

# *Listeria monocytogenes*: physiology and metabolism

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## SUMMARY

*Listeria monocytogenes* possess metabolic and physiological activities that affect the etiology and pathogenicity of this foodborne human pathogen. These include: ability to grow at refrigerator temperatures (5°C) as well as 40°C; facultative anaerobiosis with presence of respiratory enzymes and cofactors; ability to survive high levels of salt, nitrite, thallos and lithium salts, as well as moxalactam, bacitracin, and nalidixic acid; production of hemolysins and phospholipase; presence of catalase and superoxide dismutase for surviving macrophagic intracellular oxidative bursts; temperature-regulated motility; specific attachment to intestinal Peyer's patches and ability to cross placental and brain membrane barriers; presence of plasmids; and omnipresence in the environment. Intriguing are the apparent limitation of virulence to foods of dairy origin and coleslaw; the mechanism for the CAMP hemolysis tests; and the basis of the blue color of tryptose agar colonies seen under reflected light.

## INTRODUCTION

The physiology and metabolism of *Listeria monocytogenes* affect the etiology and the pathogenicity of this foodborne intracellular human and animal pathogen. Since microbial taxonomy is an artificially imposed classification based principally on metabolic preferences and physiological activity, the genus *Listeria* has, because of its unusual behavior, been classified during the past 40 years under various catch-all groupings with little relationship to its medical importance. Some of these physiological activities, however, have relevance to its virulence and to its growth and infectivity in food products.

## IDENTIFICATION

*Listeria monocytogenes* is presently classified [67] under Section-14, Regular non-sporing Gram (+) rods, which includes a conglomerate of seven very different genera. The principal differences between the genera are based on the shape and size of rods, motility, and anaerobiosis. Under prior taxonomic classifications, this organism could have been misidentified as a coryneform contaminant in medical isolates, since such isolates often are of different appearance than the laboratory or food strains [32]. The principal bases for identification of *Listeria* are: Gram (+) staining, regular short rods, motile, and catalase (+). In actuality, old cultures may stain Gram (-), with coccoid forms often present, particularly in fresh isolates from diseased animals.

The motility is quite dependent upon incubation temperature, often being motile only between 15–30°C, and the catalase production is dependent upon medium and cultural conditions.

The present method for identification of *Listeria* is based on biotyping, or separation by biological differences. *Listeria* can be distinguished from the related taxons *Brochothrix*, *Erysipelothrix*, *Lactobacillus*, and *Kurthia* through differences in motility, anaerobic growth, catalase production, growth temperature, and acid production from glucose (Table 14.11 in [67]). All the above taxons have been isolated from meat and meat products, and many also have been isolated from milk products and other foods. Consequently, the above five tests are critical for the initial genus placement.

Once the organism has been assigned to the genus *Listeria*, additional biotyping is necessary to further classify the species. Although the present taxonomy in Bergey's Manual (Table 14.12 in [67]) lists eight species as belonging to the genus, more recent research would indicate that the genus may be subdivided into two groups containing a total of seven species. An excellent recent report by Feresu and Jones [24] presents an examination of 187 strains of *Listeria* and related Gram (+) bacteria using 140 characterizations. Data were analyzed by computer to develop 20 phenons, useful for classification. One phenon contains five species of *Listeria*, with the other three species assigned to other phenons. The latter three were also indicated by Rocourt [59] who determined that *Listeria denitrificans* is sufficiently different in metabolic and physiological activity to be transferred out of the genus

*Listeria* to a new genus, *Jonesia*, as *Jonesia denitrificans*. The other two species, *L. grayi* and *L. murrayi*, differed sufficiently from the remaining species (*monocytogenes*, *innocua*, *seeligeri*, *welshimeri*, and *ivanovii*) to be in a separate subgroup. Three species of the genus *Listeria* produce  $\beta$ -hemolysis, two are significantly pathogenic to animals, and only one (*monocytogenes*) is pathogenic to humans.

The identification of species of an isolated *Listeria* organism is made by determination of growth in rhamnose and xylose media, by  $\beta$  hemolysis on sheep blood agar, and by the CAMP test which measures an increased hemolysis in the presence of the organisms *Staphylococcus aureus* or *Rhodococcus equi* (Table 1). It should be noted that the five species listed in Table 1 are all of recent origin. Until 1961, the sole organism in the genus *Listeria* was '*L. monocytogenes*'. In 1961, *L. denitrificans* was added (and removed in 1988); *L. grayi* was recognized in 1966; and *L. murrayi* in 1971. The remainder were added after 1980 [60]. As a result, much of the earlier research on '*L. monocytogenes*' may have been conducted with other species. Presently, biotyping for *L. monocytogenes* induces the following positive tests [67, 81]: catalase, alkaline phosphatase, beta-galactosidase, beta hemolysis, methyl red and Vokes-Proskauer (VP) tests, reduction of 0.04% tellurite and tetrazolium salts, growth in 10% and 40% bile, 6.5% NaCl, and 0.025% thallos salts. With carbohydrate media the species ferments glucose, fructose, mannose, galactose, cellobiose, trehalose, and rhamnose. There is delayed fermentation on maltose, sucrose, lactose, dextrin, and the polyhydric alcohols glycerol and sorbitol.

Table 1

Microbiological characteristics of *Listeria* subgroup I

Strain	Growth on rhamnose	Growth on xylose	Hemolysis of blood	CAMP with <i>S. aureus</i>	CAMP with <i>R. equi</i>	(GC)% DNA
<i>monocytogenes</i>	+	–	+	+	–	37–39
<i>innocua</i>	V	–	–	–	–	36–38
<i>welshimeri</i>	V	+	–	–	–	36
<i>seeligeri</i>	–	+	+	+	–	36
<i>ivanovi</i>	–	+	+	–	+	37–38

V = variable in tests.

It hydrolyzes and uses amygdalin, esculin, and salicin. *L. monocytogenes* is negative on indole production and utilization of citrate, starch, arabinose, inositol, and most sugar alcohols, including mannitol. It does not digest gelatin or hydrolyze milk or casein. It is partially inhibited by 0.02% azide and cyanide, and does not reduce nitrates or produce hydrogen sulfide. With enzyme production, it is negative for urease, coagulase, arginine decarboxylase, and the decarboxylases for glutamate, lysine, and ornithine, as well as phenylalanine deaminase and arginine dihydrolase. It does not have cytochrome C oxidase, and is negative for tributyrinase. Allegedly, it is negative for lecithinase, although it has been reported to contain a potent phospholipase C [44] which has been implicated in intracellular virulence mechanisms.

Serotyping has been used with biotyping of *L. monocytogenes*. Table 14.13 of Bergey's Manual [67] lists 15 *O* or somatic antigens and 5 *H* or flagellar antigens for the five *Listeria* species, which have been used to separate the serotypes; the *L. murrayi* subgroup contain unique *O* and *H* antigens [76]. The remaining species are serotyped into 16 serovars, with some cross-reactivities between them. *L. monocytogenes* falls into inclusive serovars #1/2, #3, #4, and #7 with serotype #5 being *L. ivanovi* and serotype #6 being one of the other three. Serotype #1/2b and serotype #4c or #4d may also be *L. seeligeri* and serotype #4ab may also be *L. innocui*. In cell wall analytical studies (*vide infra*), *O* antigen I appears to be correlated with a rhamnose containing epitope, *O* antigen VI with glucose components, and *O* antigens IX with galactose components of the teichoic acids. It is unusual that *L. seeligeri*, which cannot metabolize rhamnose in the medium, can synthesize it for teichoic acids in serotype 1/2b.

Many of the serological and biotypical reactions occur with substances at the cell surface. In this aspect, *L. monocytogenes* might be classified by structure and composition as a Gram (+) organism, but behaving in many of its virulence characteristics as a Gram (-) pathogen. A major difference between the two groups is the thickness and composition of the cell wall [2, 63]. Gram (-) organisms have a wall 2–10 nm thick, consisting of an inner plasma

membrane of proteins and lipid bilayer, an intervening periplasmic space, a thin interwoven peptidoglycan, followed by an outer membrane which may include protrusions from the plasma membrane and inclusions of flagella and amphipathic lipo-polysaccharides and glycocalyx. Other amphipathic components of the Gram (-) cell wall include phospholipid, proteins, glycolipids, and lipoproteins. In contrast, the Gram (+) cell wall has a thickness of 25–50 nm and a much thicker interconnected peptidoglycan layer. Arising from the peptidoglycan layer and continuing to the external surface are teichoic acids, defined as an alditol in a linear sequence flanked by phosphodiester. The alditol in *L. monocytogenes* is ribitol, and various sugar molecules may be attached to the ribitol through the OH linkage [25, 26]. *L. monocytogenes* also contains lipoteichoic acids [36], which arise from the inner lipid bilayer membrane and which continue to the surface. Lipoteichoic acids are the principal amphipathic component in *L. monocytogenes*, and can form micellar compounds with other cellular components.

Peptidoglycans, also known as murein or mucopeptides, are composed of glycan chains which are composed of alternating  $\beta$  1–4 linked *N*-acetyl glucosamine and *N*-acetyl muramyl residues, cross-linked through the muramyl residues by an interpeptide link composed of alternating D and L amino acids [26]. *Listeria* contain diaminopimelic acid (DMA) in place of lysine found in many peptidoglycans.

Assay of teichoic acids of the genus *Listeria* [26] indicated essentially a 1:1 relationship between molar content of ribitol and phosphorus with varying contents of glucosamine and other sugars. All *Listeria* contain glucosamine attached to the ribitol, except for serovar #7 which has no apparent carbohydrates present. Serovar #3 contains only glucosamine with no attached carbohydrates, while serovars #1/2 have attached rhamnose. The remaining serovars #4 through #6 have various combinations of glucose and galactose attached to the glucosamine. Serovars #4 and #6 incorporate the glycosyl residues into the chain, alternating with the ribitol phosphates [25, 26, 74, 75]. The rather complex at-

tachment of the teichoic acid to the peptidoglycan is also through the muramyl residues, but at the #6 carbon it is attached through a phosphorylated chain containing glycerol and acetylated sugars and which appears to be specific for the genus *Listeria* [26]. Lipoteichoic acids are connected through non-acetylated sugars and phosphatidic acid to fatty acid residues, particularly the odd numbered *iso* and *anteiso* varieties. Only 8 fatty acids are present, and their relative content has been proposed as a means of distinguishing LM from other clinical isolates [21, 62].

Methods other than biotyping and serotyping have been proposed by various investigators in an attempt to resolve the virulence properties of *L. monocytogenes* with other characteristics. These methods include: phage typing [57], ribosomal RNA typing [72], DNA probes [18, 42], restriction endonuclease typing [22], monoclonal antibody-based ELISA [6], continuous cell lines [23], and biochemical tests [58]. Other proposed methods have included isoenzyme typing, bacteriocin typing, and electrophoretic grouping [57], but most of these methods, as with the biotyping and serotyping do not always distinguish virulent from avirulent strains.

#### METABOLISM

Glucose is fermented at 37°C by the classical Embden-Meyehoff anaerobic glycolysis pathway to yield pyruvate and lactate, with the production of up to 6 mol ATP per mol of glucose [2, 49]. Recent studies by Pine et al. [55] indicate that under anaerobiosis, *L. monocytogenes* produces essentially only lactic acid (circa 80% of the yield under aerobic conditions) from both hexoses and pentoses, although the organisms frequently lyse under extended anaerobiosis.

*L. monocytogenes* is rhamnose (+) but is xylose (-). Rhamnose, found free in poison sumac and as a component in many bacteria, is a derivative of mannose in which the #6 primary alcohol group becomes a methyl group. As a consequence, this position cannot be phosphorylated to yield fructose

1,6-diphosphate. The metabolism of rhamnose is believed to proceed through an isomerization of the #1 and #2 carbons through a keto-enol reaction to produce rhamnulose, which can then be phosphorylated on the #1 alcohol position to rhamnulose-1-phosphate, and metabolized through the phosphoketolase pathway. Rhamnose is a component of one *L. monocytogenes* serotype, (#1/2), as well as one serovar of *L. seeligeri*, yet the latter is apparently unable to metabolize rhamnose! Xylose, a pentose, is not fermented by *L. monocytogenes* or *L. innocua* but is fermented by the other three species excluding the *murrayi-grayi* subgroup. Metabolism would involve phosphorylation to xylose-5-phosphate, to dismutation to triose phosphate in the phosphoketolase pathway. The metabolic basis for these differences between the virulent *L. monocytogenes*, and the other avirulent *Listeria* strains is unknown.

*L. monocytogenes* has aerobic metabolism and possesses cytochromes a, b, b<sub>1</sub>, and d [67]. It has definite requirements for riboflavin, thiamine, biotin, and lipoic acid, indicating that it can synthesize nicotinamide (for NAD and NADP), but requires the cofactors for FAD, TPP, and the decarboxylases [57, 80]. Although the aerobic metabolism is normally a complete citric acid cycle [2], Trivett and Meyer in 1971 [73] indicated that the aerobic pathway in *L. monocytogenes* is split without the normal conversion of alpha-ketoglutarate to succinate through the a-ketoglutarate dehydrogenase complex. Pine et al. [55] noted that aerobic metabolism by *L. monocytogenes* produced both lactic and acetic acids, with small amounts of isovaleric, isobutyric, and isohydroxy acids. Energy is produced through oxidative phosphorylation through reduced NAD from isocitrate and malate conversions, and from FADH<sub>2</sub> from succinate conversion. The acetyl-CoA to a-ketoglutarate pathway is oxidative and the oxalacetate to succinate pathway is reductive in nature, assuming that the oxalacetate is produced from pyruvate. This split citrate pathway also provides a means for synthesis of glutamate and aspartate. There has been no evidence that the glyoxylate pathway enzymes isocitrate lyase or malate synthase are present, nor has there

been evidence of an Entner-Doudoroff pathway at 37°C. In other organisms, metabolism may shift with lowered temperatures from Embden-Meyerhoff to Entner-Doudoroff pathways [2]. Since *L. monocytogenes* can grow under refrigerator temperatures (4°C), its metabolic characteristics under different atmospheric and temperature conditions should be investigated. Metabolic shifts might also explain the temperature dependence of motility observed. At low temperatures, insufficient ATP may be produced for flagellar motion, whereas at temperatures above 30°C, other reactions use the ATP produced. Interestingly, isolates from diseased animals in addition to having coccoid forms often require a conditioning period in the cold before being adapted to growth on laboratory media [32].

Aerobic metabolism may proceed through substrate regulation. In a study of oxidation of glucose by resting cells of a single strain of *L. monocytogenes* grown in different levels of glucose, Friedman and Alm [28] found that the level of glucose in the prior medium affected the products formed. The strain grown in a prior medium of peptone C and 1% glucose took up only 150 microliters of oxygen after 60 min incubation, but all of the glucose carbons could be accounted by pyruvate, lactate, carbon dioxide, and acetoin. Acetoin is the substance that is measured in the VP test and is responsible for the buttermilk odor often noted with *Listeria* cultures. In contrast, prior media with 0.6% and 0.2% glucose took up 220 and 260 microliters oxygen during the same period, but only 74 and 54% of the glucose could be accounted for by the four products analyzed. The highest glucose levels also apparently reduced levels of dehydrogenase and catalase as well as virulence when the cultures were injected into mice.

#### ISOLATION AND DETECTION

The isolation of *L. monocytogenes* involves the use of selective and differentiative media, the former being media on which the organism can grow in preference to other organisms, the latter being media which separate the organisms by reactions

defining certain biochemical traits [12, 45]. Many of these media use antibiotics to which *L. monocytogenes* is resistant, usually nalidixic acid, bacitracin, or moxalactam [67]. The resistance to these antibiotics [2] does not appear to be by any common mechanism (Table 2) and some resistance may be conferred by the presence of plasmids [27, 54] which may carry the antibiotic resistance genes.

Another agent often used in isolation media is esculin [67, 81], a glucoside found in the leaf and bark of horse chestnut trees. Hydrolysis of esculin or of salicin, a glucoside found in willow branches, releases the glucose for metabolism and leaves the aglycone, both of which are *o*-diphenols or catechols. Catechols can form coordinate compounds with transition metals, such as iron, and this reaction is used by many bacteria in the formation of iron-binding siderophores. In this assay, however, the released catechol is used as an indicator of hydrolysis by forming dark precipitates with the iron salts in the medium. In experiments in our laboratory, leaves or needles were removed from horse chestnut, willow, tulip poplar, Alaskan sequoia, and maple trees and examined for the presence of esculin positive colonies. A number were isolated

Table 2  
Sensitivity of *Listeria monocytogenes* to antibiotic mechanisms

Antibiotic	Mechanism	Sensitivity
Ampicillin	W	+
Chloramphenicol	P	+
Erythromycin	P	+
Neomycin	P	+
Tetracycline	P	+
Cephalosporin	P	+
Polymyxin	M	-
Nalidixic acid	D, R	-
Bacitracin	W	-
Sulfonamides	F	-
Penicillin G	W	-
Cefolaxime	W	-
Acriflavin	D	-

M = membrane transport inhibition; D = DNA synthesis inhibition; R = RNA synthesis inhibition; W = Cell wall synthesis inhibition; F = Folic acid antagonist; P = Protein synthesis inhibition.

which were also found to be Gram (+) and tellurite reducing, but with further studies were not identified as *Listeria*. Although *Listeria* is a saprophyte, it is probable that some of the organisms isolated from vegetation in earlier years would not now be identified as *Listeria*.

A physical method often employed for isolation of *Listeria* from plates containing other organisms is the Henry method of illumination [67]. When the plate is illuminated from below by a light source reflected off a mirror at a 45° angle, many *Listeria* colonies appear light blue when observed from above. This color is not particularly noticeable under direct lighting, which would seem to indicate the production by light scattering, rather than absorption or reflection, similar to the blue color of the sky. Electron micrographs of *Listeria* have not shown any capsule or unusual particles in the cell surface composition that might be scattering light but since formulae for evaluating light scattering parameters include a factor for differential refractivity, it is possible that the lipoteichoic acids may be a factor because of the higher refractivity of lipid components. The measurement in our laboratory using specular reflectance instrumentation has indicated that *Listeria* colonies do show a definite increased reflectance in the 400–500 nm 'blue' region and reduced reflectance in the 600–700 nm 'red' region. Specific negative peaks of reflectance were noted at 400 and 440 nm and 550 and 570 nm; the reason, however, is unknown.

*L. monocytogenes* apparently requires only 11 elements for growth (compared to 23 for humans), although the need for exceedingly low requirements for trace elements cannot be eliminated. These required elements are hydrogen, carbon, nitrogen, oxygen, sodium, magnesium, phosphorus, sulfur, chloride, potassium, and iron. The need for calcium has not been shown for minimal growth. Additionally, *L. monocytogenes* can tolerate three normally toxic metals: lithium, thallium, and tellurium. The resistance to uptake of lithium and thallos salts (as well as to high levels of sodium chloride and bile salts) must be by a very selective cell membrane, by an energy-requiring active transport system, or by both. Lithium ions are quite small and might be ex-

pected to enter sodium or potassium ion channels. Thallos salts usually act by denaturing proteins through a heavy metal sulfhydryl inactivation; its basis for inactivity here is unknown. Tellurium salts are tolerated by organisms that can reduce the tellurium containing anion to the metallic state, a reduction potential of about 600 mv. Although this might occur through electron transfer with oxygen radical reactions, it more likely results through specific enzymatic reactions similar to those found in *Streptococcus faecalis*, some staphylococci, *Corynebacterium diphtheriae*, *Mycobacteria*, and *Thermus thermophilus* [14]. This reduction requires a specific enzyme, NADH, and a NADH generating system, and may be related to the enzyme system noted by Cowart [16] that reduced iron from ferritin.

Three investigators [57, 73, 80] have reported the development of synthetic media for the growth of *L. monocytogenes*. One medium did not contain added iron, and two did not contain added calcium. These two elements are of interest in foods, since dairy products contain calcium in millimolar concentrations and iron in micromolar levels, whereas meat contains iron in millimolar levels and calcium at micromolar levels. Iron is definitely required for aerobic growth and for the production of catalase and cytochromes [10, 13, 17, 31, 66, 67, 78, 79], as well as for hemolysin [10, 15], and its omission from one medium as probably counteracted by low levels in the added components. The media all contained glucose as the organic energy source. There is an apparent definite requirement for four amino acids (cysteine, leucine, isoleucine, and valine) with a questionable need for methionine, arginine, histidine, tryptophan, and glutamine. Apparently, increased amounts of cysteine reduce the need for methionine (and thiamine), all of which contain sulfur. Ammonium salts also apparently replace glutamine as an amino nitrogen source, and possible also tryptophan. The vitamins are definite requirements and are essential for the pyruvate dehydrogenase in aerobic metabolism [2]. The synthetic media, however, do not support extended growth in agitated aerobic cultures, and require addition of protein hydrolysates to prevent lysis [73].

*L. monocytogenes* has been reported [67] to be

resistant to high concentrations of nitrite in the medium. In most reactions, however, the active component is not the nitrite anion but nitrous acid which is formed at lower pH values by reaction with hydrogen ion [7]. The concentration of nitrous acid can be calculated roughly at any pH with the Henderson-Hasselbach equation ( $pK_a$  of nitrous acid is 3.86), although the concentration of other cations in the medium is quite important from mass action. In a study by Shahamat et al. [68], the MIC's of nitrite on *L. monocytogenes* at different pH values and salt concentrations ranged from a high of 30 000 parts per million (ppm) or 30 parts per thousand (ppt) to less than 50 ppm (0.05 ppt), a  $10^3$  fold range. The application of the Henderson-Hasselbach equation to these data (Table 3) indicated that nitrous acid as the active factor showed much less variation in activity (54 ppm to below 1 ppm). At the concentrations permitted in cured meats of nitrite and salt and its pH of 6.0, nitrite would be only slightly less inhibitory than it is for *C. botulinum* [7].

#### INFECTIVITY AND VIRULENCE FACTORS

Only recently has listeriosis in humans been recognized as a possible foodborne intracellular infection [46, 51, 64]. The disease symptoms normally do not resemble those of foodborne pathogens (diarrhea, dysentery), but are more similar to viral infections (meningitis, abortion, occasional increased monocytes) [51, 64], although there appears to be genes in *L. monocytogenes* homologous for the dysentery-producing cholera toxin [71]. Virulence depends upon attachment of an organism to the tissue cell, entering the host, multiplying, resisting host defenses, and damaging host mechanisms [64]. For intracellular infection of a cell or tissue, the pathogen must first approach close enough either through cellular motility or through fluid transport to form an attachment [8, 33, 63]. Initial steps involve a variety of postulated mechanisms including electrostatic attraction, hydrophobicity, hydrophilicity, and van der Waal's forces [33]. Following the initial approach and attachment, the organism might

Table 3  
Effect of nitrite and nitrous acid at different pH values, temperatures, and NaCl %

Temperature	pH	Percent sodium chloride							
		0%		3%		5.5%		8%	
37°C	7.4	25.0	7.3	10.0	10.0	10.0	10.0	5.0	5.0
	6.5	3.0	6.9	5.0	11.5	3.0	6.9	1.5	3.5
	5.5	0.8	17.9	1.0	22.4	0.5	11.2	0.05	1.1
	5.0	0.4	27.0	0.5	33.8	0.05	3.4	—	—
22°C	7.4	30.0	8.7	10.0	2.9	10.0	2.9	7.0	2.0
	6.5	4.0	9.2	5.0	11.5	3.0	6.9	1.5	3.5
	5.5	1.0	22.4	1.0	22.4	1.0	22.4	0.05	1.1
	5.0	0.8	54.0	0.5	33.8	0.05	3.4	—	—
4°C	7.4	10.0	2.9	3.0	0.9	2.0	0.6	—	—
	6.5	1.0	2.3	0.6	1.4	0.3	0.7	—	—
	5.5	0.4	0.9	0.1	2.2	0.05	0.05	—	—
	5.0	—	—	—	—	—	—	—	—

The left hand column in each salt concentration represents minimum inhibitory concentrations (MIC) of sodium nitrite in parts per thousand (PPT). The right hand column represents the nitrite inhibitory concentration transformed through the Henderson-Hasselbach equation to indicate the content of nitrous acid in parts per million (PPM).

form attachment pili or fimbriae or some surface attachment biochemical, such as a glycocalyx. *L. monocytogenes* does not have fimbriae, pili, or capsules, but does possess the amphipathic lipoteichoic acids, which have been reported to attach to fibronectin on tissues [61]. Once the *L. monocytogenes* has been attached to a tissue or cell surface (in animal gastrointestinal tracts, usually the enterocytes or Peyer's patches), it may form a biofilm [33, 63] thereby allowing protected growth on the cell surface, it may enter the cell and become intracellular, or it may be phagocytosed by macrophages. There is presently no evidence for biofilm formation in *L. monocytogenes*, although it does become attached to stainless steel surfaces [35]. Studies on attachment of five organisms to different hydrophobic polymers were conducted by Absalom et al. [1]. Of the organisms examined, *L. monocytogenes* showed the highest hydrophobicity, which may be a reflection of the lipoteichoic acid content. Assuming that *L. monocytogenes* through hydrophobic or other mechanisms has attached to the tissue cell, it now must become intracellular and resist host defenses. *L. monocytogenes* contains at least three substances that are apparently implicated in these actions – catalase, superoxide dismutase (SOD), and  $\alpha$ -listeriolysin.

Macrophage engulfment is the animal's protection against many bacterial invaders. Macrophages have mechanisms for producing various oxygen containing compounds such as singlet oxygen, peroxide, and superoxide anion that can inactivate enzymes and result in microbial death [3, 5, 31, 78, 79]. These substances along with hydrolytic enzymes are usually active within the phagolysosome of the macrophage. *L. monocytogenes* contains highly active catalase to inactivate peroxide, and superoxide dismutase (SOD) to dismutate superoxide anion [78, 79]. Peroxidases have not been reported, but catalase in certain concentrations can behave as a peroxidase with an electron acceptor.

Many researchers have concentrated on the hemolysin of *L. monocytogenes* as the principal virulence factor, in that non-hemolytic strains of *Listeria* were not virulent. Apparently, the  $\beta$ -hemolysin by *L. monocytogenes* has been isolated and charac-

terized [53].  $\beta$ -Hemolysis occurs with red blood cells, especially those from sheep, and is also involved in the CAMP test. Red blood cells contain a cholesterol-sphingomyelin complex, containing about 45 mol % sphingomyelin. This may be the component acted upon in the  $\beta$ -hemolysis and the CAMP tests. With *L. monocytogenes*, this lytic action is potentiated in the presence of *S. aureus* which is known to produce a potent sphingomyelinase known as  $\beta$ -toxin. *L. invanovi*, conversely, produces a hemolysin which is not potentiated by *S. aureus*, but is by growth factors from *Rhodococcus equi* (the equi factor) [34, 69]. *L. monocytogenes* is not potentiated by the equi factor, which apparently is cholesterol oxidase or a cholesterol degrading enzyme [77]. In group B streptococci, the CAMP factor has been isolated as an extracellular protein of 23.5 kDa which apparently interacts and lyses cell membranes pretreated with sphingomyelinase or certain other phospholipases [65]. More recently, the CAMP factor in group B streptococci has been found to bind to the Fc site on IgG and IgM molecules, and function in a receptor non-immune reaction [39]. Whether this occurs in *L. monocytogenes* is presently unknown, but might account for uptake by phagocytes through a lectin-like opsonophagocytosis [52].

The principal hemolysin studied by most researchers working with *L. monocytogenes* has been an extracellular protein that is inhibited by oxidation but activated by sulfhydryls and binds to cholesterol. This hemolysin (called  $\alpha$  listeriolysin or listeriolysin O) is a pore forming cytolysin of 58 kDa. It is essential for pathogenicity in mouse cells, but its role in human virulence is unknown [29, 56]. Mengaud et al. [48] determined the amino acid sequence of listeriolysin and showed that it contained 504 amino acids with but one cysteine residue. It is quite similar in amino acid composition to streptolysin O and to pneumolysin, especially to a conserved sequence of 11 amino acids containing the cysteine residue which appears necessary for the binding to cholesterol; it differs in that *L. monocytogenes* hemolysin is active at pH 5.5 rather than pH 7.0. The present indications are that hemolysin is definitely involved in intracellular growth at least

in rat or mouse phagosomes and tissue culture enterocytes, but is not involved in entry into the cells [11, 29, 40, 43]. Other factors in addition to the intact gene for hemolysin production appear to be required. *L. monocytogenes* also produces tumor necrosis factor (TNF) during intracellular growth in the macrophage which may be related to nitrite formation from arginine by the macrophage which limits iron use by the tumor cells [37].

One may postulate a possible hypothesis for virulence of *L. monocytogenes* which involves growth in appropriate foods to allow production of virulence factors. Ingestion of food containing sufficient *L. monocytogenes* permits the organisms to pass through the stomach (particularly gastric contents with low acid levels) where they can contact the enterocytes or Peyer's patches within the intestine. Attachment may be from specific components or by a hydrophobic mechanism. The CAMP factor and phospholipases allow cell surface disruption, lectin-opsonization, and attachment to immunoglobulins, with subsequent phagocytosis by macrophages. Following internalization within the macrophage phagolysosome, the catalase and SOD protect the organism from oxidative destruction, while the  $\alpha$ -listeriolysin binds to the vacuole cholesterol, disrupting the phagolysosomal membrane, and releasing and *L. monocytogenes* into the cytoplasm where it can grow and multiply. Through some mechanism unknown [47, 50], the *L. monocytogenes* can then cross endoepithelial cells and capillaries and be transported within the body to the brain, liver, spleen, RE system, and uterus. Isolation of another extracellular protein of about 60 kDa has been reported [41] which is not the hemolysin but produces filamentous colonies and elongation of the cell and apparently is important in virulence.

#### ENVIRONMENTAL EFFECTS ON VIRULENCE

Environmental and processing conditions can have a large effect upon the growth and possibly virulence of *L. monocytogenes* [20]. The temperature and pH [30], in addition to the composition of the food, can be quite important in foodborne outbreaks. Because many human outbreaks of

listeriosis apparently are connected with a 4b serotype, it is interesting that *L. monocytogenes* isolated from meat products have never been 4b, whereas milk borne *L. monocytogenes* often is [4, 10, 19]. It has been noted that 4b contains both glucose and galactose, both components of milk lactose, but these two are also present in meat collagen [8]. Scott A (serotype 4b) when added to sterile meat does not grow at refrigerator temperatures, but neither is it killed off. In sterile milk, however, it multiplies rapidly [10]. In previous studies we examined the effect of various heating temperatures on the release of low-molecular weight components of *L. monocytogenes* in various ionic media [9]. Thermal stress and cellular death caused an increased release of these components into the medium. At the higher temperatures, the cellular release apparently plateaus after initial release, which may have resulted from cell wall denaturation rather than the denaturation of specific enzymes. As previously mentioned, meat and milk differ in their content of iron and calcium. Iron is an important factor in human disease and microbial infectivity [13]. More recently, we have examined the effect of iron level and aeration on the production of catalase and hemolysin [10, 66]. High-iron media and aeration increased growth of *L. monocytogenes* and increased production of several catalases, as determined by SDS-PAGE analysis. Reduced aeration increased hemolysin activity, but growth and catalase production were reduced. A portion of the catalase appears to be on the outer surface of the *L. monocytogenes* cell, and can be removed by appropriate treatments [66]. Dallmier and Martin [17] reported recently on the activities of catalase and SOD in different strains of *L. monocytogenes* and upon their inactivation by heating. Of the strains examined, Scott A (serotype 4b) had the highest activity of both enzymes, and the enzymes remained active to temperatures slightly above 50°C. The activity of these enzymes and recent findings by Smith and Archer [70] on heat injury indicate that underprocessing of products by heat can result in injured cells which can repair and become virulent [38].

## IMPLICATIONS FOR FURTHER RESEARCH

1. There should be a reexamination of the metabolic pathways and growth requirements using identified virulent and avirulent strains with different substrates, temperatures, and atmospheres. These data should be related to virulence, production of the hemolysin(s) and CAMP factor, SOD, and catalases.
2. The relationship of biotype to serotype and the relationship of both to virulence and growth media should be examined.
3. Why do isolates from humans and/or animals differ from food isolates in appearance, and why do they often require adaptation before growth in the laboratory?
4. What is the role of the recently identified cholera-like toxin to virulence?
5. Why is serotype 4b so common in human outbreaks, and why is it seldom found in meat or meat products?
6. Is there a relationship of food type or metal ion content to virulence or growth?
7. Of what importance to *L. monocytogenes* is its resistance to lithium, thallium, or tellurite salts, or are these reflections of other biochemical systems?

## REFERENCES

- 1 Absalom, D.R., F.V. Lamberti, Z. Policova, W. Zingg, C.J. van Oss and A.W. Neumann. 1988. Surface thermodynamics of bacterial adhesion. *Appl. Environ. Microbiol.* 46: 90-97.
- 2 Atlas, R.M. 1984. *Microbiology*. Fundamentals and Applications. Macmillan Publishing Co, New York, NY.
- 3 Badwey, J.A. and M.L. Karnovsky. 1980. Active oxygen species and the functions of phagocytic leukocytes. *Ann. Rev. Biochem.* 49: 695-726.
- 4 Barza, M. 1985. Listeriosis and milk. *New Eng. J. Med.* 312: 438-440.
- 5 Beaman, L.V. and B.B. Beaman. 1984. The role of oxygen and its derivatives in microbial pathogenesis and host defense. *Ann. Rev. Microbiol.* 38: 27-48.
- 6 Beck, L.T., B.J. Robison, P. Fahey, R.S. Flowers, J.A. Mattingly and R. Durham. 1988. A monoclonal antibody-based enzyme immunoassay (ELISA) for detection of *Listeria* in foods and environmental samples. *Abstr. Ann. Meet. Am. Soc. Microbiol.* P-34, p.
- 7 Benedict, R.C. 1980. Biochemical basis for nitrite-inhibition of *Clostridium botulinum* in cured meat. *J. Food Prot.* 43: 877-891.
- 8 Benedict, R.C. 1988. Microbial attachment to meat surfaces. 41st Reciprocal Meat Conference Proceedings, Laramie, WY, 1-6.
- 9 Benedict, R.C., F.J. Schultz and C.A. Kunsch. 1987. A spectrophotometric diffusion rate method to estimate thermal death of *Listeria monocytogenes* in ionic media. *Absts. 47th Annual IFT Meeting, Las Vegas, NV*, abst. 120.
- 10 Benedict, R.C. and F.J. Schultz. 1988. An effect of iron level and food type on virulence factors of *Listeria monocytogenes* strain Scott A. 48th Annual IFT Meeting, New Orleans, LA, abst 315.
- 11 Berche, P., J.-L. Gaillard and S. Richard. 1988. Invasiveness and intracellular growth of *Listeria monocytogenes*. *Infection* 16: Suppl. 2. S145-S148.
- 12 Buchanan, R.L., H.G. Stahl and D.L. Archer. 1987. Improved plating media for simplified, quantitative detection of *Listeria monocytogenes* in foods. *Food Microbiol.* 4: 269-275.
- 13 Bullen, J. 1981. The significance of iron in infection. *Revs. Infect. Dis.* 3: 1127-1138.
- 14 Chiong, M., E. Gonzalez, R. Barra and C. Vasquez. 1988. Purification and biochemical characterization of tellurite-reducing activities from *Thermus thermophilus* HB8. *J. Bacteriol.* 170: 3269-3273.
- 15 Cowart, R.E. and B.G. Foster. 1981. The role of iron in the production of hemolysin by *Listeria monocytogenes*. *Current Microbiol.* 6: 287-290.
- 16 Cowart, R.E. and B.G. Foster. 1985. Differential effects of iron on the growth of *Listeria monocytogenes*: Minimum requirements and mechanism of acquisition. *J. Infect. Dis.* 151: 721-730.
- 17 Dallmier, A.W. and S.E. Martin. 1988. Catalase and superoxide dismutase activities after heat injury of *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 54: 581-582.
- 18 Datta, A.R., B.A. Wentz, D. Shook and M. Truckness. 1988. Gene probes for detection of *Listeria monocytogenes*. *Abstr. Ann. Meet. Am. Soc. Microbiol.* P-32, p.
- 19 Donnelly, C.W. and E.H. Briggs. 1986. Psychrotrophic growth and thermal inactivation of *Listeria monocytogenes* as a function of milk composition. *J. Food Prot.* 49: 994-998.
- 20 Doyle, M.P. 1988. Effect of environmental and processing conditions on *Listeria monocytogenes*. *Food Technol.* 41: 169-171.
- 21 Eerola, E. and O.-P. Lehtonen. 1988. Optimal data processing procedure for automatic bacterial identification by gas-liquid chromatography of cellular fatty acids. *J. Clin. Microbiol.* 26: 1745-1753.
- 22 Facinelli, B., C. Casolari and U. Fabio. 1988. Restriction endonuclease analysis of the chromosomal DNA of *Listeria monocytogenes* and other species of the genus *Listeria*. *Abstr. Ann. Meet. Am. Soc. Microbiol.* C-176, p.

- 23 Farber, J.F. and J.I. Speirs. 1987. Potential use of continuous cell lines to distinguish between pathogenic and nonpathogenic spp. *J. Clin. Microbiol.* 25: 1463–1466.
- 24 Feresu, S.B. and D. Jones. 1988. Taxonomic studies on *Brochothrix*, *Erysipelothrix*, *Listeria* and atypical *Lactobacilli*. *J. Gen Microbiol.* 134: 1165–1183.
- 25 Fiedler, F. 1988. Biochemistry of the cell surface of *Listeria* strains: A locating general view. *Infection* 16: Suppl. 2, S92-S97.
- 26 Fiedler, F. and G.J. Ruhland. 1987. Structure of *Listeria monocytogenes* cell walls. *Bull. Inst. Pasteur.* 85: 287–300.
- 27 Flamm, R.K. 1986. Molecular genetics of *Listeria monocytogenes*. Cloning of a hemolysin gene, demonstration of conjugation, and detection of native plasmids. Dissertation, Washington State Univ. # DA 8621990.
- 28 Friedman, M.A. and W.L. Alm. 1962. Effect of glucose concentration in the growth medium on some metabolic activities of *Listeria monocytogenes*. *J. Bacteriol.* 84: 375–376.
- 29 Gaillard, J.-L., P. Berche, J. Mounier, S. Richard, and P. Sansonetti. 1987. In vitro model of penetration and intracellular growth of *Listeria monocytogenes* in the human enterocyte-like cell line Caco-2. *Infect. Immun.* 55: 2822–2829.
- 30 George, S.M., B.M. Lund, and T.F. Brocklehurst. 1988. The effect of pH and temperature on initiation of growth of *Listeria monocytogenes*. *Lett. Appl. Microbiol.* 6: 153–156.
- 31 Godfrey, R.W. and M.S. Wilder. 1985. Generation of oxygen species and virulence of *Listeria monocytogenes*. *Infect. Immun.* 47: 837–839.
- 32 Gray, M.L. and A.H. Killinger. 1966. *Listeria monocytogenes* and listeric infection. *Bacteriol. Rev.* 30: 309–382.
- 33 Gristina, A.G. 1987. Biomaterial-centered infection: microbial adhesion versus tissue integration. *Science* 237: 1588–1595.
- 34 Groves, R.D. and H.J. Welshimer. 1977. Separation of pathogenic from apathogenic *Listeria monocytogenes* by three in vitro reactions. *J. Clin. Microbiol.* 5: 559–563.
- 35 Herald, P.J. and E.A. Zottola. 1988. Attachment of *Listeria monocytogenes* to stainless steel surfaces at various temperatures and pH values. *J. Food Sci.* 53: 1549–1552, 1562.
- 36 Hether, N.W. and L.L. Jackson. 1983. Lipoteichoic acid from *Listeria monocytogenes*. *J. Bacteriol.* 156: 809–817.
- 37 Hibbs, J.B., Jr., R.R. Taintor, and Z. Vavrin. 1987. Macrophage cytotoxicity: Role for L-arginine deiminase and imino nitrogen oxidation to nitrite. *Science* 235: 473–476.
- 38 Humphrey, T.J. 1988. Peroxide sensitivity and catalase activity in *Campylobacter jejuni* after injury and during recovery. *J. Appl. Bacteriol.* 64: 337–343.
- 39 Jurgens, D., B. Sterzik, and F.J. Fehrenbach. 1987. Unspecific binding of Group B streptococcal coccytolysin (CAMP factor) to immunoglobulins and its possible role in pathogenicity. *J. Exp. Med.* 165: 720–732.
- 40 Kathariou, S., J. Rocourt, H. Hof, and W. Goebel. 1988. Levels of *Listeria monocytogenes* hemolysin are not directly proportional to virulence in experimental infections of mice. *Infect. Immun.* 56: 534–536.
- 41 Kathariou, S. and W. Goebel. 1988. Identification of *Listeria monocytogenes* variants deficient in a major extracellular 60K protein. *Abstr. Ann. Meet. Am. Soc. Microbiol.* D-56, p.
- 42 Klingler, J., D. Croan, P. Flynn, A. Johnson, W. Whipple, M. Ottaviani, G. Mock, N. Curran, M. Kimball, M. Curiale, and J. Lawrie. 1988. DNA probe hybridization assay for identification of *Listeria* species in food and environmental samples. *Abstr. Ann. Meet. Am. Soc. Microbiol.* Abst. P-33.
- 43 Kuhn, M., S. Kathariou, and W. Goebel. 1988. Hemolysin supports survival but not entry of the intracellular bacterium *Listeria monocytogenes*. *Infect. Immun.* 56: 79–82.
- 44 Leighton, I., D.R. Threfall, and C.L. Oakley. 1975. Phospholipase C activity in culture filtrates from *Listeria monocytogenes* boldy In: *Problems of Listeriosis* (M. Woodbine, ed.), pp. 239–241, University Press, Leicester, U.K.
- 45 Leighton, J. 1979. Use of selective agents for the isolation of *Listeria monocytogenes*. *Med. Lab. Sci.* 36: 283–288.
- 46 Marth, E.H. 1987. Disease characteristics of *Listeria monocytogenes*. *Food Technol.* 41: 165–168.
- 47 Melchior, P.P. and C.L. Wells. 1988. The abilities of intestinal *Listeria monocytogenes* and *Escherichia coli* to translocate to the mesenteric lymph nodes and to survive in mononuclear phagocytes. *Abstr. Ann. Meet. Am. Soc. Microbiol.* B-27, p.
- 48 Mengaud, J., M.-F. Vicente, J. Chenevert, J.M. Pereira, C. Geoffroy, B. Gicquel-Sanzey, F. Banquero, J.-C. Perez-Diaz, and P. Cossart. 1988. Expression in *Escherichia coli* and sequence analysis of the listeriolysin O determinant of *Listeria monocytogenes*. *Infect. Immun.* 56: 766–772.
- 49 Miller, I.L. and S.J. Silverman. 1960. Glucose metabolism of *Listeria monocytogenes*. *Bact. Proc. Abst.* P5, p. 103.
- 50 Moulder, J.W. 1985. Comparative biology of intracellular parasitism. *Microbiol. Rev.* 49: 298–337.
- 51 Nieman, R.E. and Lorber, B. 1980. Listeriosis in adults: A changing pattern. Report of eight cases and review of the literature, 1968–1978. *Rev. Infect. Dis.* 2: 207–227.
- 52 Ofek, I. and N. Sharon. 1988. Lectinophagocytosis: a molecular mechanism of recognition between cell surface sugars and lectins in the phagocytosis of bacteria. *Infect. Immun.* 56: 539–547.
- 53 Parrisius, J., S. Bhakdi, M. Roth, T. Tranum-Jensen, W. Goebel, and H.P.R. Seeliger. 1986. Production of listeriolysin in beta-hemolytic strains of *Listeria monocytogenes*. *Infect. Immun.* 51: 314–319.
- 54 Perez-Diaz, J.C., M.F. Vicente and F. Baquero. 1982. Plasmids in *Listeria*. *Plasmid* 8: 112–118.
- 55 Pine, L., G.B. Malcolm, J.B. Brooks and M.I. Daneshvar. 1988. Physiological studies on the utilization of sugars for growth and survival by *Listeria* species. *Abstr. Ann. Meet. Am. Soc. Microbiol.* D-41, p.
- 56 Portnoy, D.A., P. Suzanne Jacks and D.J. Hinrichs. 1988. Role of hemolysin for the intracellular growth of *Listeria monocytogenes*. *J. Exp. Med.* 167: 1459–1471.

- 57 Ralovich, B. 1984. Listeriosis Research. Present Situation and Perspective. Akademiai Kiado, Budapest.
- 58 Robisin, B.J. T. Donlevy, S. Keelan and R.S. Flowers. 1988. Use of Micro-ID and selected biochemical tests to rapidly identify *Listeria* sp. and *Listeria monocytogenes*. Abstr. Ann. Meet. Am. Soc. Microbiol. P-35, p.
- 59 Rocourt, J. 1988. Taxonomy of the Genus *Listeria*. Infection 16 Suppl. 2. S89-S90.
- 60 Rocourt, J.F. Grimont, P.A.D. Grimont and H.P.R. Seeliger. 1982. DNA relatedness among serovars of *Listeria monocytogenes* sensu lato. Current Microbiol. 7: 383-388.
- 61 Rylance, H.J., R.C. Wallace and W. Marr. 1988. The nature of the substance in acidic streptococcal extracts reacting with fibrinogen. Letters in Appl. Microbiol. 7: 5-7.
- 62 Sasser, M. and M. Roy. 1988. Fatty acid analysis for rapid identification of the species of *Listeria* and related bacteria. Abstr. Ann. Meet. Am. Soc. Microbiol. P-44, p.
- 63 Savage, D.C. and Fletcher, M., eds. 1985. Bacterial Adhesion. Mechanisms and physiological significance. Plenum Press, NY., NY.
- 64 Schlech, W.F. III. 1987. Virulence characteristics of *Listeria monocytogenes*. Food Technol. 41: 176-178.
- 65 Schneewind, O., K. Friedrich, and R. Lutticken. 1988. Cloning and expression of the CAMP factor of group B streptococci in *Escherichia coli*. Infect. and Immun. 56: 2174-2179.
- 66 Schultz, F.J., R.C. Benedict and D.K. Brauer. 1988. *Listeria monocytogenes*: Selective extraction of catalase. Poster presentation P-12, Comprehensive Conference on *Listeria monocytogenes*. Rohnert Park, CA.
- 67 Seeliger, H.P.R. and D. Jones. (1986) Genus *Listeria* Pirie 1940, 383. In Bergey's Manual of Systematic Bacteriology, Volume 2. (P.H.A. Sneath, ed.), pp. 1235-1245, Williams and Wilkins, Baltimore, MD.
- 68 Shahamat, M., A. Seaman and M. Woodbine. 1980. Influence of sodium chloride, pH and temperature on the inhibitory activity of sodium nitrite on *Listeria monocytogenes*. In Microbial Growth and Survival in Extremes of Environment. (G.N. Norris and J.E.L. Corry, eds.), pp. 227-237, Academic Press, London.
- 69 Skalka, B. and J. Smola. 1983. Selective diagnostic medium for pathogenic *Listeria* spp. J. Clin. Microbiol. 18: 1432-1433.
- 70 Smith, J.L. and D. Archer. 1988. Heat-induced injury in *Listeria monocytogenes*. J. Ind. Microbiol. 3: 1051-110.
- 71 Stephen, M., B. McCardell and J. Madden. 1988. DNA: DNA homology between the genes for cholera toxin and *Listeria monocytogenes* DNA. Abstr. Ann. Meet. Am. Soc. Microbiol. P-42, p.
- 72 Swaminathan, S., L.M. Graves, G.M. Carlone and B.D. Plikaytis. 1988. Molecular epidemiology of *Listeria monocytogenes* by ribosomal RNA typing. Abstr. Ann. Meet. Am. Soc. Microbiol. D-61, p.
- 73 Trivett, T.L. and E.A. Meyer. 1971. Citrate cycle and related metabolism of *Listeria monocytogenes*. J. Bacteriol. 107: 770-779.
- 74 Uchikawa, K.-I., I. Sekikawa, and I. Azuma. 1986. Structural studies on teichoic acids in cell walls of several serotypes of *Listeria monocytogenes*. J. Biochem. (Japan) 99: 315-327.
- 75 Ullmann, W.W. and J.A. Cameron. 1969. Immunochemistry of the cell walls of *Listeria monocytogenes*. J. Bacteriol. 98: 486-493.
- 76 Vazquez-Boland, J.A., L. Dominguez Rodriguez, J.F. Fernandez Carayzabal, J.L. Blanco Cancelo, E. Gomez-Lucia, V. Briones Dieste and G. Suarez Fernandez. 1988. Serological studies on *Listeria grayi* and *Listeria murrayi*. J. Appl. Bacteriol. 64: 371-378.
- 77 Watanabe, K., H. Shidizu, H. Aihara, R. Nakamura, K.-I. Susuki, and K. Komagata. 1986. Isolation and identification of cholesterol-degrading *Rhodococcus* strains from food of animal origin and their cholesterol oxidase activities. J. Gen. Appl. Microbiol. 32: 137-147.
- 78 Welch, D.F. 1987. Role of catalase and superoxide dismutase in the virulence of *Listeria monocytogenes*. Ann. Inst. Pasteur/Microbiol. 138: 265-268.
- 79 Welch, D.F., C.P. Sword, S. Brehm, and D. Dusanic. 1979. Relationship between superoxide dismutase and pathogenic mechanisms of *Listeria monocytogenes*. Infect. Immun. 23: 863-872.
- 80 Welshimer, H.J. 1963. Vitamin requirements of *Listeria monocytogenes*. J. Bacteriol. 85: 1156-1159.
- 81 Wetzler, T.F., N.R. Freeman, M.L.V. French, L.A. Renkowsky, W.C. Eveland and O.J. Carver. 1968. Biological characterization of *Listeria monocytogenes*. Health Lab. Sci. 5: 46-62.