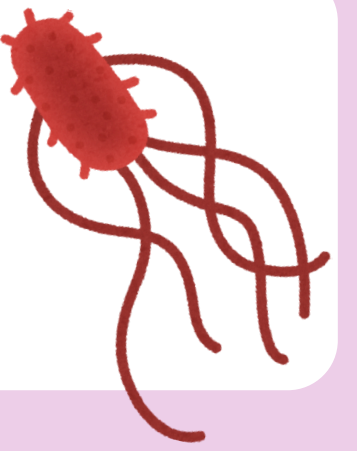


Listeria Monocytogenes

Bioinformatic Characterization of the Genome and Proteome

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Abstract

This case study explores a DNA fragment, which was determined to be *Listeria Monocytogenes* using BLAST. This project aims to use bioinformatics tools to analyze the genome and proteome of *L. monocytogenes* to uncover how its genes and key proteins, such as PrfA and CcpA, contribute to its pathogenicity and global health impact.

Introduction

Listeria monocytogenes is a gram-positive, catalase-positive, facultative intracellular rod that can grow at refrigerator temperatures [29]. It is a major cause of foodborne illness, commonly linked to contaminated deli meats and unpasteurized dairy products. The bacterium survives in diverse environments such as soil, water, sewage, animals, and food processing plants, and can infect both humans and animals [13].

In humans, it causes listeriosis, which may lead to meningitis, sepsis, encephalitis, spontaneous abortion, or mild gastroenteritis, particularly in pregnant women, the elderly, and immunocompromised individuals [29]. Transmission occurs mainly through ingestion of contaminated food, responsible for 85–95% of cases, and can also occur transplacentally or during birth; nosocomial and person-to-person spread are rare [22].

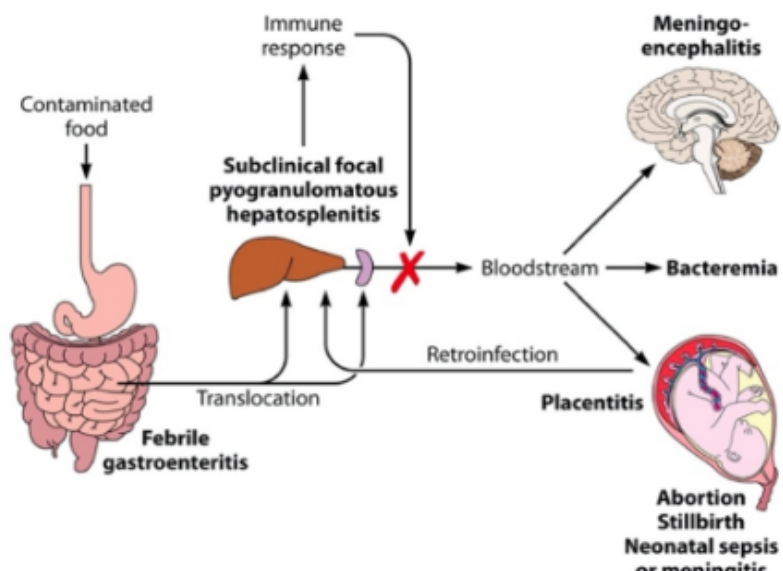


Figure 1. Transmission of *L. monocytogenes* bacteria. Transmission happens via ingestion of contaminated food or transplacentally during pregnancy [23].

Methods

NCBI

The NCBI webserver was first used to determine the organism, using a nucleotide BLAST. This was done using the standard databases and optimized for highly similar sequences.

The webserver was also used to perform an ORFfinder, using the settings: minimal ORF length 75 nt, genetic code 1. standard, and "ATG" and alternative initiation codons.

KBase

The KBase webserver was used to perform a gene annotation. This was done following the instructions of the KBase documentation guide. Prokka (Annotate Assembly and Re-annotate Genomes with Prokka – v1.14.5) was used to annotate the sequence.

Protein analysis

To analyze the protein structures, AlphaFold, InterPro, TMHMM, and the PDB webserver were used. This was done for two different genes.

In silico overexpression

The *ccpA* gene from *L. monocytogenes* was amplified using primers designed with NCBI Primer-BLAST and Benchling, incorporating restriction sites to generate sticky ends. Complementary sticky ends were introduced into the pBAD vector using the same restriction enzymes. The recombinant plasmid was transformed into *Escherichia coli* DH5- α . This allowed production of the CcpA protein in a bacterial system.

Results

Blast of unknown sequence

Description	Total score	Query cover	E-value	Accession number
Listeria monocytogenes strain FDA00006667 chromosome	37010	100%	0.0	CP044430.2
Listeria monocytogenes strain OB040119 chromosome	37010	100%	0.0	CP053630.1
Listeria monocytogenes strain J1817, complete genome	37010	100%	0.0	CP006599.1
Listeria monocytogenes strain OB030029 chromosome	37010	100%	0.0	CP053628.1
Listeria monocytogenes strain J1776	37010	100%	0.0	CP006598.1

Table 1: BLAST results. The DNA fragment is located on the *L. monocytogenes* genome

Gene annotation of *L. monocytogenes*

Gene	strand	start	stop	product
ahpC	+	9381	9926	Alkyl hydroperoxide reductase C
aroA	+	12556	13641	Protein AroA(G)
ccpA	+	13876	14883	Catabolite control protein A
ezrA	+	18161	19876	Septation ring formation regulator EzrA
msrC	-	17378	17866	Free methionine-R-sulfoxide reductase
murC	+	7893	9236	UDP-N-acetylmuramate-L-alanine ligase
pepS	-	9967	11079	Aminopeptidase PepS
rpsD	-	16564	17166	30S ribosomal protein S4
tyrS	+	15205	16464	Tyrosine-tRNA ligase
ysdC	+	1769	2842	Putative aminopeptidase YsdC
ytnP	+	64	840	putative quorum-quenching lactonase YtnP
ytpP	+	3168	3479	Thioredoxin-like protein YtpP

Table 2: KBase results fragment 2

Many proteins were found, no pathogenic proteins were found in all 3 fragments, therefore further analysis was done with *ccpA* found in fragment 2 and PrfA, not found in any fragment, due to its importance in pathogenicity.

Pathogenicity

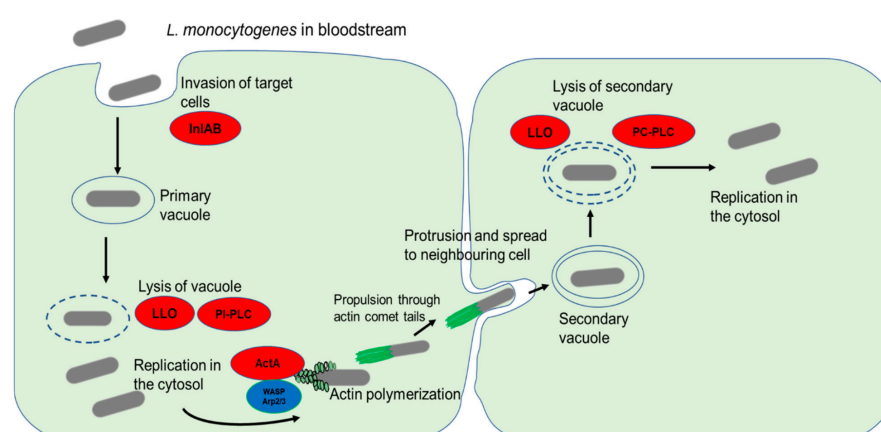


Figure 2 *L. monocytogenes* invasion and intracellular spread. Surface proteins InlA and InlB enable host cell entry, after which LLO and PI-PLC lyse the primary vacuole, releasing bacteria into the cytosol for replication. ActA recruits VASP and the Arp2/3 complex to drive cell-to-cell spread, with LLO and PC-PLC lysing the secondary vacuole to infect adjacent cells [32].

Transmission pathway

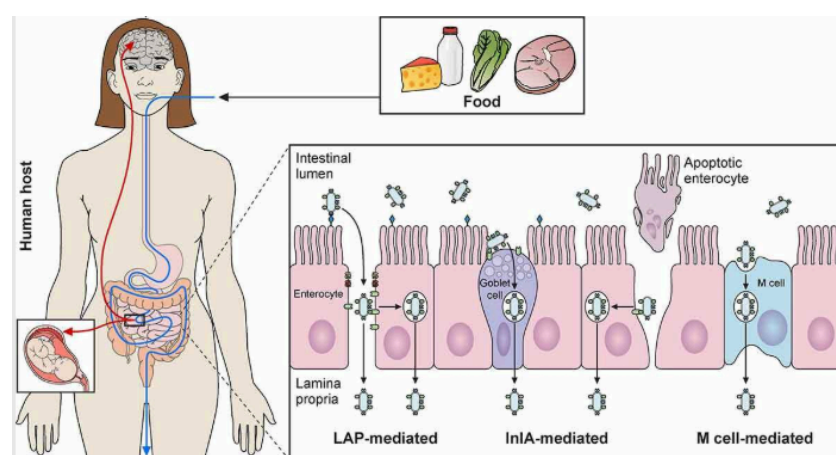


Figure 3 The transmission pathway of *Listeria monocytogenes* through oral infection [12].

Protein structures of *ccpA* and *prfA*

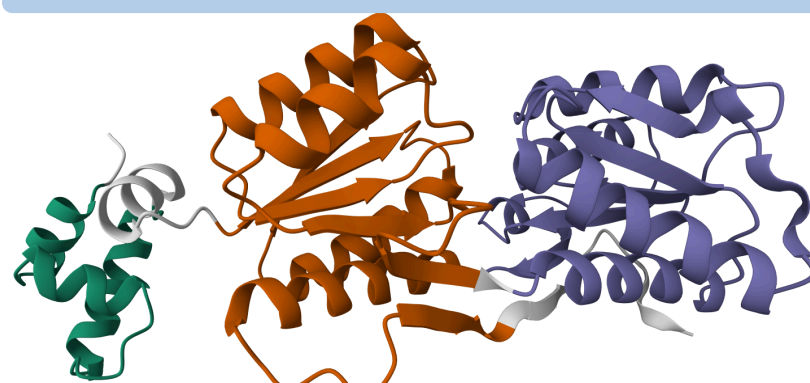


Figure 4. The 3D structure of the CcpA protein. The different colours represent different domains, using Interpro, one DNA binding site was identified as a helix-turn-helix (green) as well as two Rossmann like folds (red, purple) which assists in cofactor binding.

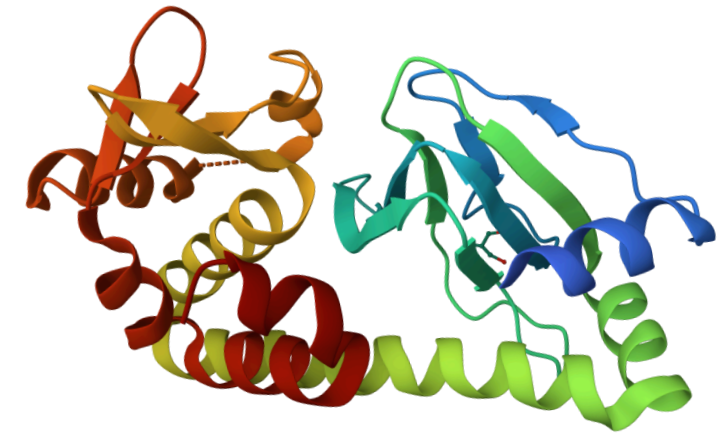


Figure 5. The 3D structure of the PrfA protein. The colours represent the location of the N-terminal (blue) and C-terminal (red). Analysis of the protein sequence in Interpro revealed a helix-turn-helix important in DNA binding and a jelly roll fold which binds cofactors.

In silico vector design

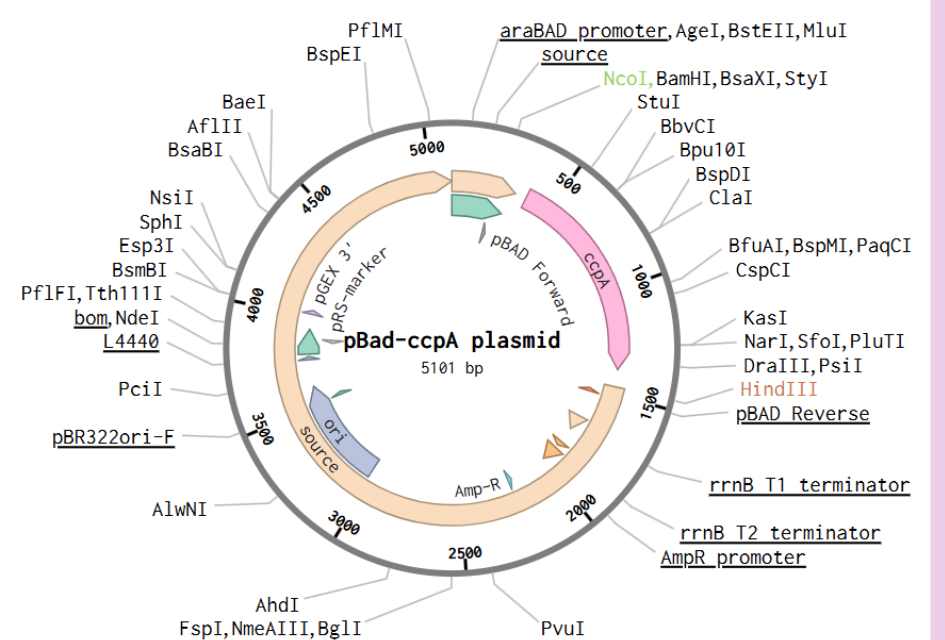


Figure 6. The pBAD plasmid containing the *ccpA* gene, indicated in pink. The restriction enzymes used are highlighted in green and brown.

Global health

Even though listeriosis is a relatively rare disease with 0.1 to 10 cases per million per year [4; 38], *Listeria monocytogenes* is still among the top causes of foodborne illness-related hospitalizations in North America and Europe [18].

There is no human vaccine [24], so global health strategies rely on safe food handling and avoidance of high-risk foods [9]. Treatment typically involves ampicillin, though antibiotic resistance is a growing concern [33]. Research into new therapeutic targets about PrfA inhibitors and FDA-approved drugs is ongoing, but shows promise. [19; 11; 27].

Conclusion

A detailed analysis of *Listeria monocytogenes* was performed using various bioinformatic tools and resources. This revealed that it is a foodborne pathogen capable of surviving and replicating at low temperatures, making it a big cause for foodborne outbreaks.

Analysis of the *ccpA* and *prfA* genes and their structures showed insights into the organism's metabolic regulation and virulence factors. Moreover, a construction of an overexpression vector for *ccpA* was made to study how the protein works and to explore it as a possible target for drug design. Lastly, Research about *Listeria monocytogenes*' pathogenic mechanisms, revealed that *L. monocytogenes* infects via the transmission pathway.

Overall, Listeriosis' global health impact, with it specifically being risky for vulnerable groups, highlights the importance of food safety and proper therapies.

References

