Scientific Research Publishing

Advances in Infectious Diseases, 2025, 15(3), 578-593
https://www.scirp.org/journal/aid
ISSN Online: 2164-2656
ISSN Print: 2164-2648

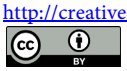
Gene Expression Profiling of *Corynebacterium pseudotuberculosis* under Host Cell Stress in Caseous Lymphadenitis

Flávia Martins Silva[1*], Suzana Telles da Cunha Lima[2], Roberto José Meyer Nascimento[1]

[1]Institute of Health Sciences, Laboratory of Immunology and Molecular Biology, Federal University of Bahia, Salvador, Brazil
[2]Institute of Biology of UFBA, Bioprospecting and Biotechnology Laboratory, Federal University of Bahia, Salvador, Brazil
Email: *fafanutribio@gmail.com, stcunhalima@ufba.br, rmeyer@ufba.br

How to cite this paper: Silva, F.M., da Cunha Lima, S.T. and Meyer Nascimento, R.J. (2025) Gene Expression Profiling of *Corynebacterium pseudotuberculosis* under Host Cell Stress in Caseous Lymphadenitis. *Advances in Infectious Diseases*, **15**, 578-593.
https://doi.org/10.4236/aid.2025.153044

Received: July 31, 2025
Accepted: September 21, 2025
Published: September 24, 2025



Abstract

Corynebacterium pseudotuberculosis is a bacterium that causes Caseous Lymphadenitis (CLA) in goats and sheep. It is a chronic infectious disease that affects meat, milk, and wool production in several countries. CLA has no effective treatment, highlighting the vaccination schedule as the best control strategy. Uncovering the antigenic properties of proteins may provide valuable insights into pathogenicity, as well as factors associated with the antimicrobial resistance of *C. pseudotuberculosis*. These proteins are displayed on the cell surface, and in bacteria, their exposure is continuous, intensifying markedly under stress. Heat Shock Proteins (HSPs) have been shown to play an important role in increasing microbial resistance under stress conditions during infection. In addition, they are immunologically important and recognized by the host, therefore capable of inducing a strong cellular and humoral immune response in mammals. HSPs are highly immunogenic dominant bacterial antigens identified during the infection cycle and can be used as vaccine components or, due to their properties, as carriers of other antigens. This review reveals a profile of potential transcriptional genes involved in the resistance and adaptability of *C. pseudotuberculosis* to adverse conditions imposed by the host's immune system. Strains of *C. pseudotuberculosis* can alter gene expression as a form of adaptation to cellular stress conditions, possibly encoding proteins associated with bacterial survival in hostile environments. Understanding these genes may provide insights into the pathogenicity and virulence of this bacterium and support their use as targets for treatment and vaccine development in future studies.

Keywords

Caseous Lymphadenitis, *C. pseudotuberculosis*, Heat Shock Protein,

Resistance, Stress

1. Introduction

The goat livestock production chain involves more than one million rural establishments in the Brazilian Northeast, having a relevant socioeconomic function, whether directly to feed families or through the generation of income. Economic activity of strategic importance in this region has been directed to the production of leather, meat, milk, and their derivatives [1] [2]. Due to this upward economic movement, the occurrence of diseases in these animals deserves further studies targeted at enhancing productivity and profitability through effective disease control and prevention [3].

Despite all research on the pathogenesis of caseous lymphadenitis caused by *C. pseudotuberculosis*, there is still inadequate information on the molecular mechanisms of bacterial pathogenesis and immunological prophylaxis against the infection. Identifying novel antigens may facilitate vaccine development and the formulation of innovative diagnostic tools for the detection of *C. pseudotuberculosis* in ovine and caprine samples [4].

The discovery of the antigenic properties of proteins can help in understanding the pathogenicity, as well as factors associated with C. *pseudotuberculosis* resistance to antimicrobials. Studies on Heat Shock Proteins (HSPs), or chaperones, have demonstrated the important role of these molecules in increasing microbial resistance under stress conditions during infection. HSPs are considered immunologically essential because they are recognized by the host and are therefore capable of inducing strong cellular and humoral immune responses in mammals [5]. Studies have shown that these HSPs can be used as effective strategies to induce an immune response and as antigenic molecules for the development of vaccines against diseases [6]-[8].

At the intracellular phase in the host, *C. pseudotuberculosis* faces several types of stresses inside the phagolysosome, characterized by the presence of low pH, high oxidative potential, thermal shock, nitrosative, osmotic, and other conditions. However, resistance to different environmental stresses is associated with the ability of *C. pseudotuberculosis* to survive in the host [7] [9].

Therefore, the bacteria adapt to different stress conditions imposed by the host during infection. This review examines the expression of potential transcriptional genes in *C. pseudotuberculosis* that may shed light on the mechanisms underlying its virulence, resistance, and pathogenicity.

2. Methods

In the current review, findings were extracted from PubMed, NCBI, Scopus, Web of Science, and Google Scholar databases from early January 2012 to late May 2023 (**Figure 1**), searching for keywords including "caseous lymphadenitis", "stress",

"genes", "*C. pseudotuberculosis*", "resistance" and HSPs. These articles were subsequently filtered to ensure relevance to the scope of this review.

The articles were initially selected based on their title and abstract. The inclusion and exclusion criteria were then applied, and finally, the selected articles were read in full, as shown in Figure 1. The inclusion criteria were full texts, studies written in English, and addressing *Corynebacterium pseudotuberculosis*, tolerance, adaptability, and resistance factors. Studies unrelated to caseous lymphadenitis were excluded. After reading the selected articles, relevant information that met the objective of the literature review was extracted.

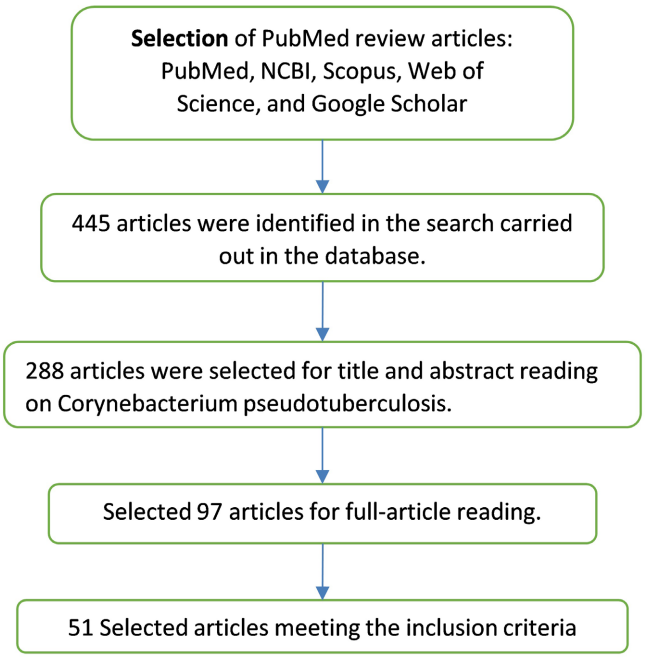


Figure 1. Activity flowchart.

3. Microbiological characteristics of *Corynebacterium pseudotuberculosis*

Corynebacterium pseudotuberculosis is a Gram-positive bacterium that is part of a general group of the Actinobacteria class that includes the genus *Mycobacterium*, *Nocardia*, and *Rhodococcus*. It is the etiologic agent responsible for cases of Caseous Lymphadenitis (CL) affecting small ruminants (sheep and goats) all over the world. *C. pseudotuberculosis* is an intracellular facultative anaerobic pathogen that exhibits pleomorphic forms, such as coccoid and filamentous rods, characterized as a Gram-positive coccobacillus. These microorganisms are characterized by a layer of mycolic acids, known as the mycomembrane, which overlies a complex cell wall with a high DNA content and confers significant resistance to environmental stress. In addition, cell membrane components are crucial for the interaction of these bacteria with host cells associated with the synthesis and interaction of mycolic acid as components of the immune system of the genus *Corynebacterium* [10] [11].

As biochemical characteristics, catalase and urease ferment glucose and ribose without gas production, and about 80% of the isolates ferment maltose, but not lactose, mannose, or sucrose. They do not produce spores and are immobile, possess *pili*, and are capable of producing biofilm [12].

Strategically, this microorganism has a cell wall organization, characterized mainly by the presence of an immense complex polymer composed of peptidoglycan, arabinogalactan, and mycolic acids. This is related to the potential to survive for several weeks in the environment, which contributes to the ability to spread within a herd. The genomes of several species of this group have already been completely sequenced, especially due to the considerable medical, veterinary, and biotechnological importance of these organisms. Transmission between sheep or goats occurs mainly through contamination of superficial wounds, which can appear during common procedures, such as shearing or castration, or through injuries to the animal's body caused by other traumatic events [13] [14].

3.1. Virulence Determinants

C. pseudotuberculosis possesses virulence mechanisms that are fundamental for adhesion, invasion, colonization, dissemination, and evasion of the host immune system. The ability to survive and adapt through various strategies to its environment is a hallmark of this microorganism. Once successfully established within a host, it is able to replicate within phagocytic cells, invading the immune system with ease. As a result, chronic infections can last for a long time in the animal [12].

There are two known virulence factors of *C. pseudotuberculosis*: phospholipase D and mycolic acids. Phospholipase D (PLD) is a potent exotoxin produced by *C. pseudotuberculosis* and was considered the main virulence factor for these bacteria. Phospholipases are glycophospholipids that play an important role in signal transduction and inflammatory response in eukaryotic cells. Mycolic acids are components of the waxy cell wall, synthesized by the sequential action of three enzymatic units: fatty acid synthase I (FAS-I), fatty acid synthase II (FAS-II), and polyketide synthase (Pks13). These are α-alkyl of very long chain, β-hydroxy fatty acids. All are potential factors contributing to the viability and initial virulence of the infection in the host [12]-[15].

The complete genome sequence of *C. pseudotuberculosis* isolates allowed the identification of SpaC and NanH as genes that encode proteins considered potential virulence factors. SpaC is a pilus-like protein that can likely make initial contact with host cell receptors to allow ligand-receptor interactions and facilitate virulence and intracellular invasion. NanH is an extracellular neuraminidase (sialidase) belonging to a class of glycosyl hydrolases that catalyze the removal of terminal sialic acid residues from a variety of glycoconjugates and may contribute to the recognition of sialic acids exposed on the cell surface [16] [17].

The *SodC* protein (superoxide dismutase), encoded in the *C. pseudotuberculosis* genome, is located extracellularly. This zinc-dependent enzyme probably protects the outer surface of the bacterial cell against superoxide generated externally by

mammalian host cells. In *Mycobacterium tuberculosis*, *SodC* contributes to the resistance of this microorganism against oxidative products generated by activated macrophages. The protective activity of Cu, Zn-SODs has been linked to virulence in other bacteria such as Neisseria meningitides and *Hemophylus ducreyi* [17] [18].

As an important part of cellular signaling mechanisms, protein kinases/serine and threonine present in bacteria are molecules that are part of complex signaling pathways and play diverse physiological roles in the processes of development, metabolism, cell division, cell wall synthesis, central metabolism, and virulence [17].

3.2. Biofilm Production

Biofilm microbial communities demonstrate coordinated behavior with the formation of complex three-dimensional structures and functionally heterogeneous bacterial communities. This phenotypic heterogeneity, or localized specialization in biofilms, demonstrates populations of bacteria that exhibit differences in surface molecule expression, antibiotic resistance, nutrient utilization, and virulence factors [16] [17].

Thus, a biofilm is an organized aggregate of microorganisms within an extracellular polymeric matrix, composed partly of proteins, surrounded by some nutrients such as polysaccharides, important minerals, and essentially water (97%), which promotes the flow of nutrients [19] [20].

C. pseudotuberculosis has the ability to form biofilms that develop strategies to ensure its survival and adaptation to stressful environments. This leads to the formation of a cohesive and strong community of cells that have intercellular communication. This biofilm possibly forms an organized aggregate of microorganisms that live within a self-produced matrix of extracellular polymeric substances (EPS) attached to a biotic or abiotic surface. Biofilm formation can increase microbial resistance against radiation, temperature, pH, high salinity, and high pressure [21].

In extreme environments, bacteria regulate the expression of a series of biofilm-forming genes through QS (quorum sensing), signaling based on nucleotide messengers, to provide the microorganism with the ability to become resistant to these environments. Then, they adhere to the epithelial surface and begin to multiply, emitting chemical signals that facilitate communication with other bacterial cells. This leads to the formation of cellular aggregates with low motility that progressively increase in thickness, eventually forming layers exceeding 10 μm, at which point they reach maturity [22].

C. pseudotuberculosis Quorum Sensing (QS) is defined as a cell density-dependent bacterial intercellular communication involved in gene expression (e.g., virulence genes for exoenzymes, exopolysaccharides) and the consequent altered behavior of biofilm cells, including resistance to stress conditions [23].

It is important to emphasize that *C. pseudotuberculosis* forms biofilms that de-

velop strategies to ensure their survival and adaptation to stressful environments. However, this adaptability condition depends on chemicals produced in biofilm formation that are associated with cellular signaling through molecules that are incorporated into an extracellular matrix. This matrix increases resistance to antibiotics and host immune responses, making conventional therapeutic approaches less effective [24] [25].

Understanding the role of genetic factors in biofilm formation in *C. Pseudotuberculosis* is essential to understanding the challenges of pathogenesis, antibiotic resistance, and diagnostic evasion. Genetic determinants play a key role in orchestrating the formation of these biofilms, influencing virulence, resistance, and adaptability to the host environment [22] [25].

Despite the growing recognition of the importance of biofilms in *C. Pseudotuberculosis* and their genetic basis, there are still gaps in our understanding of the precise mechanisms driving their formation, their impact on disease progression, and the development of new therapeutic and diagnostic strategies. Therefore, a comprehensive exploration of the genetic factors shaping biofilms in *C. Pseudotuberculosis* is needed, providing insights that can support the development of targeted interventions to combat caseous lymphadenitis more effectively in Brazil [18].

4. Stress Resistance Mechanism

Gene processes and molecules are involved in resistance to stressful conditions. These include stress proteins and transcription factors, efflux pumps, altered membrane composition, energy metabolism, chemical detoxification, and chaperone synthesis and accumulation. Microbial organization and modulation determine a successful and tolerated response to stress, involving one or a few genes, with new genomic and biological tools aiding the understanding of resistance mechanisms [23].

In *C.pseudotuberculosis*, the modulation of gene expression at the transcriptional level is carried out by a class of proteins that constitute dissociable subunits of RNA polymerase, the sigma (σ) factors. The alternative σ factors of bacterial RNA polymerase provide a means of rapidly regulating gene expression in response to various changes in the extracellular environment [26].

Potential pathogenicity islands of *C. pseudotuberculosis*, a group of virulence genes, are essential for distinguishing pathogenic from nonpathogenic species. They are present in large numbers and contain species-identifying virulence elements. The formation of the virulence factors they contain may help increase the adaptability of strains to different host environments. This increased adaptability is demonstrated by the discovery of genes expressing proteins for iron uptake, production of fimbrial subunits, insertion elements, and secreted toxins, acquired through horizontal gene transfer, contributing to our understanding of how this species can resist environmental adversities [18] [27].

Genetic composition of pathogenicity islands can shed light on the lifestyle of

this bacterium, since they include virulence genes that mediate mechanisms of adhesion, invasion, colonization, proliferation in the host, and adaptation to the immune system. Furthermore, pathogenicity islands are characterized as unstable regions that can be affected by insertions and deletions, influencing bacterial adaptability to new environments and hosts. In *C. pseudotuberculosis*, σ factors are the main regulators of gene expression at the transcriptional level and, therefore, play an important role in the bacterial adaptive response to different environmental stimuli. These factors form a holoenzyme with the core RNA polymerase and direct it to specific promoters, resulting in the activation of selected genes. Most bacteria possess several different σ factors that allow them to maintain basal gene expression as well as modulation in response to specific environmental signals [18].

The PhoPR (transmembrane sensory protein histidine kinase) system regulates the expression of several genes involved in metabolic, virulence, and resistance processes in several intracellular bacterial pathogens. These are regulatory proteins that function in bacteria as sensory and adaptive factors in response to a wide range of environmental stimuli. However, two-component systems, such as PhoP/PhoQ (intracellular response regulatory protein), control the transcription of key virulence genes essential for survival in host cells in several intracellular bacterial pathogens, including *C. pseudotuberculosis sp.*, *Salmonella sp.*, *Shigella sp.*, *Yersinia sp.*, and *Mycobacterium tuberculosis*. This makes it particularly remarkable that many microorganisms can tolerate significant fluctuations in the conditions necessary for their survival and growth [10] [27].

Environmental change often requires the expression of new genes, activated by exposure to these conditions, a phenomenon called the stress response. These findings have significant applications across diverse areas, including infection biology, food microbiology, and biotechnology. Understanding how microorganisms respond to stresses caused by the immune system, antibiotics, antifungals, and disinfectants could lead to a better understanding of infectious diseases, including the identification of targets for future drug and vaccine development [28].

5. HSPs

HSPs (Heat Shock Proteins), also known as chaperones, are highly conserved proteins. Prokaryotic and eukaryotic cells synthesize heat shock proteins constitutively as a result of stressful conditions, including adverse environmental circumstances (radiation, acidity, osmotic changes, temperature changes). Studies show that HSPs have the capacity for intracellular interaction with many proteins, playing a role in folding and translocation, repair, and formation of complexes [29].

Especially in bacteria, surface expression of HSPs and proteases is presumably essential to overcome changes involving protein denaturation. They are controlled by a specific sigma factor (rpoH gene), which provides a means of rapidly regulating HSP gene expression in response to various changes in the bacterial extracellular environment [30] [31].

Bacterial HSPs are both protective and exert pathogenic activities in mammals depending on the infection. The most studied bacterial HSPs are HtpG, GroEL and HSP60, HSP70 (DnaK), HSP25, HSP90, Hsp65, Hsp10, HspR, Cpl-B and DnaJ. They exhibit varying molecular weights and have been shown to be highly immunogenic, capable of inducing antibody production, inflammatory responses, and activation of macrophages and T cells [7] [8].

GroEL chaperone (HSP60) is essential for preserving cell growth under many adverse environmental conditions. It assists in several cellular processes, including DNA replication, mutagenesis on UV exposure, RNA transcription, and translocating proteins across membrane barriers and secretion. Studies with HSP60 analyzed the synthesis of specific serum antibodies in the host, demonstrating that bacterial HSP can be a biomarker to monitor immune status. Thus, the dual role of HSP60 as an immune modulator and a biomarker provides an opportunity to modulate immunity for therapeutic purposes and monitor the immune response in health and disease [32] [33].

Assessing differential gene expressions of HSPs is useful for understanding the mechanisms of adaptation during host infection. Different transcripts may be expressed among strains C. *pseudotuberculosis* with diverse characteristics during infection. Certainly, it plays an important role in the adaptations and modifications required for the development of a successful infection by this pathogen. Factors that regulate these genes respond to specific environmental or cellular signals to stimulate or inhibit transcription or translation, modifying the rate of synthesis of gene products, which is important for the physiological and biochemical adaptation required [20].

6. Abiotic Stress

The immune response established for this bacterium is complex and involves both innate and adaptive immune response mechanisms. Macrophages and neutrophils stand out at the beginning of the infection and establish a protective response to primary and secondary infection. During the infection process, *C. Pseudotu- berculosis* alters its gene expression to resist different types of stress and avoid the host's immune system. The bacteria are phagocytosed by macrophages, which are drained into local lymph nodes. Experiments with lymph node drainage have shown that a large layer of macrophages and lymphocytes is formed around the necrotic tissue. Once internalized, bacteria avoid the cellular immune response mechanisms and can survive and multiply rapidly inside the macrophage phagosome [34].

To comprehend the functional role of macrophage response to infection by *C. pseudotuberculosis*, it is important to emphasize that in the course of maturation, phagosomes acquire an arsenal of antimicrobial characteristics. In phagosome acidification, there is an increase in the synthesis of ATPases that acidify the phagosome lumen, creating a hostile environment that prevents microbial growth, not only directly impairing bacterial metabolism but also favoring the H+ gradient gen-

erated by this ATPase. This gradient is used to destroy essential nutrients present in the phagosomal lumen and can be used by this microorganism [30]. ATPase also facilitates the generation of superoxide (O_2), generating reactive oxygen species. However, bacterial defense mechanisms include modification of their surface to resist or break down antimicrobial peptides, and expression of enzymes such as catalase. This enzyme converts reactive species into less harmful compounds or prevents recruitment of protein complexes that synthesize Reactive Nitrogen Species (RNS) or Reactive Oxygen Species (ROS) [35].

Factors that contribute to the success of the bacterium are the ability to resist potentially bactericidal host defenses and elimination by an activated immune system. This host-imposed resistance to abiotic stresses is in part due to the low permeability of the bacterial cell envelope to many toxic molecules. Furthermore, it depends on detoxifying reactive oxygen and nitrogen molecules produced by the host, repairing the damage these molecules cause, and maintaining a neutral intrabacterial pH in acidic environments [36] [37].

6.1. Oxidative Stress

Activated macrophages express two enzymes, phagocyte oxidase (NOX2/gp91 phox) and inducible Nitric Oxide Synthase (iNOS), which generate Reactive Oxygen İntermediates (ROI) and Reactive Nitrogen İntermediates (RNI), respectively. After phagocytosis, the preformed NOX2 subunits assemble into an active enzyme complex that transfers electrons across the membrane from cytosolic NADPH to molecular oxygen. This produces superoxide anions (O_2), which dismutase into hydrogen peroxide (H_2O_2) and therefore generate toxic hydroxyl radicals. iNOS is induced by interferon (IFNγ) and produces nitrite and nitrate via nitric oxide. Under acidic conditions, as in the phagosomes of IFNγ-activated macrophages, nitrite forms nitrous acid, which dismutes into Nitric oxide (NO) and another toxic radical, nitrogen dioxide [8] [19].

Superoxide Dismutase (SOD) is a key enzyme for resistance to oxidative stress. Within granulomas, *C. pseudotuberculosis* is subjected to prolonged oxidative stress due to persistent activation of macrophages and other immune cells. The oxidative factor of the immune cell seriously compromises the survival and pathogenicity of bacterial pathogens. The presence of SODC activates the resistance of *C. Pseudotuberculosis* to oxidative stress and helps in the understanding of its survival strategies within the host, but may also provide a theoretical basis for the development of new treatment strategies, thus more effectively controlling infections caused by this pathogen [16].

6.2. Acid Stress

High concentrations of protons, which define acidic environments, present a particular challenge for unicellular organisms, since the protonation of biological molecules can affect their charge, structure, and function, which has potentially harmful consequences for the cell. Therefore, bacterial cells generally have home-

ostatic and protective mechanisms to counteract the inhibitory effects of low pH [9] [23].

Changes in the cell membrane help to sustain cellular activities under acidic conditions. Membrane-bound H^+—ATPase regulates the pH of cells by pumping protons out of the cytoplasm. Therefore, control of H^+—ATPase levels and its activity results in greater acid tolerance, providing a constant intracellular envi- ronment for cell growth and metabolism [24] [36] [38].

Studies of *C. pseudotuberculosis* (Cp1002) under acid stress conditions have demonstrated the induction of genes involved in cell adhesion and oxidation-reduction processes. The genes induced during these biological processes encode secreted proteins containing LPxTG motifs, which are highly virulent and may represent important vaccine candidates against caseous lymphadenitis [23] [25].

6.3. Osmotic Stress

Strategies employed to overcome salt stress are driven by the molecular mechanisms of osmoadaptation, which describe the physiological and genetic manifestations of adaptation. Studies using the Solid 3 Plus platform demonstrated that *C. pseudotuberculosis* strains 258 and 1002 were significantly reduced during stress induction. This platform allows massively parallel sequencing of clonally amplified DNA/RNA fragments bound to beads. It is a methodology based on sequential ligation with fluorophore-labeled oligonucleotides and can be used for sequencing whole genomes, exomes, and transcriptomes. Through transcriptome analysis, it was indicated that gene regulation involves Transcription Factors (TFs), which are regulatory proteins that activate or repress the expression of their Target Genes (TGs). These TFs belong to the TetR family, which generally increases the expression of genes involved in drug resistance, antibiotic biosynthesis, pathogenicity, virulence, quorum sensing, and catabolic pathways. Knowledge about Transcription Factors (TFs) in *C. pseudotuberculosis* is still scarce [10] [39].

The srtA gene was differentially expressed in *C. pseudotuberculosis* 258 under acid, osmotic, and thermal stress. This srtA gene encodes a group of enzymes called sortase, present in gram-positive bacteria and involved in the ability to cause various diseases. Sortase A is a surface protein that is bound to the cell wall, allowing for greater bacterial adhesion. These genes were experimental candidates in assays with *C. pseudotuberculosis* for osmotic, acid, and thermal stresses [16] [40].

6.4. Temperature Stress

Under heat shock stress, hspR, dnaK, and grpE genes were differentially expressed in microorganisms such as *Salmonella* spp., *Escherichia coli*, *Yersinia_enterocolitica*, and/or *Listeria monocytogenes*. This research detailed the behavior of these microorganisms under the influence of temperature and duration of thermal shock. It showed that maximum thermotolerance was obtained after heating for 20 min at 48˚C [27] [41] [42].

The hspR gene, which encodes a TF, belongs to the TetR family that, in general,

regulates the expression of genes involved in drug resistance, antibiotic biosynthesis, pathogenicity, virulence, quorum detection, and catabolic pathways, and is known to regulate the response to heat shock. In addition, it regulates genes that maintain the structure of proteins in various cells. Likewise, with *C. pseudotuberculosis* strains 1002B and 258, experiments show that they can regulate four genes: dnaK, grpE, clpB, and clgR. These genes are involved in bacterial virulence, thermal and osmotic conditions, participating in the stress resistance system, in addition to regulating the expression of repair genes and proteins involved in cellular integrity [10] [16] [42].

7. Conclusions

Research shows that *C. pseudotuberculosis* can survive inside macrophages and resist aggressive host conditions, establishing infection and inducing typical clinical symptoms. One of the characteristics of resistance is the lipid cover recognized as an electron-dense layer that is found on the outside of the cell wall which plays an important role, suggesting that it provides a protective barrier against the antibacterial mechanisms of the host. This morphology helps to establish a regulatory mechanism that allows adaptation to environmental stimuli [43].

By surviving inside macrophages for more than 48 hours, the bacteria are released as a result of a process that leads to death by phagocytes, although this property varies between different strains. These effects may be associated with the outer lipid layer in the *C. pseudotuberculosis* cell wall and other antigenic components that attenuate the production of nitric oxide by macrophages. However, bacterial growth becomes uncontrolled within the host cell, which tries to restrict and limit the infection through the formation of pyogranulomas, which are characterized by the encapsulation of cells infected by *C. pseudotuberculosis* [44] [45].

Changes in environmental conditions affect the pathogenic properties of microorganisms that lead to the synthesis of HSPs (heat shock), which biochemically change their molecular structure [45]. Especially in bacteria, the expression of HSPs and proteases is essential to overcome the changes that involve the denaturation of proteins. They are controlled by a specific sigma factor, which provides a means of rapidly regulating the gene expression of HSPs in response to various changes in the bacterial extracellular environment. However, it is still not clear what these proteins would be in *C. pseudotuberculosis* [34] [46].

Functional genomics approaches focused on pathogen interactions with macrophages further enhance our understanding of pathogenicity. *Corynebacterium pseudotuberculosis* is able to survive and grow in animal macrophages in order to spread within the host, similarly to *C. diphtheriae*, which is able to survive inside human macrophages, ensuring growth and survival [47]. Since proteins exposed and secreted on the cell surface play an essential role in the host-pathogen interaction, these proteins represent a potential target in the formulation of drugs, biomarkers, and vaccines [48] [49].

An immune response was observed after administration of the *C. Pseudotu-*

berculosis DNA-hsp60 vaccine in mice, utilizing genes encoding HSPs to induce immunity. The results indicated that the DNA-hsp60 vaccine generated IgG1 and IgG2a responses when administered to BALB/c mice, but with a tendency toward a Th1-type immune response after 30 days of the first immunization. The protective efficacy of DNA-hsp65 vaccines has also been extensively studied for *Mycobacterium tuberculosis*. Another example was the *M. leprae* DNA-hsp65 vaccine, which induced protective immunity against tuberculosis challenge in a murine model. Similarly, the M. avium DNA-hsp65 vaccine induced a strong protective immune response in lambs and protected against infection with tuberculosis of the *M. avium* subspecies [11] [19] [50].

C. pseudotuberculosis strains can alter gene expression as a form of adaptation under stress conditions. These genes possibly encode proteins that are related to the survival of the bacteria under adverse conditions [35] [51]. The expression of potential genes (Hsp65, Hsp10, HspR, Dna-J, Cpl-B) may support the tolerance of *C. pseudotuberculosis* to the hostile conditions encountered during infection. These genes may be related to the regulation of metabolic functions, growth, virulence, and immunomodulation. Thus, they may contribute to the understanding of new immunization strategies and drugs for controlling caseous lymphadenitis [8].

The use of the molecular biology tool can help with the identification of these genes associated with the propagation and persistence of *C. pseudotuberculosis* during the infection process. It may also suggest possible paths for studies related to the development of vaccines, diagnoses, and therapies that can help to minimize the damage of the disease.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] IBGE (2023) PPM 2023. Produção da pecuária municipal 2023. Agência IBGE Notícias.

[2] Nascimento, M.I.d.S.S., Ferreira, F.F.d.S., Albuquerque, H.J.O., Nascimento, T.L.F., Albuquerque, H.O., Cabral, A.M.D., *et al.* (2022) Insights dos principais produtos oriundos da caprinovinocultura no Nordeste brasileiro. *Research, Society and Development*, **11**, e41811528264. https://doi.org/10.33448/rsd-v11i5.28264

[3] Farias, A.M., Alves, J.R.A., Alves, F.S.F., Pinheiro, R.R., Faccioli-Martins, P.Y., Lima, A.M.C., *et al.* (2018) Soroprevalência da infecção por Corynebacterium pseudotuberculosis em caprinos no Nordeste brasileiro utilizando técnica de imunoabsorção enzimática (ELISA-indireto). *Pesquisa Veterinária Brasileira*, **38**, 1344-1350. https://doi.org/10.1590/1678-5150-pvb-5282

[4] Guglielmini, J., Hennart, M., Badell, E., Toubiana, J., Criscuolo, A. and Brisse, S. (2021) Genomic Epidemiology and Strain Taxonomy of *Corynebacterium diphtheriae*. *Journal of Clinical Microbiology*, **59**, e01581-21. https://doi.org/10.1128/jcm.01581-21

[5] Shan, Q., Ma, F., Wei, J., Li, H., Ma, H. and Sun, P. (2020) Physiological Functions of Heat Shock Proteins. *Current Protein & Peptide Science*, **21**, 751-760.

https://doi.org/10.2174/1389203720666191111113726

[6] Pisu, D., Huang, L., Narang, V., Theriault, M., Lê-Bury, G., Lee, B., *et al.* (2021) Single Cell Analysis of *M. tuberculosis* Phenotype and Macrophage Lineages in the Infected Lung. *Journal of Experimental Medicine*, **218**, e20210615. https://doi.org/10.1084/jem.20210615

[7] Bolhassani, A. and Agi, E. (2019) Heat Shock Proteins in Infection. *Clinica Chimica Acta*, **498**, 90-100. https://doi.org/10.1016/j.cca.2019.08.015

[8] Harnagel, A., Lopez Quezada, L., Park, S.W., Baranowski, C., Kieser, K., Jiang, X., *et al.* (2020) Nonredundant Functions of *Mycobacterium tuberculosis* Chaperones Promote Survival under Stress. *Molecular Microbiology*, **115**, 272-289. https://doi.org/10.1111/mmi.14615

[9] Guan, N. and Liu, L. (2019) Microbial Response to Acid Stress: Mechanisms and Applications. *Applied Microbiology and Biotechnology*, **104**, 51-65. https://doi.org/10.1007/s00253-019-10226-1

[10] Parise, D., Teixeira Dornelles Parise, M., Pinto Gomide, A.C., Figueira Aburjaile, F., Bentes Kato, R., Salgado-Albarrán, M., *et al.* (2021) The Transcriptional Regulatory Network of *Corynebacterium pseudotuberculosis*. *Microorganisms*, **9**, Article No. 415. https://doi.org/10.3390/microorganisms9020415

[11] Sá, M.C.A.d., Silva, W.M.d., Rodrigues, C.C.S., Rezende, C.P., Marchioro, S.B., Rocha Filho, J.T.R., *et al.* (2021) Comparative Proteomic Analyses between Biofilm-Forming and Non-Biofilm-Forming Strains of *Corynebacterium pseudotuberculosis* Isolated from Goats. *Frontiers in Veterinary Science*, **8**, Article ID: 614011. https://doi.org/10.3389/fvets.2021.614011

[12] Gradowski, M., Baranowski, B. and Pawłowski, K. (2020) The Expanding World of Protein Kinase-Like Families in Bacteria: Forty Families and Counting. *Biochemical Society Transactions*, **48**, 1337-1352. https://doi.org/10.1042/bst20190712

[13] Nabih, A.M., Hussein, H.A., El-Wakeel, S.A., Abd El-Razik, K.A. and Gomaa, A.M. (2018) *Corynebacterium pseudotuberculosis* Mastitis in Egyptian Dairy Goats. *Veterinary World*, **11**, 1574-1580. https://doi.org/10.14202/vetworld.2018.1574-1580

[14] Burkovski, A. (2018) The Role of Corynomycolic Acids in Corynebacterium-Host Interaction. *Antonie van Leeuwenhoek*, **111**, 717-725. https://doi.org/10.1007/s10482-018-1036-6

[15] de Oliveira Zamprogna, T., Ribeiro, D., Azevedo, V.A.C., Lara, G.H.B., Motta, R.G., da Silva, R.C., *et al.* (2020) Bacteriological, Cytological, and Molecular Investigation of *Corynebacterium pseudotuberculosis*, Mycobacteria, and Other Bacteria in Caseous Lymphadenitis and Healthy Lymph Nodes of Slaughtered Sheep. *Brazilian Journal of Microbiology*, **52**, 431-438. https://doi.org/10.1007/s42770-020-00403-0

[16] Ibraim, I.C., Parise, M.T.D., Parise, D., Sfeir, M.Z.T., de Paula Castro, T.L., Wattam, A.R., *et al.* (2019) Transcriptome Profile of *Corynebacterium pseudotuberculosis* in Response to Iron Limitation. *BMC Genomics*, **20**, Article No. 663. https://doi.org/10.1186/s12864-019-6018-1

[17] Furukawa, Y., Shintani, A. and Kokubo, T. (2021) A Dual Role of Cysteine Residues in the Maturation of Prokaryotic Cu/Zn-Superoxide Dismutase. *Metallomics*, **13**, mfab050. https://doi.org/10.1093/mtomcs/mfab050

[18] Thais de Oliveira Silva, M., Barros de Pinho, R., da Rocha Fonseca, B., Silvestre Brilhante Bezerra, F., Severo Sabedra Sousa, F., Kommling Seixas, F., *et al.* (2020) NanH and PknG Putative Virulence Factors as a Recombinant Subunit Immunogen against *Corynebacterium pseudotuberculosis* Infection in Mice. *Vaccine*, **38**, 8099-

8106. https://doi.org/10.1016/j.vaccine.2020.11.010

[19] Birhanu, A.G., Gómez-Muñoz, M., Kalayou, S., Riaz, T., Lutter, T., Yimer, S.A., *et al.* (2022) Proteome Profiling of *Mycobacterium tuberculosis* Cells Exposed to Nitrosative Stress. *ACS Omega*, **7**, 3470-3482. https://doi.org/10.1021/acsomega.1c05923

[20] da Conceição Aquino de Sá, M., Filho, J.T.R.R., Alcantara, M.E., da Costa Silva, M., dos Santos, M.M., dos Santos, A.S., *et al.* (2022) Analysis of Gtpases Rab 5 and Rab 7 Expression from Macrophages Infected with Biofilm-Producing and Non-Biofilm-Producing Strains of *Corynebacterium pseudotuberculosis*. *Brazilian Journal of Microbiology*, **53**, 447-453. https://doi.org/10.1007/s42770-021-00665-2

[21] Muhammad, M.H., Idris, A.L., Fan, X., Guo, Y., Yu, Y., Jin, X., *et al.* (2020) Beyond Risk: Bacterial Biofilms and Their Regulating Approaches. *Frontiers in Microbiology*, **11**, Article No. 928. https://doi.org/10.3389/fmicb.2020.00928

[22] Devaraj, A., Buzzo, J.R., Mashburn-Warren, L., Gloag, E.S., Novotny, L.A., Stoodley, P., *et al.* (2019) The Extracellular DNA Lattice of Bacterial Biofilms Is Structurally Related to Holliday Junction Recombination Intermediates. *Proceedings of the National Academy of Sciences*, **116**, 25068-25077. https://doi.org/10.1073/pnas.1909017116

[23] Lund, P.A., De Biase, D., Liran, O., Scheler, O., Mira, N.P., Cetecioglu, Z., *et al.* (2020) Understanding How Microorganisms Respond to Acid Ph Is Central to Their Control and Successful Exploitation. *Frontiers in Microbiology*, **11**, Article ID: 556140. https://doi.org/10.3389/fmicb.2020.556140

[24] Yin, W., Wang, Y., Liu, L. and He, J. (2019) Biofilms: The Microbial "Protective Clothing" in Extreme Environments. *International Journal of Molecular Sciences*, **20**, Article No. 3423. https://doi.org/10.3390/ijms20143423

[25] Pakharukova, N., Tuittila, M., Paavilainen, S., Malmi, H., Parilova, O., Teneberg, S., *et al.* (2018) Structural Basis for *Acinetobacter baumannii* Biofilm Formation. *Proceedings of the National Academy of Sciences*, **115**, 5558-5563. https://doi.org/10.1073/pnas.1800961115

[26] Marques da Silva, W., Seyffert, N., Silva, A. and Azevedo, V. (2021) A Journey through the *Corynebacterium pseudotuberculosis* Proteome Promotes Insights into Its Functional Genome. *PeerJ*, **9**, e12456. https://doi.org/10.7717/peerj.12456

[27] Parise, M.T.D., Parise, D., Kato, R.B., Pauling, J.K., Tauch, A., Azevedo, V.A.d.C., *et al.* (2020) CoryneRegNet 7, the Reference Database and Analysis Platform for Corynebacterial Gene Regulatory Networks. *Scientific Data*, **7**, Article No. 142. https://doi.org/10.1038/s41597-020-0484-9

[28] Wang, J., Ge, P., Lei, Z., Lu, Z., Qiang, L., Chai, Q., *et al.* (2021) *Mycobacterium tuberculosis* Protein Kinase G Acts as an Unusual Ubiquitinating Enzyme to Impair Host Immunity. *EMBO Reports*, **22**, e52175. https://doi.org/10.15252/embr.202052175

[29] Singh, P., Khurana, H., Yadav, S.P., Dhiman, K., Singh, P., Ashish, *et al.* (2020) Biochemical Characterization of Clpb Protein from *Mycobacterium tuberculosis* and Identification of Its Small-Molecule Inhibitors. *International Journal of Biological Macromolecules*, **165**, 375-387. https://doi.org/10.1016/j.ijbiomac.2020.09.131

[30] Droppa-Almeida, D., Franceschi, E. and Padilha, F.F. (2018) Immune-Informatic Analysis and Design of Peptide Vaccine from Multi-Epitopes against *Corynebacterium pseudotuberculosis*. *Bioinformatics and Biology Insights*, **12**, 1-9.

[31] Guerra, P.V., Andrade, C.M., Nunes, I.V., Gama, B.C., Tibúrcio, R., Santos, W.L.C.,

et al. (2021) Oral Tolerance Induced by Heat Shock Protein 65-Producing *Lactococcus lactis* Mitigates Inflammation in *Leishmania braziliensis* Infection. *Frontiers in Immunology*, **12**, Article ID: 647987. https://doi.org/10.3389/fimmu.2021.647987

[32] Kim, W.S., Jung, I.D., Kim, J., Kim, H.M., Kwon, K.W., Park, Y., *et al.* (2018) *Mycobacterium tuberculosis* Grpe, a Heat-Shock Stress Responsive Chaperone, Promotes Th1-Biased T Cell Immune Response via TLR4-Mediated Activation of Dendritic Cells. *Frontiers in Cellular and Infection Microbiology*, **8**, Article No. 95. https://doi.org/10.3389/fcimb.2018.00095

[33] Malik, J.A. and Lone, R. (2021) Heat Shock Proteins with an Emphasis on HSP 60. *Molecular Biology Reports*, **48**, 6959-6969. https://doi.org/10.1007/s11033-021-06676-4

[34] Taha, E.A., Ono, K. and Eguchi, T. (2019) Roles of Extracellular Hsps as Biomarkers in Immune Surveillance and Immune Evasion. *International Journal of Molecular Sciences*, **20**, Article No. 4588. https://doi.org/10.3390/ijms20184588

[35] Shi, J., Wang, Z., Wu, B., Li, X., Li, X., Tian, S., *et al.* (2019) Cofilin-1, Peroxiredoxin-1, and Galectin-3: Major Proteins Released by Macrophages Infected with *Corynebacterium pseudotuberculosis*. *Veterinary Microbiology*, **239**, Article ID: 108461. https://doi.org/10.1016/j.vetmic.2019.108461

[36] Avican, K., Aldahdooh, J., Togninalli, M., Mahmud, A.K.M.F., Tang, J., Borgwardt, K.M., *et al.* (2021) RNA Atlas of Human Bacterial Pathogens Uncovers Stress Dynamics Linked to Infection. *Nature Communications*, **12**, Article No. 3282. https://doi.org/10.1038/s41467-021-23588-w

[37] Borer, B., Tecon, R. and Or, D. (2018) Spatial Organization of Bacterial Populations in Response to Oxygen and Carbon Counter-Gradients in Pore Networks. *Nature Communications*, **9**, Article No. 769. https://doi.org/10.1038/s41467-018-03187-y

[38] Arcari, T., Feger, M., Guerreiro, D.N., Wu, J. and O'Byrne, C.P. (2020) Comparative Review of the Responses of *Listeria monocytogenes* and *Escherichia coli* to Low pH Stress. *Genes*, **11**, Article No. 1330. https://doi.org/10.3390/genes11111330

[39] Gomide, A.C.P., de Sá, P.G., Cavalcante, A.L.Q., de Jesus Sousa, T., Gomes, L.G.R., Ramos, R.T.J., *et al.* (2018) Heat Shock Stress: Profile of Differential Expression in *Corynebacterium pseudotuberculosis* Biovar Equi. *Gene*, **645**, 124-130. https://doi.org/10.1016/j.gene.2017.12.015

[40] Gomide, A.C.P., Ibraim, I.C., Alves, J.T.C., de Sá, P.G., de Oliveira Silva, Y.R., Santana, M.P., *et al.* (2018) Transcriptome Analysis of *Corynebacterium pseudotuberculosis* Biovar Equi in Two Conditions of the Environmental Stress. *Gene*, **677**, 349-360. https://doi.org/10.1016/j.gene.2018.08.028

[41] Dorey, A.L., Lee, B., Rotter, B. and O'Byrne, C.P. (2019) Blue Light Sensing in *Listeria monocytogenes* Is Temperature-Dependent and the Transcriptional Response to It Is Predominantly SigB-Dependent. *Frontiers in Microbiology*, **10**, Article No. 2497. https://doi.org/10.3389/fmicb.2019.02497

[42] Eisenreich, W., Rudel, T., Heesemann, J. and Goebel, W. (2021) Persistence of Intracellular Bacterial Pathogens—With a Focus on the Metabolic Perspective. *Frontiers in Cellular and Infection Microbiology*, **10**, Article ID: 615450. https://doi.org/10.3389/fcimb.2020.615450

[43] Corrêa, J.I., Stocker, A., Trindade, S.C., Vale, V., Brito, T., Bastos, B., *et al.* (2018) *In Vivo* and *in Vitro* Expression of Five Genes Involved in *Corynebacterium pseudotuberculosis* Virulence. *AMB Express*, **8**, 1-9. https://doi.org/10.1186/s13568-018-0598-z

[44] Traven, A. and Naderer, T. (2019) Central Metabolic Interactions of Immune Cells

and Microbes: Prospects for Defeating Infections. *EMBO Reports*, **20**, e47995. https://doi.org/10.15252/embr.201947995

[45] Silva, M.T.d.O., de Pinho, R.B., Bezerra, F.S.B., Scholl, N.R., Moron, L.D., Alves, M.S.D., *et al.* (2021) *In Silico* Analyses and Design of a Chimeric Protein Containing Epitopes of SpaC, PknG, NanH, and SodC Proteins for the Control of Caseous Lymphadenitis. *Applied Microbiology and Biotechnology*, **105**, 8277-8286. https://doi.org/10.1007/s00253-021-11619-x

[46] Gul, S., Shah, K.N., Rana, R.M., Khan, M.A., El-Shehawi, A.M. and Elseehy, M.M. (2021) Phylogenetic and Expression Dynamics of Tomato ClpB/Hsp100 Gene under Heat Stress. *PLOS ONE*, **16**, e0255847. https://doi.org/10.1371/journal.pone.0255847

[47] Roh, J.S. and Sohn, D.H. (2018) Damage-Associated Molecular Patterns in Inflammatory Diseases. *Immune Network*, **18**, e27. https://doi.org/10.4110/in.2018.18.e27

[48] Khan, A., Singh, V.K., Hunter, R.L. and Jagannath, C. (2019) Macrophage Heterogeneity and Plasticity in Tuberculosis. *Journal of Leukocyte Biology*, **106**, 275-282. https://doi.org/10.1002/jlb.mr0318-095rr

[49] Tan, J., Yi, W., Wang, Z., Ye, C., Tian, S., Li, X., *et al.* (2021) TRIM21 Negatively Regulates *Corynebacterium pseudotuberculosis*-Induced Inflammation and Is Critical for the Survival of *C. pseudotuberculosis* Infected C57BL6 Mice. *Veterinary Microbiology*, **261**, Article ID: 109209. https://doi.org/10.1016/j.vetmic.2021.109209

[50] Brundage, C. and Burmayan, A. (2021) Caseous Lymphadenitis Outbreak in a Small Ruminant Herd. *Open Veterinary Journal*, **11**, 530-534. https://doi.org/10.5455/ovj.2021.v11.i4.2

[51] Magana, M., Sereti, C., Ioannidis, A., Mitchell, C.A., Ball, A.R., Magiorkinis, E., *et al.* (2018) Options and Limitations in Clinical Investigation of Bacterial Biofilms. *Clinical Microbiology Reviews*, **31**, e00084-16. https://doi.org/10.1128/cmr.00084-16