possibilities would
214 be poor growth under highly aerobic conditions or growth and lysis under such
215 conditions.

216 Pine, Franzus, Brooks and Daneshvar. (1989) observed death and lysis of *Listeria spp.*
217 in DM whether grown aerobically or anaerobically, but only when glucose was used as a
218 carbon source. This was observed both when measuring OD values and in direct plate
219 enumeration, although the percent decrease as measured by OD was 3 to 50 times less
220 than as measured by CFU/ml on blood agar (Pine et al., 1989). Interestingly, Jones et
221 al. (1995) reported rapid autolysis of *L. monocytogenes* in shaken anaerobically grown
222 batch cultures using the DM of Trivett and Meyer (1971) with glucose as the carbon
223 source. Azizoglu and Kathariou (2010) reported that *L. monocytogenes* strain F2365
224 containing a *kat* (catalase) mutation exhibited impaired growth at 4°C statically and
225 almost no growth under shaking conditions, as compared to the wild-type strain.
226 However, growth differences were not observed between the wild-type and mutant
227 strains incubated statically at 37, 10, and 25°C, and growth using shaking incubation at
228 37, 10, or 25°C was not reported (Azizoglu & Kathariou, 2010).

229 Using elimination and serial transfer experiments, Welshimer (1963) demonstrated a
230 requirement for tested strains of *L. monocytogenes* for thiamine, biotin, α -lipoic acid,
231 and pyridoxine. Earlier confusion about these requirements, especially α -lipoic acid, was
232 apparently caused by transfer of these substances with the inocula into the test medium
233 (Welshimer, 1963). Trivett and Meyer (1971) modified Welshimer's medium while
234 attempting to elucidate whether *L. monocytogenes* possessed a completely intact citrate
235 cycle. Ralovich, Shahamat & Woodbine (1977) subsequently developed a solid MM.
236 Unlike previous work which had focused on minimal growth, Siddiqi and Khan (1982)
237 sought to define a synthetic medium which promoted "rapid and luxuriant" growth for six
238 strains of *L. monocytogenes* and allowed cell growth to an OD of at least 1.00. They
239 compared this to growth in a vitamin-free medium which supported growth to an OD no
240 greater than 0.25 and subsequently published a MM detailing the minimum amino acids
241 needed to support growth (Siddiqi & Khan, 1989).

242 Premaratne, Lin, & Johnson (1991) were unable to culture *L. monocytogenes* strains
243 Scott A, California, Ohio, V7, and ATCC 19115 in Welshimer's medium. They aimed to
244 develop a medium which promoted "good growth" of these strains. Their modified
245 medium, dubbed Modified Welshimer's Broth (MWB), supported the growth of "several
246 known isolates", 28 environmental dairy isolates, *L. ivanovii*, and *L. innocua*
247 (Premaratne et al., 1991). Lastly, they evaluated the survival (not growth) of *L.*
248 *monocytogenes* in phosphate buffered saline (PBS) as compared to their medium, their
249 medium without glucose, and their medium without glucose supplemented with chitin,
250 cell walls, or casein. Surprisingly, while *Listeria* counts declined within 15 days to
251 undetectable CFU in MWB or PBS, cell counts of 4 log, 4.5 log, 5.5 log, and 6.5 log

252 CFU were **seen** in MWB without glucose or MWB without glucose supplemented with
253 casein, bacterial cell walls, or chitin respectively.

254 Patchett, Kelly, & Kroll (1992) examined the effect of NaCl on *L. monocytogenes*'
255 intracellular solutes employing a medium they developed for this study. Their medium
256 **included** the vitamin levels from previous work of Trivett and Meyer (1971), the trace
257 minerals from Harwood and Archibald (1990), and most of the amino acids from
258 Welshimer (1963) but at differing levels. The solid medium supported growth and they
259 were able to demonstrate that the presence of NaCl in the medium did not alter visual
260 colony characteristics other than reducing colony size. Salt concentrations in the
261 medium up to 7.5% did not affect cell volume, but increased K⁺, betaine, and glutamate
262 concentrations within the cell, which are considered common reactions of bacteria to
263 osmotic stress (Patchett et al., 1992). Amezaga et al. (1995) further investigated the
264 effects of high osmolarity using a modification of the medium of Patchett et al. (1992) as
265 well as less defined media. They determined that when both glycine betaine and
266 peptone are present in the medium, *L. monocytogenes* is very salt tolerant and growth
267 was comparable to that achieved in a more complex medium such as brain heart
268 infusion (BHI) (Amezaga et al., 1995).

269 Phan-Thanh and Gorman (1997) developed a modified MWB in which *L.*
270 *monocytogenes* strain EGD grew to 9 log CFU/ml after 27 hours as compared to only 7
271 log in original MWB whereas in BHI it only took 12 hours to reach 9 log CFU/ml. Data
272 was provided only up to 27 hours, leaving the question of whether *L. monocytogenes*
273 strain EGD would have continued to increase in population levels or if there would have

274 been differences in the cell survival rates between BHI and the new medium over more
275 extended incubation periods (Phan-Thanh & Gorman, 1997). Nevertheless, this medium
276 supported faster growth under their conditions than those published previously; *L.*
277 *monocytogenes* strain EGD exhibited a doubling time of 45 minutes as compared to 88
278 minutes in the medium of Phan-Thanh and Gorman (1997).

279 Tsai and Hodgson (2003) also attempted to refine MWB. They reported that they were
280 able to “drastically” improve growth in liquid and on solid media as compared to MWB
281 (Premaratne et al., 1991; Tsai & Hodgson, 2003). The new medium was used for
282 proteome analysis of strain EGD-e by the European *Listeria* Genome Consortium.
283 However for unknown reasons it failed to support growth of *L. monocytogenes* strain
284 Scott A (Tsai & Hodgson, 2003). They were also able to demonstrate that the strains
285 used adapted physiologically to the DM. They attributed the time required for
286 physiological adaptation to the time required to initiate *de novo* synthesis of amino acids
287 not provided in the medium (Tsai & Hodgson, 2003).

288 Jones et al. (1995) compared the growth obtained for three separate *L. monocytogenes*
289 strains using static and shaken (100 rpm) batch cultures at 30°C in the media of Trivett
290 & Meyer (1971), MWB (Premaratne et al., 1991), Ralovich et al. (1977), Friedman and
291 Roessler (1961), and Welshimer (1963). These direct comparisons led them to choose
292 the medium from Trivett and Meyer (1971) for further work with continuous cultures.
293 They obtained a range of maximum specific growth rates ($\mu_{\max}$) of 0.16 to 0.31 hr⁻¹ and
294 cell yields (mg/ml) in the range of 1.55 to 4.10 and were able to attain a $\mu_{\max}$ of
295 approximately 0.30 before the bacterial culture washed out, with glucose in excess

296 between dilution rates of 0.23 and 0.30 h⁻¹. Kelly and Patchett (1996) grew *L. innocua* in
297 MWB in continuous culture and did not observe glucose in the filtrates until a dilution
298 rate of 0.14 h⁻¹ was reached, indicating that glucose was in excess at that dilution rate.

299 Romick, Fleming and McFeeters (1996) supplemented the medium of McFeeters and
300 Chen (1986), which was originally designed for *Lactobacillus plantarum*, with 4.8 μM α-
301 lipoic acid, to study the aerobic and anaerobic growth of 10 *L. monocytogenes* strains
302 (from serotypes 4, 4b, and 1a). Aerobic growth with glucose as the sole energy source
303 sustained growth to 10 log CFU/ml compared to anaerobically grown cells which
304 reached nine log CFU/ml at approximately 30 h after inoculation with 5.5 log CFU/ml. All
305 10 strains when grown aerobically with glucose produced acetate (16.6 mM) and lactate
306 (13.3 mM) with some acetoin (9.4 mM) (Romick et al., 1996). End products under
307 anaerobic conditions included 23.4 mM lactate, 0.9 mM acetate, 4.8 mM formate, and
308 3.4 mM ethanol, but no acetoin. Their medium was designed so that the stock solutions
309 for preparing this medium could be frozen and subsequently thawed, combined, diluted,
310 and filter-sterilized when needed (Romick et al., 1996).

311 It should also be noted that the media developed by Pine, Franzus and Malcolm (1986)
312 and Pine et al. (1989) have been modified by at least three other research groups, and
313 all of these modifications are often referred to as Modified Pine's Medium. Ko, Smith
314 and Smith (1994) removed the choline from Pine's medium for their work on
315 osmotolerance. While the reason for removing choline was not mentioned, it is likely
316 because previous work (Patchett et al., 1992) indicated that choline was utilized by *L.*
317 *monocytogenes* as an osmoprotectant. Becker, Çetin, Hutkins and Benson (1998)

318 modified Pine's medium by including some aspects of Premaratne's medium
319 (Premaratne et al., 1991), for the study of osmotolerance mediated by the σ^B gene.
320 Subsequently this has been referred to as Modified Pine's Medium as well (Dykes &
321 Dworaczek, 2002).

322 More recently Schneebeili and Egli (2013) published their evaluation of the individual
323 elements of the media of Welshimer (1963), Premaratne et al. (1991), Phan-Thanh and
324 Gormon (1997), and Tsai and Hodgson (2003). They used published data on the dry
325 biomass composition of bacteria and yeast to calculate the theoretical excess of each
326 nutrient in each medium, assuming glucose to be the limiting carbon source. Using this
327 analysis, they reported that the media they tested were inadequate for the range of
328 research approaches required for assessment of genomics, proteomics or metabolomic
329 responses, **and that one or more elements other than glucose were limiting**. Therefore
330 they developed a mineral medium where glucose was the only carbon source and all
331 nutrients were in excess except glucose, and thus it was the only limiting nutrient
332 (Schneebeili & Egli, 2013). They reported their medium would support the growth of all
333 *Listeria* spp. except for *L. ivanovii* (Schneebeili & Egli, 2013). They supplemented with
334 additional amino acids or 1:1000 BHI, but failed to stimulate growth of *L. ivanovii*. It is
335 possible that this is due to the PrfA protein produced by *L. ivanovii*. The protein PrfA is a
336 regulator protein which controls expression of numerous virulence factors. Research
337 has shown that the PrfA of *L. ivanovii* is more similar to the constitutively expressed *L.*
338 *monocytogenes* PrfA (usually denoted with an asterisk PrfA*) than it is to the normally
339 expressed *L. monocytogenes* PrfA (Mauder et al., 2006). This is significant because
340 research has also shown that over expression of PrfA can inhibit growth of *L.*

341 *monocytogenes* in DM where glucose is used as a sole carbon source (Marr et al.,
342 2006). Therefore, it is possible that the PrfA produced by *L. Ivanovii* is inhibiting its
343 ability to grow on glucose in DM.

344 **5. Elements of defined/minimal medium**

345 **5.1 Carbon source**

346 Miller and Silverman (1959) were one of the first research groups to systematically
347 evaluate the carbon metabolism of *L. monocytogenes*. They reported that *L.*
348 *monocytogenes* lacked the ability to use xylose, arabinose, ribose, or gluconate in place
349 of glucose (**Table 3**). *L. monocytogenes* produced pyruvate, lactate, and acetoin when
350 grown aerobically, but produced only lactate when grown anaerobically (Miller &
351 Silverman, 1959). Friedman and Alm (1962) reported on Warburg experiments using
352 three different levels of glucose as a carbon source (0.2, 0.6, and 1.0%). Pyruvate,
353 lactate, acetoin, and CO₂ accounted for all of the glucose utilized when 1% glucose was
354 in the medium; lesser amounts of these corresponding compounds **were** recovered
355 when cells were grown on the lower levels of glucose or on BHI (Friedman & Alm,
356 1962). Trivett and Meyer (1967) sought to evaluate the ability of *L. monocytogenes* to
357 metabolize erythritol due to its presence in the ruminant placenta; however their results
358 indicated that *L. monocytogenes* was unable to metabolize erythritol.

359 Trivett and Meyer (1971) designed experiments to determine if *Listeria* had the
360 enzymatic facilities for the oxidation of pyruvate, the citrate cycle, and the glyoxylate

bypass. They employed pyruvate or citrate cycle intermediaries (acetate, pyruvate, citrate, isocitrate, α -ketoglutarate, succinate, fumarate, or malate) as either sole or supplementary carbon and energy sources. Growth was neither supported by any of the intermediaries when used as the sole carbon source nor stimulated when used individually to supplement 0.5% glucose over a period of 24 hours. The authors suggested that *L. monocytogenes* has a split or incomplete conventional citric acid cycle and that carbon fixation may be possible (Trivett & Meyer, 1971). Ralovich et al. (1977) used 15 strains to assess a modified Welshimer's medium, and contrary to Trivett and Meyer (1971), they found citrate supported cell growth on glucose; however, the authors attributed this to the ability of *Listeria* to chelate iron rather than to its metabolism. Ralovich et al. (1977) also used 1% glucose, which they found to optimally promote growth despite reports that glucose levels between 0.2% and 0.6% allow for optimal glucose oxidation (Friedman & Alm, 1962; Trivett & Meyer, 1971).

Pine et al. (1989) compared the aerobic and anaerobic growth of several *Listeria* species on different sugars. They determined that all species they tested grew aerobically using glucose, and all produced lactic acid and/or acetic acid from glucose. When grown anaerobically, *Listeria* produced only 80% of the cell yield it produced when grown aerobically and only lactic acid was formed. They also demonstrated that *Listeria* spp. possess differing growth capabilities aerobically and anaerobically on glucose, galactose, lactose, rhamnose, and xylose. None of the strains were able to grow on galactose anaerobically, but the *L. ivanovii* strain apparently grew better on glucose anaerobically (OD = 1.19) than aerobically (OD = 0.75). Lastly they reported the ability of N-acetylglucosamine and glucosamine to enhance growth on lactose for *L.*

384 *monocytogenes* and *L. innocua*. Premaratne et al. (1991) determined that glucose could
385 be replaced by the amino sugars N-acetylglucosamine and N-acetylmuramic acid. Since
386 these sugars are components of bacterial cell walls, Premaratne et al. (1991)
387 **investigated** the survival of *L. monocytogenes* in MWB or phosphate buffer in the
388 presence of chitin or cell walls of *Lactococcus lactis* and **reported** that both supported
389 survival of the pathogen. They suggested that *L. monocytogenes* is able to colonize and
390 grow in cheese or fermented foods in part because it can gain carbon and energy from
391 other bacteria or fungi that may be present (Premaratne et al., 1991).

392 The virulence and stress genes of *L. monocytogenes* can be influenced by the type of
393 carbohydrate present. Park and Kroll (1993) reported that cellobiose repressed the
394 expression of two genes coding for the virulence factors listeriolysin O and
395 phospholipase C in *L. monocytogenes* NCTC 7973, and Park (1994) observed that
396 arbutin had the same effect. Park and Kroll (1994) speculated that these compounds
397 were signals to *L. monocytogenes* that the organism was present in a vegetative
398 environment and therefore repressed their virulence genes. However Milenbachs,
399 Brown, Moors and Youngman (1997) determined that glucose, cellobiose, maltose,
400 trehalose, and fructose present at 25 mM were all able to suppress the expression of
401 listeriolysin O, but did so only when present in amounts that would stimulate growth of
402 the organism.

403 **5.2 Vitamins**

Porter and Pelczar (1941) first reported investigating the vitamin requirements for *Listerella monocytogenes*. They evaluated vitamins in the B-complex family, purines, pyrimidines, coenzymes and multiple organic acids for stimulatory effects by growing 12 strains via serial sub-culture on a medium of vitamin-free, acid-hydrolyzed casein, inorganic salts, and glucose. Riboflavin and biotin were both identified as essential for growth, and thiamine was stimulatory although not a requisite. Hutner (1942) also explored the growth requirements of *Listerella monocytogenes* and confirmed the requirement for riboflavin in an ovine strain using titratable acidity as a measurement of growth. Hutner (1944) published another abstract describing the culturing of nine strains with biotin, riboflavin, and thiamine in order to identify the '*Listeria* growth factor' which appeared to be contaminating vitamin-free, acid-hydrolyzed casein, but was unable to identify it. Cury et al. (1954) also identified thiamine, and α -lipoic acid as essential in addition to riboflavin and biotin, but did not observe growth improvements with addition of pantothenic acid, pyridoxine, pyridoxal, pyridoxamine, paraminobenzoic acid, B₁₂, folic acid, leucovorino (folinic acid), cocarboxylase, DPN, ATP, xanthopterin, hydrolyzed RNA and DNA, or purine and pyrimidine bases. Patocka, Mara and Schindler (1960) reported pyridoxine as being able to replace biotin for 38 strains isolated from humans. Friedman and Roessler (1961) also confirmed that thiamine, biotin, and riboflavin were required. Details of vitamins found to be essential or stimulatory to *Listeria* spp. growth are listed in **Table 4**.

Welshimer (1963) attempted to clarify these conflicting reports about the requirement of *L. monocytogenes* for thiamine, biotin, and pyridoxine, as well as lipoic acid. He initially washed the bacterial growth from an 18-hour slant culture and transferred it to a lipoic

acid-free and thiamine-free medium to be incubated for 24 hours at 37°C. Aliquots of this culture were subsequently transferred to media supplemented with combinations of riboflavin, thiamine, lipoic acid, biotin, or pyridoxine; Welshimer concluded that all of the tested vitamins were required for growth except pyridoxine, which also could not substitute for biotin. This finding was in contrast to a previous report which found that pyridoxine could substitute for biotin when combined with riboflavin (Patocka et al., 1960). The reported discrepancies may be due to the difference in protocols or strains used. Serially passaging cells, as performed by Welshimer (1963), would essentially dilute out any carried over biotin. Welshimer (1963) found that the requirement for lipoic acid could be reduced by excessive thiamine in the medium, although lipoic acid was still necessary. Romick and Fleming (1998) determined that thiamine is necessary as a cofactor for acetolactate synthase, the enzyme required to convert pyruvate to acetoin. Similarly, lipoic acid is a required cofactor for several enzyme complexes, including α -ketoacid dehydrogenase (Spalding & Prigge, 2010). However, Ralovich et al. (1977) found only riboflavin and biotin to be essential, although they reported that the inclusion of thiamine promoted **larger colony size** on agar plates. Premaratne et al. (1991) confirmed the levels that Welshimer used, but still increased their concentrations to improve growth of *L. monocytogenes* strain Scott A.

When evaluating vitamin requirements, Siddiqi and Khan (1989) observed only riboflavin and Ca-pantothenate to be essential for all six strains, and biotin for three strains. However, two of these strains were later classified as *L. ivanovii* serotype 5 (Nadon, Woodward, Young, Rodgers, & Wiedmann, 2001). The experimental design of Siddiqi and Khan (1989) called for samples to include: 1) the complete medium, 2) **one**

with all the vitamins removed, followed by a sample for each individual vitamin added to the all-vitamins-removed-medium. Thus, samples which would contain all but one or a series of vitamins were not reported. Considering previous reports which demonstrated growth without taking into account pantothenic acid, Siddiqi and Khan (1989) left open the question as to the true requirements of these strains. They also did not observe a requirement for thiamine, which they speculated could be due to the inclusion of sulfur containing amino acids (Siddiqi & Khan, 1982). In addition, Premaratne et al. (1991) removed the riboflavin but increased the biotin to 0.5 mg/L (2.0465 μ M). Phan-Thanh and Gorman (1997) added the vitamins pyrodoxal HCl, para-aminobenzoic acid, calcium pantothenate, and nicotinamide. Schneebeli and Egli (2013) added niacin, pantothenic acid, cobalamin, pyridoxine, folic acid, 4-aminobenzoic acid and nicotinamide in addition to riboflavin, biotin, and thiamine; they added these extra vitamins because they did not negatively affect growth and they are often required for bacterial growth.

Cury et al. (1954) first reported *L. monocytogenes*' requirement for lipoic acid, demonstrating that increasing concentrations from 43.6 mM to 242 mM linearly increased growth. They also found that high levels of acetate (0.005 to 0.02 mM) or pyruvate (0.03 to 0.11 mM) mediated the effects of low lipoic acid. Oddly, they found that the strains were also able to grow well in its absence. Friedman and Roessler (1961) also failed to confirm whether lipoic acid was necessary. However, in 1963 Welshimer corrected this by investigating lipoic acid starved *L. monocytogenes* (Welshimer, 1963). Because the requirement for lipoic acid was so low, he demonstrated that Friedman and Roessler (1961) most likely retained sufficient levels of

473 the vitamin in the inoculum so as not to detect an effect in their exclusion studies.
474 Friedman and Roessler (1961) acknowledged this could be the case due to the very low
475 levels (2.4×10^{-9} to 4.4×10^{-10} mM) published by Cury et al. (1954).

476 In order to determine whether lipoic acid was indeed necessary, Welshimer (1963) first
477 prepared an 18-hour slant culture, washed the growth from it, and transferred the
478 growth to lipoic acid free and thiamine free medium to be incubated for 24 hours at
479 37°C. The author's hypothesis was that this would provide sufficient exponential growth
480 to deplete any residual lipoic acid associated with the cells even after washing, thus
481 providing a source of lipoic acid for lipoic acid-starved cells. Upon suspension of these
482 cells in media supplemented with combinations of riboflavin, thiamine, lipoic acid, biotin,
483 and pyridoxine, it was demonstrated that lipoic acid was required for growth (Welshimer,
484 1963). Previous research revealed that 8-methylthioctic acid, an analogue of lipoic acid,
485 served as an antagonist of lipoic acid for *Streptococcus faecalis* and *Tetrahymena geleii*
486 metabolism (Stokstad, 1954). Welshimer (1963) found that 8-methylthioctic acid was
487 also an antagonist of lipoic acid in *L. monocytogenes*. Welshimer (1963) discovered
488 that providing 1 µg/ml of thiamine in the medium overcame the antagonistic effect of 8-
489 methylthioctic acid when 4.8×10^{-8} mM of lipoic acid was used in the medium. However,
490 when only 3.3×10^{-9} mM of thiamine was present in the medium, 8-methylthioctic acid
491 was antagonistic to as high as 4.8×10^{-6} mM of lipoic acid (Welshimer, 1963).

492 Ralovich et al. (1977) claimed that thiamine and lipoic acid were not required for growth
493 while confirming that riboflavin and biotin were required. However, they noted that the
494 growth was slight, based solely on colony size, and therefore added thiamine and lipoic

acid to encourage vigorous growth. Considering the work of Welshimer (1963), it seems likely that Ralovich et al. (1977) transferred small quantities of each nutrient in conjunction with their inoculum because they did not include a depletion step as previously described.

Premaratne et al. (1991) quadrupled the lipoic acid in their efforts to promote better growth. Keeney, Colosi, Weber, & O’Riordan (2009) would later show that lipoate (the conjugate base of lipoic acid) is essential in nutrient limited conditions for *L. monocytogenes*’ branched-chain fatty acid metabolism, which in turn is required for cell wall construction. Larson, Hattier and McCleskey (1965) found only one strain out of 128 to require fatty acids. In this case the strain required isobutyric acid or 2-methylbutyric acid.

5.3 Amino acids and other nitrogen sources

Porter and Pelczar (1941) reported attempting to replace vitamin-free, acid-hydrolyzed casein with 18 amino acids, but determined that only 1 of the 12 strains grew, although not consistently. Adding an additional 25 amino acids and their derivatives resulted in growth by some strains. However, they did not include individual amino acid exclusion experiments which would have provided data about the requirement for each amino acid. The various nitrogen sources used for *Listeria spp.* are listed in **Table 5**.

Premaratne et al. (1991) claimed that leucine (0.00076 mM), isoleucine (0.00076 mM), arginine (0.00057 mM), methionine 0.00067 mM), valine (0.00085 mM) and cysteine (0.00083 mM) were required for growth of *L. monocytogenes* in MWB. They also stated

516 that glutamine (0.0041 mM) was required as the primary source of nitrogen. Friedman
517 and Roessler (1961) found that inorganic ammonium salts could not serve as the sole
518 nitrogen source in MM for the strains of *L. monocytogenes* which they studied.
519 However, there is evidence that *L. monocytogenes* living within mammalian host cells
520 uses ammonium rather than glutamine as a nitrogen source (Joseph et al., 2006).
521 Friedman and Roessler (1961) determined a requirement for valine, isoleucine, leucine
522 and cysteine by at least one strain. However they demonstrated the cysteine
523 requirement actually represented a requirement for organically bound sulfur, since using
524 either filtered cystine or sodium thioglycolate also promoted growth (Friedman &
525 Roessler, 1961). Ralovich et al. (1977) reported valine, leucine, isoleucine, and cysteine
526 to be essential, but that some strains also required glutamine, methionine, arginine, and
527 histidine. In addition, they did not find that sodium thioglycolate could replace cysteine,
528 but they retained it in their medium to maintain semi-anaerobic conditions to enhance
529 growth.

530 Siddiqi and Khan (1982) found that most of the six strains of *L. monocytogenes* that
531 they studied required cystine, valine, isoleucine, and leucine, while phenylalanine was a
532 stimulatory growth factor for all six strains. They also stated that tryptophan was
533 essential for growth of three strains and stimulatory for the other three. However, Tsai
534 and Hodgson (2003) demonstrated that the solid form of MWB, only methionine and
535 cysteine were essential. Although Premaratne et al. (1991) reported that *L.*
536 *monocytogenes* could not use inorganic nitrogen, Tsai and Hodgson (2003) found they
537 could replace glutamine with $(\text{NH}_4)_2\text{SO}_4$ and $(\text{NH}_4)\text{NO}_3$, but not with NaNO_3 , which
538 suggests that *L. monocytogenes* does not possess nitrate reductase.

539 The addition of 16 other amino acids (**Table 5**) enhanced growth, mostly by decreasing
540 lag phases by 10+ hours, but also by slightly decreasing the doubling times (Tsai &
541 Hodgson, 2003). They hypothesized that the long lag phase was due to the time
542 required for the organism to produce the amino acids necessary for growth (Tsai &
543 Hodgson, 2003). All of these experiments highlight the strain differences in nutrient
544 requirements for *L. monocytogenes*. However, it seems that most, if not all, strains have
545 a requirement for cysteine, methionine, isoleucine and valine. In addition, there is a
546 requirement for glutamine as a nitrogen source, although some strains appear to be
547 able to use inorganic ammonium.

548 While *L. monocytogenes* lacks extracellular peptidase activity, it has been shown to
549 transport peptides up to octapeptides across its membrane with a permease (Verheul,
550 Rombouts, & Abee, 1998) and can use proton-motive-force (PMF) to transport di- and
551 tripeptides (Verheul et al., 1995). This not only sheds light on how *L. monocytogenes*
552 gains nutrients from the environment, but provides a wider opportunity for defined media
553 composition in the future. Marquis, Bouwer, Hinriches, & Portnoy (1993) demonstrated
554 the ability of amino acid auxotrophs (phe, ade, gly, thr, phe trp & tyr, and pro) to survive
555 in cell lines which had been starved of the respective amino acids. While growth was
556 slower than the wild-type *L. monocytogenes*, the auxotrophs still succeeded in
557 producing adequate growth. This may shed light on why there is so much strain to strain
558 variation for amino acid requirements. Since much of the literature relied on OD, another
559 possibility is that some strains were falsely determined to be unable to grow without
560 certain amino acids, but were actually multiplying below the OD limit of detection.

5.4 Trace elements

Friedman and Roessler (1961) found both Mg^{++} and K^{+} to be required for at least one strain. Trivett and Meyer (1971) included iron in their medium based on Sword's work (Sword, 1966) demonstrating the stimulatory effect of it upon *L. monocytogenes* growth and its lethal dose (LD50) reduction ability. Trivett and Meyer (1971) also noted a two-thirds reduction in growth when iron was not present in their medium. Cowart and Foster (1985) later demonstrated that of the two virulent and two avirulent strains, all four were stimulated by iron inclusion, but that the two virulent strains had a greater percent increase in specific growth rate. In addition, Trivett and Meyer (1971) excluded Mn^{++} from their medium because Sword (1966) found it to be inhibitory to *L. monocytogenes* growth *in vitro*. Ralovich et al. (1977) found manganese (II), in the form of $MnCl_2$, to be inhibitory to some strains which corroborates Sword's work (Sword, 1966). Citrate was used to keep Fe^{++} , Mg^{++} , and Ca^{++} in solution (Ralovich et al., 1977), whereas Premaratne et al. (1991) used ferric citrate to provide an iron source. However, Premaratne et al. (1991) determined that hemin, hematin, and ferric nitrate could provide the required iron but protoporphyrin IX, hematoporphyrin, and ferric chloric did not supply iron. Schneebeli and Egli (2013) found a combination of trace elements to be necessary for the growth of *L. monocytogenes* (**Table 6**). They tested a medium containing no trace elements, one containing the concentration listed in Table 5 (1X) as well as 2X and 4X. Cell yield increased by a small margin with increasing levels of trace elements, but μ_{max} was highest at 1X trace elements and decreased by 38.4% at 4X trace elements. No growth was observed when the trace elements were omitted

(Schneebeli & Egli, 2013). McLaughlin, Hill, and Gahan (2011) provide a nice overview of the iron acquisition mechanisms of *L. monocytogenes*.

5.5 Buffering

Premaratne et al. (1991) noted that part of the lack of sustainable growth in Welshimer's medium might be due to the rapid decrease in medium pH. Therefore they doubled the potassium and sodium phosphate salts of Welshimer's medium in order to increase the buffering capacity (**Table 7**). Conversely, Behari and Youngman (1998), reduced the potassium and sodium salts and replaced them with 3-N-[morpholino]propanesulfonic acid (MOPS) for buffering. Tsai and Hodgson (2003) also reduced both the KH_2PO_4 and Na_2HPO_4 to 1/10th of what was used by Premaratne et al. (1991) in order to avoid the toxic effects of phosphate salts on the cells. They reported that this substitution 'drastically' improved growth in liquid and on solid media as compared to MWB. Fraser, Harvie, Coote and O'Byrne (2000) observed that the addition of peptone to the DM of Amezaga et al. (1995) was associated with an increased growth. Schneebeli and Egli (2013) also used MOPS (100 mM) as a buffering agent and reduced the phosphate concentration to 1/10 the original level in MWB and found that growth in liquid medium was better than with the phosphate buffers and allowed formation of easily visible colonies on the solidified medium. The actual impact of phosphate salts, or other buffering agents, on the growth of *Listeria spp.* is not known and worthy of some investigation. The issue may be confounded by the buffers' influence on osmotic stress and thus the bacterium's osmotic stress responses. The use of a continuous culture system would mitigate some need for buffering as the dilution would remove the acids

being produced. In addition, an ecological application being studied might actually benefit from a less buffered system.

6. Effects of media on genetic expression and phenotype

In order for *L. monocytogenes* to successfully infect humans certain virulence genes must be present and be expressed; this expression has been demonstrated in various growth media (Datta & Kothary, 1993; Mekalanos, 1992). A number of conditions in the environment control expression of these genes including temperature, pH, osmolarity and presence of some ions or sugars (Datta & Kothary, 1993; Park & Kroll, 1993). For instance, the expression of listeriolysin, a pore forming toxin necessary for *L. monocytogenes* to escape from vacuoles, is induced by heat shock and oxidative stress (Sokolovic & Goebel, 1989). *L. monocytogenes* does not produce all of its virulence factors in rich media but these factors are expressed when the organism is grown in MM (Bohne, Kestler, Uebele, Sokolovic & Goebel, 1996; Bohne, Sokolovic & Goebel. 1994; Ripio et al., 1996). Ripio et al. (1996) surveyed *L. monocytogenes* strains of different origins and determined that wild type strains did not produce hemolysin or lecithinase when cultured in a rich medium such as BHI, but did produce these virulence factors when charcoal was added to the medium. They determined that charcoal induced the transcription of PrfA, which is the regulatory protein that turns on expression of most virulence factors in *L. monocytogenes*.

Park and Kroll (1993) found that cellobiose was the only one of several mono- and disaccharides they tested that repressed expression of listeriolysin or lecithinase and

626 therefore concluded that it was a signaling molecule indicating to *Listeria* cells that they
627 were in a soil environment and did not need to produce virulence factors. However,
628 Milenbachs et al. (1997) reported that several metabolizable sugars, including glucose,
629 fructose, maltose and trehalose also down regulated some virulence genes in several
630 strains of *L. monocytogenes*, although they did find that for strain NCTC7973 only
631 cellobiose was able to repress these genes. Jaradat and Bhunia (2002) found that
632 growth in BHI or media with high concentrations of glucose suppressed the expression
633 of *Listeria* adhesion protein (LAP) when compared to growth in nutrient poor peptone
634 broth; they speculated that the growth environment influences expression of LAP which
635 could affect attachment in the host and severity of the disease process.

636 Nannapaneni, Story, Bhunia and Johnson (1998a) determined that growth conditions
637 and media affected the expression of surface epitopes on *L. monocytogenes* and thus
638 their response to monoclonal antibodies. None of the 13 serotypes they tested was
639 detected by one of the monoclonal antibodies when cells were grown in either UVM1
640 broth or Fraser broth, indicating that the epitope was not expressed under these
641 conditions. Bhunia and Johnson (1992) had developed a monoclonal antibody, EM-6EII,
642 which recognized a *Listeria* genus-specific epitope on cell-surface antigens. This
643 antibody was able to detect live cells of all *Listeria* species and all 13 serotypes of *L.*
644 *monocytogenes* by ELISA when cells were grown in BHI, *Listeria* enrichment broth, or in
645 *Listeria* repair broth, but it could only detect four of the serotypes of *L. monocytogenes*
646 when cells were grown in the UVM1 formulation of *Listeria* enrichment broth or in Fraser
647 broth (Nannapaneni, Story, Bhunia & Johnson, 1998b).

L. monocytogenes can be divided into primary genetic lineages based on multigene phylogenetic analyses (Nadon et al., 2001). More than 98% of reported human listeriosis cases are caused by serotypes within lineages I and II (1/2a, 1/2c, 1/2b, and 4b). Ta ng et al. (2015) measured the $\mu_{\max}$ of lineage I and lineage II strains when grown in a DM at 16°C. They determined that some lineage II strains could grow faster than some genetic subgroups of lineage I strains under these conditions. They suggested that this might be the basis for the persistence of some strains in processing plants, where nutrient-limited conditions may occur, which could in turn lead to a higher probability that these organisms can survive and contaminate food leading to foodborne illnesses.

7. Conclusions

Historically, defined media have been developed for fundamental microbial studies investigating bacterial genetics, particularly for organisms such as *Escherichia coli* and *Salmonella*. These organisms have the ability to synthesize their nutritive requirements from minimal ingredients such as ammonia, salts and glucose and a formulated minimal defined medium proved critical for elucidating fundamental biosynthesis and degradation pathways (Gutnick, Calvo, Klotkowski & Ames, 1969; Miller, 1972; Baker, Rubinelli, Park, Carbonero & Ricke, 2016). This was critical as individual research laboratories needed universal standard media to compare results and interpretations across independent studies. As this review indicates, although individual attempts have been made to develop similar such media for *Listeria*, these have been less generalized as the need has been more based on individual specific experimental questions rather

670 than overarching fundamental objectives. Consequently, no universal defined media
671 currently exists for *Listeria*. Considering the diverse media designed for the culture of
672 *Listeria spp.* and the widely varying results with varying strains, how should we define
673 whether a medium has the ability to sustain growth of a strain, species, or the genus?
674 Should we establish a quantitative criterion, such as a maximum doubling time? Since
675 we often use the criterion of 1 log difference when assessing treatments and effects in
676 food microbiology, the very minimum requirement should be that a medium is able to
677 support at least 1 log of growth above the inoculated concentration. Because of the
678 tenuous relationship between OD and CFU for *Listeria* in DM, this will require the use of
679 plate count techniques. Secondly and of equal importance, the medium should be able
680 to sustain growth of a number of serial transfers in batch culture or over a sustained
681 period of time in continuous culture. This will ensure that the inoculum is not carrying
682 over minute nutrients necessary for growth and giving the appearance of sufficiency.

683 Researchers should consider using a DM which has historically been used, so as to
684 provide as much comparison as possible to the literature. If a new medium is
685 necessary, then a comprehensive list of strains and species should be used and a
686 historically-used medium should be included for side-by-side comparison. To facilitate
687 choosing an appropriate medium for a particular project, use Table 2 to find a medium
688 which has been used for your environmental conditions or studied phenomenon. The
689 use of bioinformatics to identify novel metabolic genes and pathways may yield
690 interesting paths of inquiry. However, researchers should be aware of anomalies which
691 would indicate novel mechanisms not predicted by bioinformatics.

As we continue to consider questions about the ecology of *Listeria spp.* and *L. monocytogenes* and their interactions which may influence whether food products become contaminated or foster the growth of *Listeria*, the use of continuous culture with a single DM across a range of dilution rates to study a mixture of strains and species offers some interesting avenues. Co-culture or competitive inhibition experiments have provided interesting insights into the possibility of *L. innocua* overgrowth of *L. monocytogenes* in enrichment (Koo, Ndahetuye, O'Bryan, Ricke & Crandall, 2014). Environmental simulation with a DM at low dilution rates would yield interesting insights into their competition. Phyllospheres (leaf surfaces), floors, masonry, and water sources are all environments which would benefit from a common DM adjusted to model the conditions.

Acknowledgements: Author Jarvis gratefully acknowledges financial support from a Distinguished Doctoral Fellowship from the Graduate School of the University of Arkansas. Author Crandall gratefully acknowledges funding from USDA/NIFA grant # 2010-51110-21004.

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1258	Table 1: General uses for defined medium by researchers	Reference
	Assess growth rates under reproducible conditions, rather than using rich media which is variable.	Greasham & Herber, 1997
	Cause limiting conditions for single or multiple nutrients	Greasham & Herber, 1997
	Add labeled nutrients to track catabolism and anabolism	Eisenreich et al., 2006; Eylert et al., 2008
	Evaluate the production of metabolites	Eisenreich et al., 2006; Greasham & Herber, 1997
	Evaluate the effect of mutations	Mortelmans & Zieger, 2000
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1260		

1261 Table 2 Specific uses of minimal and defined media for the study of *Listeria monocytogenes*
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Medium	Acid stress	AA metabolism	Antimicrobials	Attachment	Barometric stress	BCFA	Bile stress	Biofilms	Carbon metabolism	Low temperature	Virulence factors	Osmotic stress	Iron	Proteomics	Signaling	References
Amezaga et al 1995	X	X														Abram et al., 2008; Heavin et al., 2009, 2010; Karatzas et al., 2012; Amezaga et al., 1995
IMM	X					X			X	X	X	X	X	X		Milillo et al., 2009; Phan Than & Gorman, 1998; Keeney et al., 2009; Crimmins et al., 2009; Brøndsted et al., 2003; García-del Portillo et al., 2011; Garfan et al., 2003; Dussurge et al., 2003
Esvan et al. 2000	X											X		X		Mbandi et al., 2007; Esvan et al., 2007
MWB	X	X			X		X	X	X	X	X	X	X	X		Michel et al., 2011; Eisenreich et al., 2006; Verheul et al., 1995, 1997, 1998; Wouters et al., 2005; Considine et al., 2011; Sleator et al., 2001, 2004; Chang et al., 2012, 2013; Moltz & Martin, 2005; Zhou et al., 2012; Joseph et al., 2008; Marr et al., 2006; Mertins et al., 2007; Schar et al., 2010; Stoll & Goebel, 2010; Wemekamp-Kamphuis et al., 2004; Cabrita et al., 2010; Coffey et al., 1996; Stoll et al., 2008; Beumer et al., 1994; Okada et al., 2002, 2006; Wouters et al., 2005; Mohammed et al., 2006; Rea et al., 2004;
HTM						X				X						Zhu et al., 2005
McFeeters & Chen, 1986									X							Romick & Fleming, 1998
Modified Pine's										X		X	X			Dykes & Morehaed, 2001; Dykes & Dworaczek, 2002; Angelidis & Smith, 2003
MCDB 202								X				X		X		Combrouse et al., 2013; Duche et al, 2002; Renier et al., 2013

Sterile pond microcosm	X			Mai & Conner, 2007
MEM w/ Eagle Salts		X		Böckmann et al., 1994, 1996; Engelbrecht et al., 1996; Lalic-Mülthaler et al., 2001; Sokolovic et al., et al., 1993, 1996
M9	X			Martinez-Abad et al., 2012
Dulbecco's modified Eagle's		X		Midelet-Bourdin et al., 2006
Riesenberg et al., 1991			X	Yang et al., 2006
Patchett et al., 1992		X		Patchett et al., 1992
RPMI 1640		X		Newton et al., 2005

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1281 Table 3 Carbon sources tested for utilization by *Listeria monocytogenes*

	Miller & Silverman, 1959	Friedman & Alm, 1962	Trivett & Meyer, 1967	Trivett & Meyer, 1971	Pine et al., 1989
Glucose	NS	0.2, 0.6, 1.0%	28 mM		0.25%
Gluconate	NS				
Xylose	NS				0.25%
Arabinose	NS				
Ribose	NS				
Erythritol			0.2, 0.8, 3.1, 12.5, 50, 200 mM		
Pyruvate				0.2%	
Acetate				0.2%	
Citrate				0.2%	
Isocitrate				0.2%	
α -ketoglutarate				0.2%	
Succinate				0.2%	
Fumarate				0.2%	
Malate				0.2%	

	Galactose	0.25%
	Lactose	0.25%
	Rhamnose	0.25%
1282	NS = not stated	
1283		

1284 Table 4 Vitamins found to be required for or stimulatory to *Listeria spp.* growth
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Citation	Friedman & Roessler, 1961	Welshimer, 1963	Trivett & Meyer, 1971	Ralovich et al., 1977	Siddiqi & Khan, 1982	Premaratne et al., 1991	Phan-Thanh & Gormon, 1997	Tsai & Hodgson, 2003	Patchett et al., 1992	Amezaga et al., 1995	Esvan, et al., 2000	Schneebeli & Egli, 2013
Riboflavin	13.29 ^a	2.66	2.66	10.01	13.29	1.33	13.29 ^b	1.33	2.66	2.66	10.64	132.85 pM
Thiamine-HCl	14.82	2.96	2.96	27.92	16.82	3.32	2.96	2.96	2.96	2.96	2.96	166.22 pM
Biotin	20.47	0.41	0.41	15.42	20.47	2.05	2.05	2.05	0.41	0.41	0.66	81.86 pM
α-Lipoic Acid	24.23	0.00485	0.00485			0.024	0.024	24 pM ^c	0.00485	0.00485	24.2 pM	242.24 pM
Niacin					40.61							406.14 pM
Nicotinamide					40.94		8.19					409.42 pM
Pantothenic acid					20.98		4.20					228.07 pM
Folic acid					11.33							45.31 pM
Cobalamin												36.89 pM
Pyridoxine					24.56		4.91					591.09 pM

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1287 ^a Concentration in μM unless otherwise indicated.

1288 ^b They list MWB as containing 5 mg/L (13.29 μM) riboflavin, when Premaratne et al (1991) report using 10-fold less. For
1289 purposes of this table, the values reflect what each respective publication says about itself.

1290 ^c They list MWB as containing 24 pM of α-lipoic acid, but Premaratne et al (1991) report using nM concentrations. Likely a
1291 mistransposition of digits. pM concentrations for Tsai and Hodgson (2003) reported, but may have been nM.

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1297 Table 5 Nitrogen sources used for growth of *Listeria spp.*
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Reference	Friedman & Roessler, 1961	Welshimer, 1963	Trivett & Meyer, 1971	Ralovich et al., 1977	Siddiqi & Khan, 1982	Premaratne et al., 1991	Phan- Thanh & Gormon, 1997	Tsai & Hodgson, 2003 ^c	Patchett et al., 1992	Amezaga et al., 1995	Esvan et al., 2000	Schneebeli & Egli, 2013
Source												
Casein hydrolysate		2% or the amino acids			0.5 ^b							
Alanine	8.98 ^a											
Aspartic Acid	6.0											
Glutamic acid	5.4											
Arginine	0.94	0.95	0.95			0.57	0.47		1.1	0.95		0.57
Lysine	1.1											
Histidine	0.52	1.0	1.0				0.52		1.3	1.0		0.64
Isoleucine	1.5	1.5	1.5	1.4		0.76	0.76			0.76	0.02	0.76
Leucine	0.76	0.76	0.76	0.72		0.76	0.76		0.76	0.76	0.01	0.76
Norleucine									0.76			
Methionine	1.3	1.3	1.3			0.67	0.67	0.67	0.67	0.67	0.16	0.67
Phenylalanine	0.61						0.61					
Proline	0.87											
Threonine	0.84											
Tyrosine	0.56											
Valine	1.71	1.71	1.71	1.61		0.85	0.85		1.71	0.85	0.02	0.85
Tryptophan	0.98	0.98			2.4		0.49		0.49	0.49		0.49
Cysteine	0.63	0.63	0.63	2.39	4.12	0.82	0.63	0.82		0.82	0.04	0.82

1312 Table 6 Metal ions reported as required or stimulatory for the growth of *L. monocytogenes*

1313

Citation	Friedman & Roessler, 1961	Welshimer, 1963	Trivett & Meyer, 1971	Ralovich et al., 1977	Siddiqi & Khan, 1982	Premaratne et al., 1991	Phan- Thanh & Gormon, 1997	Tsai & Hodgson, 2003	Patchett et al., 1992	Amezaga et al., 1995	Esvan et al., 2000	Schneebeili & Egli, 2013
MgSO ₄	3.25	1.66	1.66	7.64	0.20	1.66	1.66	1.7	3.32	1.62	20	6.29
MgCl ₂												0.33
MnSO ₄	0.592											
MnCl ₂												0.029
FeSO ₄	0.14			0.03							0.007	
Ferric Citrate						0.36	0.36					
FeCl ₃			0.18		0.006					0.025		0.14
ZnCl ₂										0.006		
ZnO												0.02
CuSO ₄												0.003
CuCl ₂										0.002		
CoCl ₂										0.002		0.003
Na ₂ MoO ₄										0.001		0.02
CaCl ₂				0.056						0.019	0.001	
CaCO ₃												0.4
H ₃ BO ₃												0.01
EDTA												5

EDTA·Na ₄ ·2H ₂ O		0.94
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Na-citrate	1.55
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Na ₃ -citrate·2H ₂ O	1.60	0.05
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1314 ^a All concentration in mM unless otherwise indicated

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1316 Table 7 Buffering agents used in minimal and defined media for *Listeria monocytogenes*

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Citation	Friedman & Roessler, 1961	Welshimer, 1963	Trivett & Meyer, 1971	Ralovich et al., 1977	Siddiqi & Khan, 1982	Premaratne et al., 1991	Phan- Thanh & Gormon, 1997	Tsai & Hodgson, 2003	Patchett et al., 1992	Amezaga et al., 1995	Esvan et al., 2000	Schneebeli & Egli, 2013
NaCl	42.78 ^a				85.56						500	
KCl					0.67068							
Na ₂ HPO ₄		57.76	9.38		17.61	115.49	115.49	11.55	17.42	17.42		12.8
Nitrilotriacetic acid			2.51							0.35		
MOPS								100				100.01
K ₂ HPO ₄	34.45		48.80	56.76					45.93	46.01	1050	
KH ₂ PO ₄		24.10		31.14	11.02	48.20	48.20	4.82			450	4.82
Total PO ₄	34.45	81.86	58.18									
NH ₄ Cl			9.35						18.70	18.70		
(NH ₄) ₂ SO ₄				7.13	37.84		4.54				100	53.5
Total NH ₄			9.35	14.26								
NaOH Sodium pyruvate			6.00							0.84		
											50	

1318 ^a All concentrations in mM unless otherwise indicated

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