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Listeria monocytogenes: Overview and Targeting Advances

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ABSTRACT

Listeria monocytogenes is a serious foodborne zoonotic pathogen capable of causing gastroenteritis and severe systemic infections such as septicemia, meningitis or abortion in the infected individuals what is called listeriosis. The bacterium is reported as the third leading cause of death among the foodborne pathogens preceded by nontyphoidal *Salmonella* spp. and *Toxoplasma gondii*. The power to tolerate a wide range of temperatures is considered the most prominent trait distinguishing it from the other foodborne pathogens. Within the infected host, the bacteria harbor inside macrophages and jump from cell to another without leaving the safeguarding milieu of the host's cells utilizing a set of genes including *hly* (listeriolysin O), *plcA* (phosphatidylinositol-specific phospholipase c), *plcB* (phosphatidylcholine-phospholipase C) and *actA* (actin-assembly inducing protein). In addition to the health concerns associated with antibiotics, treatment failure likely occurs among listeriosis-infected persons especially with the inability of most antibiotics to access intracellular replicative niches and achieve the optimum therapeutic concentrations within the infected cells. Recently, one novel choice, peptide nucleic acid (PNA), has been emerged to target this bacterium as a model of targeting intracellular pathogens with antisense agents. PNA is a one of the DNA analogues which works via specific inhibition of bacterial gene expression.

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Introduction

Among the currently recognized ten species of genus *Listeria* (*L. monocytogenes* , *L. ivanovii, L. seeligeri, L. innocua, L. grayi, L. welshimeri, L. marthii, L. rocourtiae, L. weihenstephanensis* and *L. fleischmannii*), *L. monocytogenes* is the major human pathogen (Graves *et al*., 2010; Leclercq *et al*., 2010; Liu, 2013). The organism is a Gram-positive, facultative anaerobic, non-spore forming, able to grow over a wide range of pHs (4.4-9.4), water activity (0.90-0.97) and salt concentrations (up to 25% at 4ºC) (Beverly, 2004). Motility of the organism is limited to 10-25°C and cannot form the peritrichous flagellae at 37°C (Galsworthy *et al*., 1990). *L. monocytogenes* was documented for the first time as a foodborne pathogen in 1981(Schlech *et al*., 1983) and since that date, it has been continued to emerge as a one of the main foodborne pathogens resulting in several outbreaks. Among the thirteen currently known *L. monocytogenes* serotypes, 1/2a, 1/2c, 1/2b and 4b serovars (of lineages I and II) are responsible for more than 98% of infections from the documented human cases (Gianfranceschi *et al*., 2009; Smith *et al*., 2013; Tan *et al*., 2015). Recently, novel approaches such as peptide nucleic acid (PNA) have been utilized to target pathogenic organisms including *L. monocytogenes* (Alajlouni and Seleem, 2013; Kuriakose *et al*., 2013; Nepal *et al*., 2015). PNA was introduced to the field of life science in 1991 (Nielsen

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et al., 1991) as an artificial DNA mimic able to form specific, strong and stable complexes with RNA and DNA and thus inhibiting gene expression (Nielsen, 2004). Given their unique mode of action in silencing expression of essential genes, PNAs hold the promise to act as novel therapeutic alternatives to conventional antibiotics.

Discovery and evolution of L. monocytogenes: Historical background

The causative agent of listeriosis was discovered and named as Bacterium monocytogenes more than ninety years ago in University of Cambridge, England by Everitt Murray, a South African bacteriologist, and his colleagues. When they observed a Gram-positive bacillus organism is able to cause infection and deaths in laboratory rabbits and Guinea pigs and was associated with a marked mononuclear leucocytosis (Murray *et al*., 1926). In 1929, the bacterium was first described as a cause of severe invasive infections in humans (Nyfeldt, 1929). Eleven years later, it was named *Listeria* after Joseph Lister; Pioneer of septic surgery (Pirie, 1940). In the same year, Paterson (1940) presented the serotyping as a first discriminatory method for *Listeria* subtypes. Based on O (somatic) and H (flagellar) antigens, he classified *Listeria* into five somatic and four flagellar antigens.

The genetic determinants responsible for *L. monocytogenes* serotypes have been extensively studied in the recent years. As early as 1989, the first phylogenetic classification of *L. monocytogenes* by multilocus enzyme electrophoresis (MLEE) typing was conducted and it was found that the bacterium can be grouped in two distinct evolutionary groups, termed lineage I and II (Piffaretti *et al*., 1989). This initial observation was also confirmed by two independent studies performed in the same year (Bibb *et al*., 1989) as well as in the following year (Bibb *et al*., 1990). However, in 1995, the analysis of the data obtained from partial sequencing of the genes flaA (flagellin), *hly*, *iap* (p60), and 23S rDNA revealed the existence of a third lineage (III) (Rasmussen *et al*., 1995). This existence was further supported by a study conducted two years later (Wiedmann *et al*., 1997). Later on 2001, Nadon *et al*. (2001) demonstrated a correlation between serotyping, ribotyping, and lineage assignment. They found that lineage 1 includes isolates of serotypes 1/2b, 3b, 3c, and 4b, whereas lineage II included isolates of serotypes 1/2a, 3a, and 1/2c, and isolates of serotypes 4a and 4c were specified to lineage III. Moreover, based on partial *sigB* and *actA* sequencing, Roberts *et al*. (2006) showed that lineage III includes three genetically and phenotypically distinct subgroups (i.e. IIIA, IIIB and IIIC) and the most prominent phenotypic feature was the inability of all subgroup IIIB and IIIC isolates to utilize rhamnose, unlike typical *L. monocytogenes*. Two years later, the subgroup IIIB was reported as the fourth evolutionary lineage of *L. monocytogenes* (Ward *et al*., 2008).

L. monocytogenes genomics

Though the first DNA sequence appeared early in 1977 for the Bacteriophage [phi]X174 [5386 base pairs (bp)] as published elsewhere (Sanger *et al*., 1977), the first bacterium, *Haemophilus influenza* was not fully sequenced until 1995 (Fleischmann *et al*., 1995) and since then genome sequences have been published for a myriad of pathogens.

The first whole genome sequencing of *L. monocytogenes* has emerged in 2001 and it was found that *L. monocytogenes* EGD-e (serovar 1/2a) genome size is ~ 3.0 Mb, containing 39% G+C content and the total number of genes and protein-coding genes is 3835 and 2853, respectively. The organism has a cluster of virulence genes, involving *prfA* (positive regulatory factor A), *inlA* (internalin A), *inlB* (internalin B), *hly* (listeriolysin O), *plcA* (phosphatidylinositol-specific phospholipase c), *plcB* (phosphatidylcholine-phospholipase C), *actA* (actin-assembly inducing protein) and *mpl* (zinc metalloproteinase), encoding for particular proteins that contribute in definite steps in the infection process. The genome has 331 (11.6% from total genes) transporter genes and 88 from which are responsible for glucose metabolism indicating the essentiality of such carbohydrate in the growth of *L. monocytogenes*. The bacterium was also found to encode 209 transcriptional regulators, which represents 7.3% of the predicted coding sequences (Glaser *et al*., 2001) and this high ratio of regulatory genes, explains the ingenuity of *L. monocytogenes* to survive and grow in a wide range of arduous environmental conditions (Glaser *et al*., 2001; Liu *et al*., 2004). The successful full genome sequencing of *L. monocytogenes* EGD-e by Glaser *et al*., in 2001 provided new insights re-

garding the virulence of the bacterium and has opened the door for several *L. monocytogenes* strains of diverse sources to be partially or completely sequenced by other listeriologists in the following years (Doumith *et al*., 2004; Weinmaier *et al*., 2013; Wu *et al*., 2015). In fact, the later DNA sequencing studies have elucidated remarkable genetic diversity across the individual strains of *L. monocytogenes*, with 25-140 genes being strain-specific (Doumith *et al*., 2004; Hain *et al*., 2012, 2006; Kuenne *et al*., 2013).

L. monocytogenes RNA polymerase (RNAP)

Gene expression is a critical process for the viability of all organisms. Bacterial RNA polymerase (RNAP) is considered the principal enzyme of gene expression (Bai *et al*., 2011; Borukhov and Nudler, 2008). It exists in two forms; core and holoenzyme (Borukhov and Nudler, 2008). In *L. monocytogenes*, the core RNAP complex consists of four catalytic subunits (β', β, α, and ω) which encoded by *rpoC*, *rpoB*, *rpoA* and *rpoZ*, respectively (Glaser *et al*., 2001). Initiation of transcription requires association of sigma factors to the core to form the active complex of the enzyme (RNAP holoenzyme) (Borukhov and Nudler, 2008). Moreover, the enzyme is evolutionarily conserved, shares high similarity between bacterial strains, and is different from the eukaryotic homologues (Bai *et al*. 2011).

Intracellular lifestyle of L. monocytogenes

L. monocytogenes has the power to enter and survive into both phagocytic and nonphagocytic cells, such as epithelial cells, hepatocytes and endothelial cell (Lecuit and Cossart, 2002; Lecuit, 2005). Following internalization by an approximately half hour, it invades the host cytosol with the aid of listeriolysin O (LLO) and two phospholipases (e.g. phosphatidylinositol-specific phospholipase C and a broad-spectrum phospholipase C) (Tilney and Portnoy, 1989; Portnoy *et al*., 2002; Bonazzi and Cossart, 2006).

The cytolytic toxin LLO which encoded by the *hly* gene is considered the main virulence factor of *L. monocytogenes* required in the intracellular life cycle. It makes pores in vacuolar membranes of the phagosomes causing a passive flux of ions and macromolecules. This leads to a quick vacuolar lysis and bacterial release into the neutral pH and nutrient-rich host cytosol (Gaillard *et al*., 1987; Portnoy *et al*., 2002). Beside LLO, *L. monocytogenes* produces two important phospholipases, PI-plc (encoded by *plcA*) and PC-plc (encoded by *plcB*). They are well-known virulence factors functioning in cooperation with LLO so as to help the bacteria to escape from the vacuoles inside the host cells. Once released in the cytosol, *Listeria* can proliferate and then distribute to the adjacent cells via formation of actin tail (Mengaud *et al*., 1991; Schuppler and Loessner, 2010).

L. monocytogenes as an emerging foodborne pathogen

L. monocytogenes is an ubiquitous organism and can be found in a wide variety of raw and processed foods such as fish and poultry (Hussein *et al*., 2011; Jamali *et al*., 2013; Montero *et al*., 2015), milk and dairy products, various meats and meat products such as beef, pork and fermented sausages (Meloni, 2015; Montero *et al*., 2015).

The first evidence of foodborne transmission of *L. monocytogenes* was documented in 1981 in Canada when 34% died out of 41 confirmed cases of listeriosis associated with consumption of contaminated coleslaw (Schlech *et al*., 1983). Since that date, the pathogen has been implicated in several foodborne outbreaks with high case fatality rates (Schlech, 2000). Recently, in 2011, cantaloupes contaminated with *L. monocytogenes* caused the deadliest foodborne disease outbreak in the United States in nearly 90 years with 33 deaths and one miscarriage out of 147 confirmed human cases (McCollum *et al*., 2013), this outbreak was followed by several outbreaks in the recent four years (CDC, 2015). Moreover, the bacterium was reported as the third leading cause of death attributed to food poisoning pathogens (Scallan *et al*., 2011).

The burden and management of listeriosis

It was indicated that the presence of the bacteria in the human or animal feces is not a proof indication of illness since between 2% and 6% of healthy humans and animals harbor *L. monocytogenes* in their gastrointestinal tract and act as transient carriers for the organism without showing clinical signs (Gellin and Broome, 1989; Rocourt, 1996). However, *L. monocytogenes* possesses a public

health impact as a causative agent of listeriosis which considered one of the emerging foodborne zoonoses (Oevermann *et al*., 2010; Dhama *et al*., 2013) primarily contracted by ingestion of contaminated food products such as raw meat, dairy products, vegetables, and seafood (Allerberger and Wagner, 2010) and triggering primarily pregnant women, neonates, geriatric population, and those with weakened immune systems (Elinav *et al*., 2014).

The clinical manifestations of listeriosis in humans vary from mild gastroenteritis to invasive life-threatening illness depending on the infected person. For instance, in healthy people, the disease can be manifested as a self-limiting febrile gastroenteritis after consumption of heavily contaminated food (Ooi and Lorber, 2005). While, the aged and immunocompromised individuals show the invasive form of the disease that can be manifested by more severe clinical symptoms like septicemia, meningitis, and meningoencephalitis. Moreover, this serious invasive form can also occur in pregnant women and their newborns leading to abortion, premature birth and neonatal meningitis (Schuchat *et al*., 1991; Vázquez-Boland *et al*., 2001).

Effective treatment of diseases produced by intracellular zoonotic pathogens like *Mycobacterium, Brucella, Salmonella* and *Listeria* is a daunting task due to inability of most antibiotics to access intracellular replicative niches and attain the optimum therapeutic concentrations within the infected cells (Schuchat *et al*., 1991; Vázquez-Boland *et al*., 2001; Seleem *et al*., 2009a, 2009b). These challenges have sparked efforts to target intracellular bacteria utilizing different novel approaches such as peptide nucleic acid (Alajlouni and Seleem, 2013; Kuriakose *et al*., 2013; Nepal *et al*., 2015).

Peptide nucleic acid (PNA)

History and features of PNA

Peptide nucleic acid was introduced to the field of life science by Peter E. Nielsen and others, University of Copenhagen, Denmark in 1991 (Nielsen *et al*., 1991). It is a nucleic acid mimic, has the standard nucleobases (Adenine, Thymine, Guanine and Cytosine), in which the sugar phosphate backbone of natural nucleic acid is replaced by pseudopeptide backbone (polyamide) usually formed from N-(2-amino-ethyl)-glycine units (Egholm *et al*., 1992; Nielsen and Egholm, 1999). The purine and pyrimidine bases are linked to the pseudo peptide backbone by methylene carbonyl linkages (Nielsen, 1999). PNA has a number of unique traits, making it a promising antigene and antisense therapeutic agent; First, PNA structure is free from the phosphate group (uncharged) thus, it is able to form extraordinary strong complexes with the negatively charged RNA and DNA without electrostatic repulsion (Demidov and Frank-Kamenetskii, 2004). It was found that PNA-DNA duplexes possess higher thermal stability than the corresponding DNA-DNA duplexes due to the absence of electrostatic repulsion (Ray and Nordén, 2000). Moreover, the presence of pseudo-peptide backbone makes it resistant towards nucleases and proteases of cells allowing *in vitro* and *in vivo*extended life span for PNA comparing to DNA or RNA (Demidov *et al*., 1994; Mishra and Samal, 2011). Unlike primers which act as a substrate to DNA polymerase (Berdis 2009), PNA due to the lack of the free 3′ hydroxyl group, cannot be recognized by such enzyme (Orum *et al*., 1993; Pellestor and Paulasova, 2004).

Mode of action

PNAs can convey their action by two unique strategies e.g. antigene strategy and antisense strategy (Good and Nielsen, 1997; Mishra and Samal, 2011) as illustrated in Fig.1. First, PNA is able to recognize and hybridize to complementary sequences in the gene of interest within the genomic DNA resulting in extra stable PNA-DNA complex which interferes with the transcription of that gene (antigene effect) either by blocking the action of the RNA polymerase (Hanvey *et al*., 1992) or by arresting the further transcription elongation thereby producing truncated RNA transcripts (Mishra and Samal, 2011). Secondly, PNA functions via recognition and binding to the reserve mRNA forming stable RNA-PNA complexes which cause steric blocking of either RNA processing, transport into the cytoplasm, or translation (antisense effect) and cannot act as substrates for RNase H (Mologni *et al*., 1998).

It was demonstrated that the rate of DNA-PNA binding can be dramatically increased during DNA transcription, especially when the PNA sequence is designed to be complementary to the nontemplate

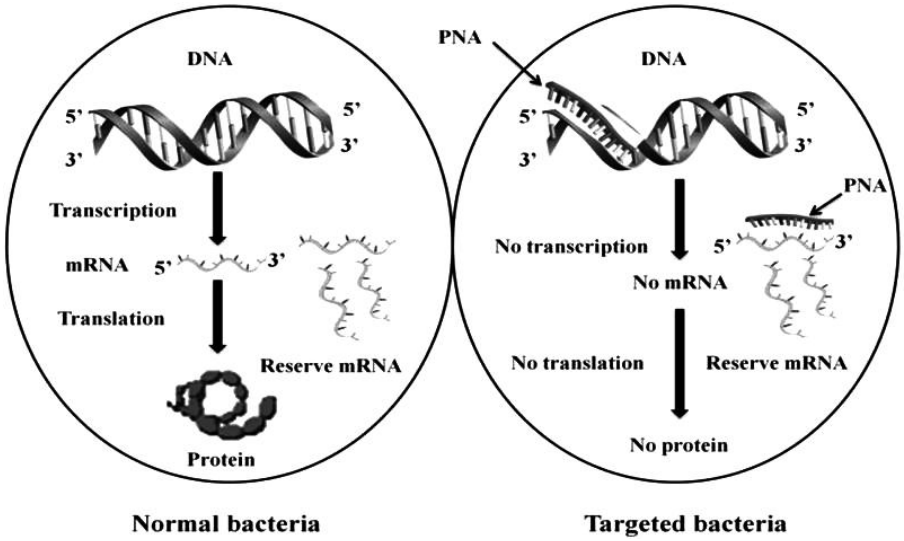

Fig. 1. The dual strategy of PNA in silencing bacterial genes. Regular steps of gene expression in normal bacteria (Left). Blocking of gene expression transcription (antigene) and translation (antisense) in targeted bacteria (Right).

strand (mRNA-like strand) rather than to the template strand (Larsen and Nielsen, 1996).

Being accessible for ribosome assembly, the translation start codon region including the upstream ribosomal binding site, Shine-Dalgarno, is considered the most sensitive region for inhibition by PNAs comparing to other regions in the nucleic acid (Dryselius *et al*., 2003; Rasmussen *et al*., 2007).

Intracellular PNA delivery

The high molecular weight as well as the non-ionic feature of the PNA constitute a major challenge for the successful utilization of the PNA to target intracellular pathogens (Soofi and Seleem, 2012) since their uptake is controlled by the selective permeability of cellular membranes (De Coupade *et al*., 2005; Munyendo *et al*., 2012). To overcome the uptake obstacle of PNA, many cell penetrating peptides (CPPs) have been utilized over the last 25 years to successfully deliver PNAs to their targets (Shiraishi and Nielsen, 2014, 2011).

Cell penetrating peptides (CPPs)

CPPs are positively charged short peptide residues holding the merit to transport molecules across the cell membranes (Bechara and Sagan, 2013). They constitute a promising tool for overcoming the antimicrobials bioavailability problems as they are able to unlock intracellular and even intranuclear targets helping in an efficient intracellular delivery of various antimicrobial agents including PNA (De Coupade *et al*., 2005; Munyendo *et al*., 2012). The synthetic (KFF)3K (where K is Lysine and F is Phenylalanine) which developed in 1996 (Vaara and Porro, 1996) is the commonly used CPP and was successfully able to deliver PNAs into *L. monocytogenes* (Alajlouni and Seleem, 2013).

L. monocytogenes RNA polymerase as a potential antisense target

RNAP is a crucial enzyme to the life of all organisms including bacteria and any possible mean that can interfere with its function could possess a direct negative effect on bacterial viability (Bai *et al*., 2011). Furthermore, RNAP subunits encoded by *rpoA, rpoB* and *rpoD* genes have received a considerable attention as antisense targets especially in intracellular pathogens like *S. enterica* (Bai *et al*., 2012; Soofi and Seleem, 2012), *B. suis* (Rajasekaran *et al*., 2013) and *L. monocytogenes* (Alajlouni and Seleem, 2013).

Regarding the latter bacteria, Alajlouni and Seleem (2013) targeted *L. monocytogenes* F4244 using two different PNAs specific for *rpoA* ((KFF)3K-O-cgatcattcaaa) and *rpoD* ((KFF)3K-O-tcataactgcc)) and they found that both PNAs at 40 µM were able to completely clear the bacteria at pure culture media by 8 hours. Moreover, they found that both PNAs at 15 µM possessed antimicrobial effect against the intracellular bacterial count inside murine macrophage cells when applied for 24 hours with a 1.29 and 0.39 log10 CFU bacterial reduction, respectively.

Listeria-infected Caenorhabditis elegans and PNA efficiency

C. elegans is a small free-living soil nematode with a length around 1 mm. The worm feeds on microorganisms; mostly bacteria (Hart and Chao, 2010). It was first introduced as a laboratory animal model for studying development and behavior by Sydney Brenner in the mid- 1960s (Brenner, 1974). Recently, the worm has been extensively adopted as a whole animal model for exploring the host-pathogen interactions (Moy *et al*., 2006; Zhang and Hou, 2013). For the first time, Alajlouni and Seleem (2013) have used infected *C. elegans* as a whole animal model for screening the PNAs efficacy. They evaluated the capability of the two abovementioned PNAs targeting *L. monocytogenes rpoA* and *rpoD* genes to treat Listeria- infected *C. elegans* for 24 hours. Although the anti-*rpoD* PNA was not able to induce significant effect, significant reduction accounting for 37% and 72% of the intracellular bacterial growth was achieved by the anti-*rpoA* PNA when applied at 15 μM and 30 μM, respectively.

Future prospects

In the era of the spread of zoonotic diseases and lack of effective treatment strategies to keep pace the increasing antimicrobial resistance, PNAs hold a future promise as effective alternatives to the traditional antibiotics. Based on the *in vitro* and *in vivo*results achieved (Alajlouni and Seleem, 2013), we suggest that *rpoA* gene is an encouraging target for the development of antisense therapeutics for effective targeting of the intracellular zoonotic pathogens like *L. monocytogenes*. However, further improvement in terms of intracellular delivery of such agents and foundation of convenient PNA design basics is still required to achieve more success. Focusing on the development of appropriate antisense therapeutics specific for *L. monocytogenes* could play an important role in the creation of a knowledge-based design that can be utilized for targeting other intracellular zoonotic pathogens such as *Mycobacterium, Brucella,* and *Salmonella.*

References

Alajlouni, R.A., Seleem, M.N., 2013. Targeting *Listeria monocytogenes rpoA* and *rpoD* genes using peptide nucleic acids. Nucleic acid therapeutics 23, 363–367.

Allerberger, F., Wagner, M., 2010. Listeriosis: A resurgent foodborne infection. Clinical Microbiology and Infection 16, 16-23.

Bai, H., You, Y., Yan, H., Meng, J., Xue, X., Hou, Z., Zhou, Y., Ma, X., Sang, G., Luo, X., 2012. Antisense inhibition of gene expression and growth in gram-negative bacteria by cell-penetrating peptide conjugates of peptide nucleic acids targeted to *rpoD* gene. Biomaterials 33, 659–667.

Bai, H., Zhou, Y., Hou, Z., Xue, X., Meng, J., Luo, X., 2011. Targeting bacterial RNA polymerase: promises for future antisense antibiotics development. Infectious disorders drug targets 11, 175–187.

Bechara, C., Sagan, S., 2013. Cell-penetrating peptides: 20 years later, where do we stand? FEBS letters 587, 1693–702. doi:10.1016/j.febslet.2013.04.031

Berdis, A.J., 2009. Mechanisms of DNA polymerases. Chemical reviews 109, 2862–79. doi:10.1021/cr800530b

Beverly, R.L., 2004. The control, survival and growth of *Listeria monocytogenes* on food products. Doctor of Philosophy Thesis. Louisiana State University. Available online at [WWW Document]. URL http://etd.lsu.edu/docs/available/etd-11102004-072203/unrestricted/Beverly_dis.pdf

Bibb, W.F., Gellin, B.G., Weaver, R., Schwartz, B., Plikaytis, B.D., Reeves, M.W., Pinner, R.W., Broome, C. V., 1990. Analysis of clinical and food-borne isolates of *Listeria monocytogenes* in the United States by multilocus enzyme electrophoresis and application of the method to epidemiologic investigations. Applied and Environmental Microbiology 56, 2133–41.

Bibb, W.F., Schwartz, B., Gellin, B.G., Plikaytis, B.D., Weaver, R.E., 1989. Analysis of *Listeria monocytogenes* by multilocus enzyme electrophoresis and application of the method to epidemiologic investigations. International Journal of Food Microbiology 8, 233–9.

Bonazzi, M., Cossart, P., 2006. Bacterial entry into cells: A role for the endocytic machinery. FEBS Letters 580, 2962-2967.

Borukhov, S., Nudler, E., 2008. RNA polymerase: the vehicle of transcription. Trends in Microbiology 16, 126–34.

Brenner, S., 1974. The genetics of Caenorhabditis elegans. Genetics 77, 71–94.

CDC, 2015. *Listeria* Outbreaks. Available online at http://www.cdc.gov/listeria/outbreaks/index.html.

De Coupade, C., Fittipaldi, A., Chagnas, V., Michel, M., Carlier, S., Tasciotti, E., Darmon, A., Ravel, D., Kearsey, J., Giacca, M., Cailler, F., 2005. Novel human-derived cell-penetrating peptides for specific subcellular delivery of therapeutic biomolecules. The Biochemical Journal 390, 407–418.

Demidov, V. V., Frank-Kamenetskii, M.D., 2004. Two sides of the coin: Affinity and specificity of nucleic acid interactions. Trends in Biochemical Sciences 29, 62-71.

Demidov, V. V., Potaman, V.N., Frank-Kamenetskil, M.D., Egholm, M., Buchard, O., Sönnichsen, S.H., Nlelsen, P.E., 1994. Stability of peptide nucleic acids in human serum and cellular extracts. Biochemical Pharmacology 48(6): 1310-1313.

Dhama, K., Rajagunalan, S., Chakraborty, S., Verma, A.K., Kumar, A., Tiwari, R., Kapoor, S., 2013. Food-borne

pathogens of animal origin-diagnosis, prevention, control and their zoonotic significance: a review. Pakistan Journal of Biological Sciences 16, 1076–85.

Doumith, M., Cazalet, C., Simoes, N., Frangeul, L., Jacquet, C., Kunst, F., Martin, P., Cossart, P., Glaser, P., Buchrieser, C., 2004. New Aspects Regarding Evolution and Virulence of *Listeria monocytogenes* Revealed by Comparative Genomics and DNA Arrays. Infection and Immunity 72, 1072–1083.

Dryselius, R., Aswasti, S.K., Rajarao, G.K., Nielsen, P.E., Good, L., 2003. The translation start codon region is sensitive to antisense PNA inhibition in Escherichia coli. Oligonucleotides 13, 427–433.

Egholm, M., Buchardt, O., Nielsen, P.E., 1992. Peptide nucleic acids (PNA). Oligonucleotide analogs with an achiral peptide backbone. Journal of the American Chemical Society 114, 1895–1897.

Elinav, H., Hershko-Klement, A., Valinsky, L., Jaffe, J., Wiseman, A., Shimon, H., Braun, E., Paitan, Y., Block, C., Sorek, R., Nir-Paz, R., 2014. Pregnancy-associated listeriosis: clinical characteristics and geospatial analysis of a 10-year period in Israel. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 59, 953–61.

Fleischmann, R.D., Adams, M.D., White, O., Clayton, R.A., Kirkness, E.F., Kerlavage, A.R., Bult, C.J., Tomb, J.F., Dougherty, B.A., Merrick, J.M., 1995. Whole-genome random sequencing and assembly of Haemophilus influenzae Rd. Science 269, 496–512.

Gaillard, J.L., Berche, P., Mounier, J., Richard, S., Sansonetti, P., 1987. In vitro model of penetration and intracellular growth of *Listeria monocytogenes* in the human enterocyte-like cell line Caco-2. Infection and Immunity 55, 2822–2829.

Galsworthy, S.B., Girdler, S., Koval, S.F., 1990. Chemotaxis in *Listeria monocytogenes*. Acta Microbiologica Hungarica 37, 81–85.

Gellin, B.G., Broome, C. V, 1989. Listeriosis. Journal of the American Medical Association 261, 1313–20.

Gianfranceschi, M.V., D'Ottavio, M.C., Gattuso, A., Bella, A., Aureli, P., 2009. Distribution of serotypes and pulsotypes of *Listeria monocytogenes* from human, food and environmental isolates (Italy 2002-2005). Food Microbiology 26, 520–526.

Glaser, P., Frangeul, L., Buchrieser, C., Rusniok, C., Amend, A., Baquero, F., Berche, P., Bloecker, H., Brandt, P., Chakraborty, T., Charbit, A., Chetouani, F., Couvé, E., de Daruvar, A., Dehoux, P., Domann, E., Domínguez-Bernal, G., Duchaud, E., Durant, L., Dussurget, O., Entian, K.D., Fsihi, H., García-del Portillo, F., Garrido, P., Gautier, L., Goebel, W., Gómez-López, N., Hain, T., Hauf, J., Jackson, D., Jones, L.M., Kaerst, U., Kreft, J., Kuhn, M., Kunst, F., Kurapkat, G., Madueno, E., Maitournam, A., Vicente, J.M., Ng, E., Nedjari, H., Nordsiek, G., Novella, S., de Pablos, B., Pérez-Diaz, J.C., Purcell, R., Remmel, B., Rose, M., Schlueter, T., Simoes, N., Tierrez, A., Vázquez-Boland, J.A., Voss, H., Wehland, J., Cossart, P., 2001. Comparative genomics of *Listeria* species. Science 294, 849–852.

Good, L., Nielsen, P.E., 1997. Progress in developing PNA as a gene-targeted drug. Antisense & Nucleic Acid Drug Development 7, 431–7.

Graves, L.M., Helsel, L.O., Steigerwalt, A.G., Morey, R.E., Daneshvar, M.I., Roof, S.E., Orsi, R.H., Fortes, E.D., Milillo, S.R., den Bakker, H.C., Wiedmann, M., Swaminathan, B., Sauders, B.D., 2010. *Listeria* marthii sp. nov., isolated from the natural environment, Finger Lakes National Forest. International Journal of Systematic and Evolutionary Microbiology 60, 1280–8.

Hain, T., Ghai, R., Billion, A., Kuenne, C.T., Steinweg, C., Izar, B., Mohamed, W., Mraheil, M.A., Domann, E., Schaffrath, S., Kärst, U., Goesmann, A., Oehm, S., Pühler, A., Merkl, R., Vorwerk, S., Glaser, P., Garrido, P., Rusniok, C., Buchrieser, C., Goebel, W., Chakraborty, T., 2012. Comparative genomics and transcriptomics of lineages I, II, and III strains of *Listeria monocytogenes*. BMC genomics 13, 144.

Hain, T., Steinweg, C., Chakraborty, T., 2006. Comparative and functional genomics of *Listeria* spp. Journal of Biotechnology 126, 37–51.

Hanvey, J.C., Peffer, N.J., Bisi, J.E., Thomson, S.A., Cadilla, R., Josey, J.A., Ricca, D.J., Hassman, C.F., Bonham, M.A., Au, K.G., 1992. Antisense and antigene properties of peptide nucleic acids. Science (New York, N.Y.) 258, 1481–1485. doi:10.1126/science.1279811

Hart, A.C., Chao, M.Y., 2010. From Odors to Behaviors in Caenorhabditis elegans. (CRC Press: Boca Raton, FL). http://www.ncbi.nlm.nih.gov/books/NBK55983/

Hussein, A., Othman, R.E.E., Sayed, S.M.A., Hassanein, R., Abushahba, F.N.M., 2011. Occurrence of *Listeria monocytogenes* in poultry, fish & their products as well as its public health hazard on women., in: Animal Hygiene and Sustainable Livestock Production. Proceedings of the XVth International Congress of the International Society for Animal Hygiene, Vienna, Austria, 3-7 July 2011, Volume 2. pp. 987–992.

Jamali, H., Chai, L.C., Thong, K.L., 2013. Detection and isolation of *Listeria* spp. and *Listeria monocytogenes* in ready-to-eat foods with various selective culture media. Food Control 32, 19–24.

Kuenne, C., Billion, A., Mraheil, M.A., Strittmatter, A., Daniel, R., Goesmann, A., Barbuddhe, S., Hain, T., Chakraborty, T., 2013. Reassessment of the *Listeria monocytogenes* pan-genome reveals dynamic integration hotspots and mobile genetic elements as major components of the accessory genome. BMC genomics 14, 47.

Kuriakose, J., Hernandez-Gordillo, V., Nepal, M., Brezden, A., Pozzi, V., Seleem, M.N., Chmielewski, J., 2013. Targeting intracellular pathogenic bacteria with unnatural proline-rich peptides: Coupling antibacterial activity with macrophage penetration. Angewandte Chemie - International Edition 52, 9664–9667.

Larsen, H.J., Nielsen, P.E., 1996. Transcription-mediated binding of peptide nucleic acid (PNA) to double-stranded DNA: sequence-specific suicide transcription. Nucleic Acids Research 24, 458–63.

Leclercq, A., Clermont, D., Bizet, C., Grimont, P.A.D., Le Flèche-Matéos, A., Roche, S.M., Buchrieser, C., Cadet-Daniel, V., Le Monnier, A., Lecuit, M., Allerberger, F., 2010. *Listeria* rocourtiae sp. nov. International Journal of Systematic and Evolutionary

Microbiology 60, 2210–4.

Lecuit, M., 2005. Understanding how *Listeria monocytogenes* targets and crosses host barriers. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 11, 430–6.

Lecuit, M., Cossart, P., 2002. Genetically-modified-animal models for human infections: the *Listeria* paradigm. Trends in Molecular Medicine 8, 537–542.

Liu, D., 2013. Molecular approaches to the identification of pathogenic and nonpathogenic listeriae. Microbiology insights 6, 59–69.

Liu, D., Ainsworth, A.J., Austin, F.W., Lawrence, M.L., 2004. Use of PCR primers derived from a putative transcriptional regulator gene for species-specific determination of *Listeria monocytogenes*. International Journal of Food Microbiology 91, 297–304.

McCollum, J.T., Cronquist, A.B., Silk, B.J., Jackson, K. a, O'Connor, K. a, Cosgrove, S., Gossack, J.P., Parachini, S.S., Jain, N.S., Ettestad, P., Ibraheem, M., Cantu, V., Joshi, M., DuVernoy, T., Fogg, N.W., Gorny, J.R., Mogen, K.M., Spires, C., Teitell, P., Joseph, L. a, Tarr, C.L., Imanishi, M., Neil, K.P., Tauxe, R. V, Mahon, B.E., 2013. Multistate outbreak of listeriosis associated with cantaloupe. The New England Journal of Medicine 369, 944–53.

Meloni, D., 2015. Presence of *Listeria monocytogenes* in Mediterranean-Style Dry Fermented Sausages. Foods 4, 34–50.

Mengaud, J., Braun-Breton, C., Cossart, P., 1991. Identification of phosphatidylinositol-specific phospholipase C activity in *Listeria monocytogenes*: a novel type of virulence factor? Mol Microbiol 5, 367–372.

Mishra, S.C., Samal, L., 2011. Peptide Nucleic Acid. Acta Informatica Medica 19, 118–123.

Mologni, L., leCoutre, P., Nielsen, P.E., Gambacorti-Passerini, C., 1998. Additive antisense effects of different PNAs on the *in vitro* translation of the PML/RARalpha gene. Nucleic Acids Research 26, 1934–1938.

Montero, D., Bodero, M., Riveros, G., Lapierre, L., Gaggero, A., Vidal, R.M., Vidal, M., 2015. Molecular epidemiology and genetic diversity of *Listeria monocytogenes* isolates from a wide variety of ready-to-eat foods and their relationship to clinical strains from listeriosis outbreaks in Chile. Frontiers in Microbiology 6, 384.

Moy, T.I., Ball, A.R., Anklesaria, Z., Casadei, G., Lewis, K., Ausubel, F.M., 2006. Identification of novel antimicrobials using a live-animal infection model. Proceedings of the National Academy of Sciences of the United States of America 103, 10414–10419.

Munyendo, W.L., Lv, H., Benza-Ingoula, H., Baraza, L.D., Zhou, J., 2012. Cell Penetrating Peptides in the Delivery of Biopharmaceuticals. Biomolecules.

Murray, E.G.D., Webb, R.A., Swann, M.B.R., 1926. A disease of Rabbits characterised by a large mononuclear Leucocytosis, caused by a hitherto undescribed bacillus Bacterium monocytogenes. The Journal of Pathology and Bacteriology 29, 407–39.

Nadon, C.A., Woodward, D.L., Young, C., Rodgers, F.G., Wiedmann, M., 2001. Correlations between molecular subtyping and serotyping of *Listeria monocytogenes*. Journal of Clinical Microbiology 39, 2704–2707.

Nepal, M., Thangamani, S., Seleem, M.N., Chmielewski, J., 2015. Targeting intracellular bacteria with an extended cationic amphiphilic polyproline helix. Organic & biomolecular chemistry 13, 5930–5936.

Nielsen, P.E., 2004. PNA Technology. Molecular biotechnology 26, 233–248. Nielsen, P.E., 1999. Peptide Nucleic Acid. A Molecule with Two Identities. Accounts of Chemical Research 32, 624–630.

Nielsen, P.E., Egholm, M., 1999. An introduction to peptide nucleic acid. Current Issues in Molecular Biology 1, 89–104.

Nielsen, P.E., Egholm, M., Berg, R.H., Buchardt, O., 1991. Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide. Science 254, 1497–500.

Nyfeldt, A., 1929. Etiologie de la mononucleose infectieuse. Comptes Rendus des Seances de la Societe de Biologie, 101, 590-591. http://www.oalib.com/references/14542835

Oevermann, A., Zurbriggen, A., Vandevelde, M., 2010. Rhombencephalitis Caused by *Listeria monocytogenes* in Humans and Ruminants: A Zoonosis on the Rise?. Interdisciplinary Perspectives on Infectious Diseases 2010, 632513.

Ooi, S.T., Lorber, B., 2005. Gastroenteritis due to *Listeria monocytogenes*. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 40, 1327–32.

Orum, H., Nielsen, P.E., Egholm, M., Berg, R.H., Buchardt, O., Stanley, C., 1993. Single base pair mutation analysis by PNA directed PCR clamping. Nucleic Acids Research 21, 5332–6.

Paterson, J.S., 1940. The antigenic structure of organisms of the genusListerella. The Journal of Pathology and Bacteriology 51, 427–436.

Pellestor, F., Paulasova, P., 2004. The peptide nucleic acids (PNAs), powerful tools for molecular genetics and cytogenetics. European Journal of Human Genetics 12, 694–700.

Piffaretti, J.C., Kressebuch, H., Aeschbacher, M., Bille, J., Bannerman, E., Musser, J.M., Selander, R.K., Rocourt, J., 1989. Genetic characterization of clones of the bacterium *Listeria monocytogenes* causing epidemic disease. Proceedings of the National Academy of Sciences of the United States of America 86, 3818–3822.

Pirie, J.H.H., 1940. Listeria: Change of Name for a Genus Bacteria. Nature 145, 264-264.

Portnoy, D.A., Auerbuch, V., Glomski, I.J., 2002. The cell biology of *Listeria monocytogenes* infection: the intersection of bacterial pathogenesis and cell-mediated immunity. The Journal of Cell Biology 158, 409–414.

Rajasekaran, P., Alexander, J.C., Seleem, M.N., Jain, N., Sriranganathan, N., Wattam, A.R., Setubal, J.C., Boyle, S.M., 2013. Peptide nucleic acids inhibit growth of Brucella suis in pure culture and in infected murine macrophages. International Journal of Antimicrobial Agents 41, 358–362.

Rasmussen, L.C.V., Sperling-Petersen, H.U., Mortensen, K.K., 2007. Hitting bacteria at the heart of the central dogma: sequence-specific inhibition. Microbial Cell

Factories 6, 24.

Rasmussen, O.F., Skouboe, P., Dons, L., Rossen, L., Olsen, J.E., 1995. *Listeria monocytogenes* exists in at least three evolutionary lines: evidence from flagellin, invasive associated protein and listeriolysin O genes. Microbiology 141, 2053–61.

Ray, A., Nordén, B., 2000. Peptide nucleic acid (PNA): its medical and biotechnical applications and promise for the future. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 14, 1041–60.

Roberts, A., Nightingale, K., Jeffers, G., Fortes, E., Kongo, J.M., Wiedmann, M., 2006. Genetic and phenotypic characterization of *Listeria monocytogenes* lineage III. Microbiology 152, 685–693.

Rocourt, J., 1996. Risk factors for listeriosis. Food Control 7, 195–202.

Sanger, F., Nicklen, S., Coulson, A.R., 1977. DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences of the United States of America 74, 5463–5467.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R. V, Widdowson, M.-A., Roy, S.L., Jones, J.L., Griffin, P.M., 2011. Foodborne illness acquired in the United States--major pathogens. Emerging Infectious Diseases 17, 7–15.

Schlech, W.F., 2000. Foodborne listeriosis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 31, 770–775.

Schlech, W.F., Lavigne, P.M., Bortolussi, R.A., Allen, A.C., Haldane, E.V., Wort, A.J., Hightower, A.W., Johnson, S.E., King, S.H., Nicholls, E.S., Broome, C.V., 1983. Epidemic Listeriosis-Evidence for Transmission by Food. New England Journal of Medicine 308, 203-206.

Schuchat, A., Swaminathan, B., Broome, C. V, 1991. Epidemiology of human listeriosis. Clinical Microbiology Reviews 4, 169–183.

Schuppler, M., Loessner, M.J., 2010. The Opportunistic Pathogen *Listeria monocytogenes*: Pathogenicity and Interaction with the Mucosal Immune System. International Journal of Inflammation 2010, 704321.

Seleem, M.N., Jain, N., Pothayee, N., Ranjan, A., Riffle, J.S., Sriranganathan, N., 2009a. Targeting Brucella melitensis with polymeric nanoparticles containing streptomycin and doxycycline. FEMS Microbiology Letters 294, 24–31.

Seleem, M.N., Munusamy, P., Ranjan, A., Alqublan, H., Pickrell, G., Sriranganathan, N., 2009b. Silica-antibiotic hybrid nanoparticles for targeting intracellular pathogens. Antimicrobial agents and chemotherapy 53, 4270–4.

Shiraishi, T., Nielsen, P.E., 2014. Cellular delivery of peptide nucleic acids (PNAs). Methods in Molecular Biology 1050, 193–205.

Shiraishi, T., Nielsen, P.E., 2011. Peptide nucleic acid (PNA) cell penetrating peptide (CPP) conjugates as carriers for cellular delivery of antisense oligomers. Artificial DNA, PNA & XNA 2, 90–9.

Smith, J.L., Liu, Y., Paoli, G.C., 2013. How does *Listeria monocytogenes* combat acid conditions? Canadian Journal of Microbiology 59, 141–52.

Soofi, M.A., Seleem, M.N., 2012. Targeting essential genes in Salmonella enterica serovar typhimurium with antisense peptide nucleic acid. Antimicrobial Agents and Chemotherapy 56, 6407–9.

Tan, W., Wang, G., Pan, Z., Yin, Y., Jiao, X., 2015. Complete Genome Sequence of *Listeria monocytogenes* NTSN, a Serovar 4b and Animal Source Strain. Genome Announcements 3, e01403–14.

Tilney, L.G., Portnoy, D.A., 1989. Actin filaments and the growth, movement, and spread of the intracellular bacterial parasite, *Listeria monocytogenes*. The Journal of Cell Biology 109, 1597–608.

Vaara, M., Porro, M., 1996. Group of peptides that act synergistically with hydrophobic antibiotics against gram-negative enteric bacteria. Antimicrobial agents and Chemotherapy 40, 1801–5.

Vázquez-Boland, J.A., Kuhn, M., Berche, P., Chakraborty, T., Domínguez-Bernal, G., Goebel, W., González-Zorn, B., Wehland, J., Kreft, J., 2001. *Listeria* pathogenesis and molecular virulence determinants. Clinical Microbiology Reviews 14, 584–640.

Ward, T.J., Ducey, T.F., Usgaard, T., Dunn, K.A., Bielawski, J.P., 2008. Multilocus genotyping assays for single nucleotide polymorphism-based subtyping of *Listeria monocytogenes* isolates. Applied and Environmental Microbiology 74, 7629–7642.

Weinmaier, T., Riesing, M., Rattei, T., Bille, J., Arguedas-Villa, C., Stephan, R., Tasara, T., 2013. Complete Genome Sequence of *Listeria monocytogenes* LL195, a Serotype 4b Strain from the 1983–1987 Listeriosis Epidemic in Switzerland. Genome Announcements 1, 8–9.

Wiedmann, M., Bruce, J.L., Keating, C., Johnson, A.E., McDonough, P.L., Batt, C.A., 1997. Ribotypes and virulence gene polymorphisms suggest three distinct *Listeria monocytogenes* lineages with differences in pathogenic potential. Infection and Immunity 65, 2707–16.

Wu, Y., Zheng, J., Wang, Y., Li, S., Jin, H., Li, Z., Bi, D., Sun, M., Liu, M., 2015. Draft Genome Sequence of *Listeria monocytogenes* LM201, Isolated from Foodstuff. Genome announcements 3, e01417–14.

Zhang, R., Hou, A., 2013. Host-Microbe Interactions in Caenorhabditis elegans. ISRN Microbiology 2013, 356451.