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Heat Tolerance and Molecular Verification of Putative Heat-Shock-Related Genes in *Lacticaseibacillus paracasei* subsp. *paracasei* BS2-PB5

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ABSTRACT

Lacticaseibacillus paracasei subsp. *paracasei* BS2-PB5 is a probiotic candidate isolated from human breast milk with potential applications in food products. The ability of probiotic bacteria to tolerate short-term exposure to elevated temperatures is an important characteristic because temperature fluctuations encountered during manufacturing, formulation, transportation, and storage may affect cell viability and functionality. This study evaluated the heat tolerance of BS2-PB5 and verified the identity of RT-PCR-amplified products generated using primers designed from putative heat-shock-related genes. BS2-PB5 cultures were incubated at 37°C, 42°C, 45°C, and 48°C for 3 h, and cell viability was determined by colony-forming unit (CFU) enumeration. Candidate heat-shock-related genes were identified from genome annotation, and sequences annotated as putative dnaJ and dnaK were selected for primer design and subsequent molecular verification.. Amplified products were visualized by agarose gel electrophoresis and subsequently analyzed by Sanger sequencing and BLASTn. BS2-PB5 remained viable at all tested temperatures, although growth decreased as temperature increased. RT-PCR amplification produced fragments of approximately 336 bp and 445 bp using the *dnaJ* and *dnaK* primer sets, respectively. However, sequence verification revealed that the amplified products did not correspond to the intended heat shock protein genes. The *dnaJ* amplicon showed high similarity to the GTP-binding protein LepA (*lepA*) gene, whereas the *dnaK* amplicon aligned primarily with chromosomal regions of *L. paracasei*. These findings highlight the importance of sequence verification in molecular studies and suggest that further investigation is required to identify genes associated with heat tolerance in BS2-PB5.

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Keywords: heat tolerance; RT-PCR; *Lacticaseibacillus*; LepA; sequence verification

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1. Introduction

Lacticaseibacillus paracasei is a Gram-positive lactic acid bacterium commonly isolated from fermented foods, the human gastrointestinal tract, and breast milk. Owing to its probiotic properties, *L. paracasei* has been widely studied for its ability to support intestinal health, modulate immune responses, inhibit pathogenic microorganisms, and contribute to the development of functional foods and dietary supplements [1], [2]. Several strains of *L. paracasei* have demonstrated the ability to survive gastrointestinal conditions and exert beneficial effects on the host, making this species an attractive candidate for industrial and probiotic applications [3]

Lacticaseibacillus paracasei is used in fermented food production and has been extensively studied as a probiotic species [4].The industrial utilization of probiotic bacteria requires tolerance to environmental stresses encountered during processing, storage, and consumption. Exposure to elevated temperatures may occur during certain processing, formulation, and storage conditions and can negatively affect bacterial viability through membrane damage, enzyme inactivation, protein denaturation, and disruption of cellular metabolism [3]. Therefore, the characterization of heat-tolerant probiotic strains is important for understanding their ability to remain viable under short-term exposure to elevated temperatures and for evaluating their technological robustness.

Bacteria employ several adaptive mechanisms to survive heat stress, including the synthesis of heat-shock-related proteins that function as molecular chaperones. These proteins assist in protein folding, prevent protein aggregation, and facilitate the recovery of damaged proteins under stressful conditions. Among the most extensively studied heat-shock-related genes are *dnaK* and *dnaJ*, which encode members of the HSP70 and HSP40 chaperone systems, respectively. These proteins play important roles in bacterial stress adaptation and cellular survival under adverse environmental conditions [5].

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Advances in whole-genome sequencing and genome annotation have facilitated the identification of genes potentially associated with stress adaptation in probiotic bacteria. Nevertheless, experimental validation remains necessary because amplification products generated through PCR-based approaches may not always correspond to the intended target sequences. Sequence verification through DNA sequencing and database comparison is therefore important to confirm target specificity and ensure the reliability of molecular analyses [6].

In a previous study conducted at the Department of Biology, Universitas Pelita Harapan (UPH), genes associated with heat-stress response were investigated in *Lactiplantibacillus plantarum* strain SU-KC1A [7]. However, information regarding heat tolerance and heat-stress-related genes in Indonesian isolates of *L. paracasei* remains limited. *Lacticaseibacillus paracasei* subsp. *paracasei* BS2-PB5, isolated from human breast milk, has been identified as a potential probiotic strain [8]. Therefore, this study aimed to evaluate the heat tolerance of BS2-PB5 under different temperature conditions and to verify the identity of RT-PCR products generated using primers designed from putative heat-shock-related genes. The findings provide preliminary information for future investigations of molecular mechanisms associated with heat tolerance in BS2-PB5.

2. Materials and Methods

2.1 Growth Medium

Trypticase Phytone Yeast (TPY) medium was used for cultivation of *Lacticaseibacillus paracasei* subsp. *paracasei* BS2-PB5 [9]. One litre of TPY broth consisted of 17.6 g Tryptic Soy Broth (TSB), 2.5 g yeast extract, 5 g glucose, 5 g peptone, 0.5 g L-cysteine, 2 g KH_2PO_4, and 1 mL Tween 80. For solid medium, 15 g/L bacteriological agar was added. Cultures were incubated under microaerophilic conditions using the candle jar method. Briefly, inoculated TPY broth and agar plates were placed in a sealed jar containing a lit candle. The jar remained sealed until the candle extinguished, thereby reducing oxygen concentration and establishing microaerophilic conditions.

2.2 Heat Tolerance Assay

A single colony of BS2-PB5 was inoculated into TPY broth and incubated for 24 h under microaerophilic conditions. Subsequently, 10% (v/v) of the overnight culture was transferred into fresh TPY broth and incubated at 37°C, 42°C, 45°C, or 48°C for up to 3 h, with 37°C serving as the control treatment. Cell viability was evaluated every hour by serial dilution and spread plating on TPY agar. A volume of 30 µL from appropriate dilutions was plated and incubated at 37°C for 48 h under microaerophilic conditions. Colony-forming units (CFU) were counted and expressed as CFU/mL using Eq. 1.

$$\textit{Bacterial Count}\left(\frac{CFU}{mL}\right) = \frac{\textit{Number of colonies x dilution factor}}{\textit{Volume plated}\ (mL)} \quad (1)$$

Each temperature treatment was conducted in duplicate. Viable cell counts are presented as mean ± standard deviation of two independent replicates.

2.3 Primer Design

The genome sequence of BS2-PB5 was previously obtained through whole-genome sequencing and annotated using the Rapid Annotation using Subsystem Technology (RAST) server [10]. Based on the RAST annotation, two sequences annotated as putative *dnaJ* and *dnaK* were selected as candidate targets for molecular verification. The corresponding nucleotide sequences were imported into Benchling software for primer design. Primer pairs were designed based on appropriate primer length, GC content, melting temperature, and predicted specificity toward the target regions. The synthesized primers were subsequently obtained from PT. Genetika Science (Indonesia). Primer sequences used in this study are shown in Table 1.

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Table 1 Primers used for RT-PCR amplification

Target gene	Primer sequence (5′–3′)
DnaK-F	CGATCCGCCAAACACCGGTTCA
DnaK-R	GGCGTTTGCTGCATGCTTTGCT
DnaJ-F	TGCTGGTCGTGGATGCTACCCA
DnaJ-R	GCACATCAAGCACAACGCCACG

2.4 RNA Extraction

Total RNA was extracted from harvested BS2-PB5 cells using the Patho Gene-spin™ DNA/RNA Extraction Kit (PathoGen, South Korea) according to the manufacturer's protocol. Briefly, bacterial cultures exposed to different temperature treatments were collected by centrifugation, and the resulting cell pellets were subjected to lysis, binding, washing, and elution steps. RNA was eluted in 30–60 µL of Elution Buffer and stored at −20°C until RT-PCR analysis.

2.5 Reverse Transcription Polymerase Chain Reaction (RT-PCR)

RT-PCR was performed using the QIAGEN OneStep RT-PCR Kit according to the manufacturer's protocol. Each reaction had a final volume of 50 µL containing 1× OneStep RT-PCR Buffer, 400 µM dNTPs, 0.6 µM of each primer, QIAGEN OneStep RT-PCR Enzyme Mix, template RNA, and RNase-free water. Amplification was carried out in a thermal cycler under the following conditions: reverse transcription at 50°C for 30 min; initial activation at 95°C for 15 min; followed by 25–40 cycles of denaturation at 94°C for 30–60 s, annealing at 50–68°C for 30–60 s, and extension at 72°C for 1 min; with a final extension at 72°C for 10 min. Amplified products were separated by electrophoresis on a 1% agarose gel and visualized under UV illumination using a 100 bp DNA ladder as a molecular size marker.

2.6 Sequence Verification and BLASTn Analysis

Representative RT-PCR products were submitted to PT. Genetika Science for Sanger sequencing. The resulting nucleotide sequences were analyzed using the Basic Local Alignment Search Tool for nucleotides (BLASTn) available through the National Center for Biotechnology Information (NCBI) database to determine sequence identity and verify whether the amplified products corresponded to the intended target genes.

3. Results and Discussion

3.1 Heat tolerance of BS2-PB5

Food products frequently undergo thermal processing to improve microbiological safety and extend shelf life. During these processes, probiotic bacteria may be exposed to elevated temperatures that can reduce cell viability and compromise their probiotic functionality. Therefore, the ability of probiotic bacteria to tolerate heat stress is an important characteristic in determining their suitability for industrial applications.

The heat tolerance of BS2-PB5 was evaluated by incubating cultures at 37°C, 42°C, 45°C, and 48°C for 3 h. Cell viability was determined by colony-forming units (CFU) enumeration at hourly intervals (**Fig. 1**).

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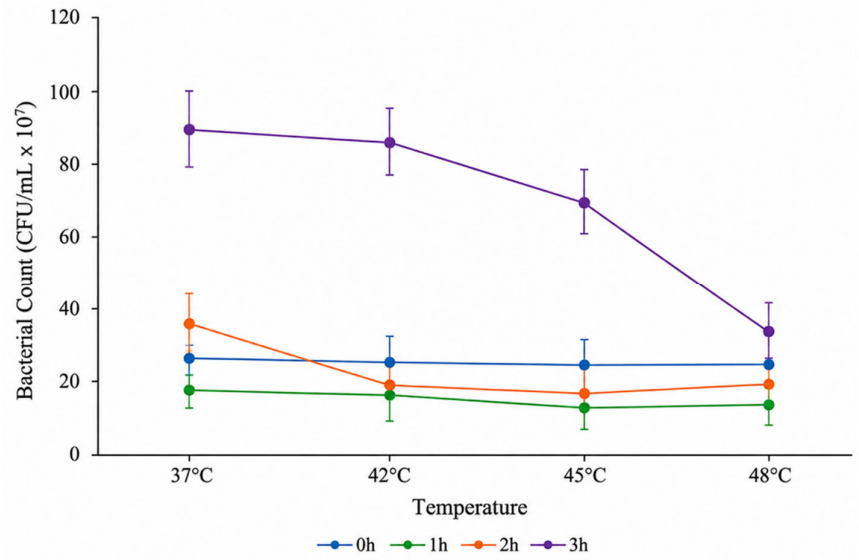


Figure 1 Growth of BS2-PB5 under different temperature treatments. Data represent the mean ± SD of two independent experiments.

BS2-PB5 remained viable throughout the incubation period at all tested temperatures. Similar growth patterns were observed across all treatments. The bacterial counts decreased after 1 h of incubation, followed by an increase after 2 h and a further increase after 3 h. After 3 h of incubation, the highest bacterial count was observed at 37°C, followed by 42°C and 45°C, whereas the lowest bacterial count was observed at 48°C. Although bacterial growth recovery occurred under all temperature conditions, the final bacterial counts tended to decrease as incubation temperature increased.

Interestingly, a temporary decline in bacterial counts after 1 h was observed not only at elevated temperatures but also in the control treatment (37°C). Because this trend occurred across all treatments, the decrease cannot be attributed solely to heat stress. The observed decline may reflect physiological adjustment following the transfer of cells from the overnight culture into fresh growth medium, during which the cells adapt to new environmental conditions before resuming active growth. Similar adaptive responses have been described in lactic acid bacteria exposed to changing environmental conditions [11]. In addition, variation associated with viable count measurements may have contributed to the fluctuation in cell numbers.

The increase in bacterial counts after 2 h and 3 h indicates that BS2-PB5 remained metabolically active and capable of growth under all tested conditions. The recovery pattern observed in the present study suggests that BS2-PB5 was able to adjust to the cultivation conditions and maintain viability throughout the incubation period. The ability of BS2-PB5 to recover growth following exposure to temperatures up to 48°C suggests that this strain possesses cellular mechanisms that support survival under thermal stress.

Although recovery was observed at all temperatures, cultures incubated at higher temperatures generally exhibited lower bacterial counts after 3 h compared with cultures incubated at 37°C. Elevated temperatures can affect bacterial growth by altering membrane fluidity, reducing protein stability, and interfering with enzymatic activity and other metabolic processes [12]. Nevertheless, viable cells were still detected after incubation at 42°C, 45°C, and 48°C, indicating that BS2-PB5 is capable of tolerating short-term exposure to elevated temperatures.

The ability of BS2-PB5 to remain viable during incubation at temperatures up to 48°C is consistent with the recognized robustness of *Lacticaseibacillus paracasei* and other lactic acid bacteria commonly used in food and probiotic applications. Previous studies have highlighted that members of the *Lacticaseibacillus* genus possess diverse stress-response mechanisms that enable adaptation to environmental challenges encountered during industrial processing, storage, and gastrointestinal transit. These mechanisms contribute to cellular survival and recovery following exposure to adverse conditions, including temperature stress [3], [5]. Therefore, the adaptation and growth recovery observed in BS2-PB5 are consistent with the general stress physiology reported for lactic acid

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bacteria[13]. This characteristic may be advantageous for probiotic applications in which bacterial cells encounter temperature fluctuations during manufacturing and storage. Further molecular investigations are required to identify the genes and pathways associated with heat tolerance in BS2-PB5.

The results indicate three distinct responses of BS2-PB5 to elevated temperatures. First, viable cells remained detectable throughout the incubation period at all tested temperatures, indicating survival under the tested conditions. Second, the temporary decline in bacterial counts after 1 h followed by recovery at later time points may reflect physiological adaptation to changes in the cultivation environment. Third, the increase in bacterial counts observed after 2 h and 3 h indicates that the surviving cells retained the ability to grow during incubation. Together, these observations suggest that BS2-PB5 was able to survive, adapt, and subsequently grow following short-term exposure to elevated temperatures.

3.2 RT-PCR amplification of putative heat-shock-related genes

Based on genome annotation results, two putative heat shock protein genes, *dnaJ* and *dnaK*, were selected for molecular analysis. Gene-specific primers were designed and used to amplify RNA-derived cDNA through RT-PCR. Following amplification, PCR products were visualized on a 1% agarose gel (**Fig. 2**). Distinct amplification products were observed in samples amplified using both primer sets, whereas no bands were detected in the negative controls. The absence of amplification in the negative controls indicates that no detectable contamination was present in the RT-PCR reactions. Single bands were detected for each target under both temperature treatments, and no apparent non-specific bands or smearing were observed. Fragment size estimation using GelAnalyzer software indicated that the products amplified using the *dnaJ* primer set were approximately 336 bp, whereas the products amplified using the *dnaK* primer set were approximately 445 bp. Similar band positions and intensities were observed for samples incubated at 42°C and 45°C, indicating consistent amplification under both treatment conditions.

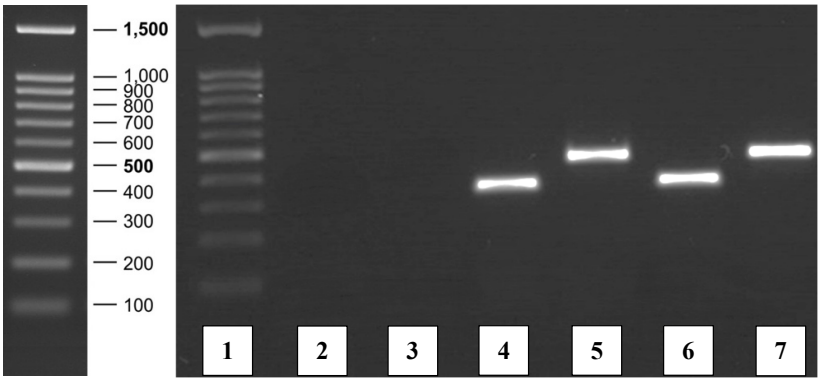


Figure 2 Electrophoretic visualization of RT-PCR products generated using *dnaJ* and *dnaK* primer sets. Lane 1: 100 bp DNA ladder; Lane 2: negative control (*dnaJ*); Lane 3: negative control (*dnaK*); Lane 4: 42°C (*dnaJ*); Lane 5: 42°C (*dnaK*); Lane 6: 45°C (*dnaJ*); Lane 7: 45°C (*dnaK*)

The detection of single amplification products with the expected size range suggested that the primer pairs successfully amplified amplification of products of the anticipated size. However, agarose gel electrophoresis provides information only on the presence and approximate size of PCR products and cannot confirm their identity [14]. Amplification products of similar size may originate from unintended genomic regions, particularly when conserved sequences are present within the genome. Therefore, sequence verification using Sanger sequencing followed by BLASTn analysis was performed to determine whether the amplified fragments corresponded to the intended target genes.

3.3 Sequence verification of RT-PCR products

To verify the identity of the amplified fragments, representative RT-PCR products were subjected to Sanger sequencing and analysed using BLASTn against sequences deposited in the NCBI database. The results are summarized in Table 2.

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Table 2 Summary of BLASTn analysis of RT-PCR amplicons generated using putative *dnaJ* and *dnaK* primer sets.

Sample	Highest-scoring BLASTn hit	Query cover (%)	Identity (%)	E-value
42°C *dnaJ*	*Lacticaseibacillus paracasei* strain YE01 chromosome / GTP-binding protein LepA (*lepA*)	95	99.34	7E−150
45°C *dnaJ*	*Lacticaseibacillus paracasei* strain YE01 chromosome / GTP-binding protein LepA (*lepA*)	96	99.33	3E−149
42°C *dnaK*	*Lacticaseibacillus paracasei* strain YE01 chromosome	93	99.50	0.0
45°C *dnaK*	*Lacticaseibacillus paracasei* strain YE01 chromosome	94	99.75	

BLASTn analysis revealed that none of the amplified products corresponded to the intended heat-shock-related genes, *dnaJ* and *dnaK*. The amplicons generated using the *dnaJ* primer set showed 95–96% query coverage and approximately 99.33–99.34% nucleotide identity with several *Lacticaseibacillus paracasei* genomes. In addition, multiple high-scoring alignments corresponded to the GTP-binding protein LepA (*lepA*) gene, suggesting that the amplified fragments were associated with the *lepA* locus rather than the intended *dnaJ* gene. Similarly, the amplicons generated using the *dnaK* primer set exhibited high similarity to chromosomal regions of *L. paracasei* strains, with query coverage ranging from 93% to 94% and nucleotide identity ranging from 99.50% to 99.75%. However, none of the highest-scoring BLASTn hits corresponded to the intended *dnaK* gene.

These findings demonstrate that amplification products within the expected size range do not necessarily represent the intended target genes. Although agarose gel electrophoresis indicated successful amplification and revealed fragments of approximately 336 bp and 445 bp, sequence verification showed that the amplified products were derived from genomic regions other than the selected heat-shock-related genes. Several factors may explain this discrepancy. One possible explanation is insufficient primer specificity, resulting in amplification of homologous chromosomal regions. In addition, sequence variation within primer-binding sites may reduce primer-target complementarity and promote amplification of unintended loci. The use of conserved genomic regions during primer design may also contribute to non-target amplification when closely related sequences are present elsewhere in the genome [15].

The identification of *lepA*-associated sequences among the highest-scoring BLASTn hits is noteworthy because LepA, also known as elongation factor 4 (EF4), is a highly conserved bacterial GTP-binding protein involved in translational processes and cellular adaptation to environmental stress [16]. However, the present results do not provide sufficient evidence to conclude that *lepA* contributes directly to heat tolerance in BS2-PB5. Additional molecular analyses would be required to investigate its potential role in thermal adaptation.

The inability to confirm amplification of *dnaJ* and *dnaK* indicates that the molecular basis underlying heat tolerance in BS2-PB5 remains unresolved. Nevertheless, BS2-PB5 remained viable at temperatures up to 48°C, suggesting that cellular mechanisms supporting thermal adaptation are present in this strain. Future studies employing redesigned primers, in silico primer specificity analysis, and quantitative gene expression approaches may help identify genes associated with heat tolerance in BS2-PB5.

4. Conclusion

Lacticaseibacillus paracasei subsp. *paracasei* BS2-PB5 remained viable following exposure to elevated temperatures of 42°C, 45°C, and 48°C, although cell growth decreased as temperature increased. RT-PCR amplification using primers designed from putative *dnaJ* and *dnaK* genes generated fragments of approximately 336 bp and 445 bp, respectively. However, Sanger sequencing and BLASTn analysis revealed that the amplified products did not correspond to the intended heat shock protein genes. The fragment amplified using the *dnaJ* primer set showed high similarity to the GTP-binding protein LepA (*lepA*) gene, whereas the fragment amplified using the *dnaK* primer set aligned primarily with chromosomal regions of *L. paracasei*. These findings demonstrate that amplification products of the expected size do not necessarily represent the intended target genes and highlight the importance of sequence verification following RT-PCR. Further studies employing optimized primer design and

quantitative gene expression analyses are required to identify genes associated with heat tolerance in BS2-PB5.

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References

[1] T. C. Pimentel, L. R. Brandão, M. P. de Oliveira, W. K. A. da Costa, and M. Magnani, "Health benefits and technological effects of Lacticaseibacillus casei-01: An overview of the scientific literature," *Trends Food Sci. Technol.*, vol. 114, pp. 722–737, Aug. 2021, https://doi.org/10.1016/J.TIFS.2021.06.030.

[2] J. Plaza-Diaz, F. J. Ruiz-Ojeda, M. Gil-Campos, and A. Gil, "Mechanisms of Action of Probiotics," 2019, https://doi.org/10.1093/advances/nmy063

[3] B. Sionek, A. Szydłowska, M. Trz, ˛ Askowska, and D. Kołoˆ Zyn-Krajewska, "The Impact of Physicochemical Conditions on Lactic Acid Bacteria Survival in Food Products," 2024, https://doi.org/10.3390/fermentation10060298

[4] P. Sornsenee *et al.*, "Probiotic Insights from the Genomic Exploration of Lacticaseibacillus paracasei Strains Isolated from Fermented Palm Sap," *Foods*, vol. 13, no. 11, 2024, https://doi.org/10.3390/foods13111773

[5] B. Li *et al.*, "Genomic Insight Into Lacticaseibacillus paracasei SP5, Reveals Genes and Gene Clusters of Probiotic Interest and Biotechnological Potential," 2022, https://doi.org/10.3389/fmicb.2022.922689

[6] K. Kadri, "Polymerase Chain Reaction (PCR): Principle and Applications," *Synthetic Biology - New Interdisciplinary Science*, Jun. 2019, https://doi.org/10.5772/INTECHOPEN.86491

[7] C. M. J. Pang, R. Dosan, H. Victor, M. Sugata, and T. J. Tan, "Analysis of heat-shock protein genes and their expression in Lactiplantibacillus plantarum SU-KC1a," *Biodiversitas*, vol. 25, no. 6, pp. 2494–2499, 2024, https://doi.org/10.13057/biodiv/d250618

[8] R. Dosan *et al.*, "Isolation and identification of Bifidobacterium species from human breast milk and infant feces in Indonesia," *Biodiversitas*, vol. 25, no. 1, pp. 337–343, 2024, https://doi.org/10.13057/biodiv/d250139

[9] T. F. Huang *et al.*, "Whole-Genome Analysis and Tolerance Assessment on Bifidobacteria-like Bacteria Isolated from Breast Milk and Infant Fecal Samples in Indonesia," *J. Trop. Biodivers. Biotechnol.*, vol. 10, no. 2, Jun. 2025, https://doi.org/10.22146/jtbb.15062

[10] R. Overbeek *et al.*, "The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST)", https://doi.org/10.1093/nar/gkt1226

[11] R. L. Bertranda, "Lag phase is a dynamic, organized, adaptive, and evolvable period that prepares bacteria for cell division," *J. Bacteriol.*, vol. 201, no. 7, Apr. 2019, https://doi.org/10.1128/JB.00697-18/ASSET/145372C7-51F7-496C-8CF6-00350CEC2C46/ASSETS/GRAPHIC/JB.00697-18-F0007.JPEG

[12] F. D. Schramm, K. Schroeder, and K. Jonas, "Protein aggregation in bacteria," *FEMS Microbiol. Rev.*, vol. 026, pp. 54–72, 2020, https://doi.org/10.1093/femsre/fuz026

[13] K. Papadimitriou *et al.*, "Stress Physiology of Lactic Acid Bacteria," *Microbiology and Molecular Biology Reviews*, vol. 80, no. 3, pp. 837–890, Sep. 2016, https://doi.org/10.1128/MMBR.00076-15/ASSET/98EF1078-BABC-42D2-9223-8715B5F0317B/ASSETS/GRAPHIC/ZMR0031624320005.JPEG

[14] C. Chang, J. Jung, A. Kwai, and E. Uy, "Agarose Gel Electrophoresis for Analysis of PCR Products," *Undergraduate Journal of Experimental Microbiology and Immunology*, vol. 4, Aug. 2025, Accessed: Jun. 28, 2026. [Online]. Available: https://ojs.library.ubc.ca/index.php/UJEMI/article/view/200819

[15] J. Ye, G. Coulouris, I. Zaretskaya, I. Cutcutache, S. Rozen, and T. L. Madden, "Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction," 2012. [Online]. Available: http://www.biomedcentral.com/1471-2105/13/134

[16] J. L. E. Heller, R. Kamalampeta, and H.-J. Wieden, "Taking a Step Back from Back-Translocation: an Integrative View of LepA/EF4's Cellular Function," *Mol. Cell. Biol.*, vol. 37, no. 12, Jun. 2017, https://doi.org/10.1128/MCB.00653-16