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Transcriptional responses of *Lactobacillus acidophilus* to Allura Red exposure: Analysis of stress and functional genes

S. Sowmiya, L. Shakila*, S. Reiana Jenifer
PG & Research Department of Zoology, Ethiraj College for Women, Chennai, India.

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ABSTRACT

Allura Red is an azo dye commonly used in food products to impart vibrant red color. Previous studies have raised considerable concerns regarding its effects on digestive health and inflammation, as chronic exposure has been shown to cause intestinal injury and inflammation in mice. However, its impact on gut-associated microorganisms remains poorly understood. This study aimed to investigate the transcriptional response of *Lactobacillus* to Allura Red exposure. *Lactobacillus* species isolated from curd samples were exposed to different concentrations of the dye, and bacterial growth, viability, and gene expression analyses were performed targeting functional genes associated with bile salt metabolism (*bshA*, *bshB*), antibiotic resistance (*vanX*), oxidative stress (*sodA*), and heat shock response (*dnaK*). Qualitative RT-PCR results demonstrated dose-dependent modulation of gene expression in *Lactobacillus*. A progressive reduction in Ct values was observed with increasing dye concentration, suggesting the upregulation of stress- and survival-related pathways. These findings indicate that Allura Red induces oxidative stress, protein damage, antibiotic stress, and membrane perturbation, thereby activating molecular defense mechanisms to restore cellular homeostasis. Collectively, these findings highlight the adaptive capacity of *Lactobacillus*, which may enhance its survival under chemically challenging conditions, such as in the gut environment exposed to food additives.

1. INTRODUCTION

Color is considered one of the most important factors in the food industry, as it enhances the visual appeal of food. The first characteristic consumers notice about food is its color, which shapes their expectations of both flavor and quality. Synthetic food dyes are manufactured by chemical reactions, and they are the most commonly used color additives in the food and pharmaceutical industries. Azo dyes are a large class of synthetic dyes characterized by the presence of an azo group (R−N=N−R′) in their molecular structure. Among these, Allura Red AC (E129) is one of the most widely used food colorants; however, its metabolic fate and potential health effects following ingestion remain incompletely understood. Following ingestion, these dyes can be biotransformed by gut microbiota, especially through azoreductase-catalyzed reduction of the azo bonds (-N=N-) to form potentially innocuous or toxic aromatic amines [1,2]. Azo dyes constitute the largest class of synthetic food color additives and are extensively manufactured and used worldwide, particularly in the United States, China, India, and the Middle East. Approximately 7×10^5 tonnes (700,000 tonnes) of these dyes are manufactured annually [3].

Allura Red (Red 40) is a Food and Drug Administration (FDA)-approved synthetic food dye widely utilized in several industries, including beverages, bakery items, dessert powders, sweets, cereals, meals, pharmaceuticals, and cosmetics. It is among the most widely consumed synthetic food colorants worldwide. Red 40 has an acceptable daily intake of 7 mg/kg body weight/day according to the FDA. Chemically, Allura Red AC is disodium 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfonatophenyl) diazenyl]naphthalene-2-sulfonate. It is chemically stable and resistant to degradation by light and air. Other synthetic food colorants such as tartrazine, Sunset Yellow FCF, Ponceau 4R, quinoline yellow, and carmoisine are also increasingly employed by manufacturers because they are inexpensive, chemically stable, highly pigmented, and produce uniform coloration [4]. Regulatory agencies have established strict limits on their permitted concentrations to protect consumer health. However, epidemiological and experimental studies have associated several of these azo dyes with behavioral alterations, most notably increased hyperactivity in children [5]. These findings raise concerns that routine dietary exposure, even within the acceptable intake range, may contribute to adverse health effects.

Numerous research studies have raised considerable concerns regarding the effects of Red 40 on gastrointestinal health and inflammation. A 2022 study published in Nature Communications demonstrated that chronic exposure to Red 40 caused intestinal injury and inflammation in mice. The study suggested that the dye altered the

*Corresponding Author
L. Shakila, PG & Research Department of Zoology, Ethiraj College for Women, Chennai, India. E-mail: shakila @ ethirajcollege.edu.in

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gut microbial composition by increasing serotonin production, thereby increasing susceptibility to intestinal inflammation [6]. Another study demonstrated that exposure to Red 40 at a dose equivalent to the human acceptable daily intake of 7 mg/kg/day altered the gut microbiota and induced mild colonic inflammation in the distal colon of mice after 10 months of exposure. The study also reported increased expression of the pro-inflammatory cytokine interleukin-6 and inducible nitric oxide synthase, both of which are established markers of colonic inflammation [7].

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [8]. However, non-viable bacteria and their structural components may also have probiotic qualities [9]. The most extensively utilized probiotic bacteria belong to the genera *Bifidobacterium* and *Lactobacillus* [10], which have health-promoting qualities such as maintaining gut barrier function and modulating the host immune system locally and systemically [11,12]. Lactic acid bacteria (LAB) constitute one of the most important groups of food-associated microorganisms, with applications in the food, probiotic, dairy, and beverage industries. These applications are largely attributed to the Generally Recognized as Safe status of LAB and their diverse physiological characteristics, which make them suitable for industrial and probiotic applications. Specific *Lactobacillus* strains have been employed for the management of gastrointestinal disorders, immunomodulation, and inhibition of colonization by pathogenic bacteria [13,14].

The human gut is a complex ecosystem inhabited by a diverse microbial community. Among these microorganisms, *Lactobacillus* species play an essential role in maintaining gut homeostasis and host health. The increasing prevalence of synthetic food dyes in processed foods raises concerns regarding their potential interactions with the human gut microbiota, particularly beneficial probiotic species such as *Lactobacillus* [15]. Allura Red is commonly used to impart a red color to a wide variety of food products; however, its metabolic fate within the gastrointestinal tract and its specific effects on probiotic bacteria remain largely unexplored [16]. This study aimed to elucidate the transcriptional responses of key probiotic genes in *Lactobacillus* species, specifically focusing on those involved in bile salt hydrolysis, antibiotic resistance, and oxidative stress response when exposed to Allura Red [16]. Furthermore, evaluating bacterial growth and viability is essential for interpreting transcriptional responses, as alterations in gene expression may reflect physiological adaptations or reduced cellular fitness following exposure to toxic compounds. The study investigated the effects of dye on the growth and viability of *Lactobacillus*. The findings from this investigation will enhance the understanding of the complex interactions between food additives and gut microbiota, providing important insights towards maintaining probiotic efficacy in food systems.

2. MATERIALS AND METHODS

2.1. Sample Collection, *Lactobacillus* Isolation, and Growth Conditions

The bacterial culture was isolated from the homemade curd prepared from the milk of an Indian native cow breed in the region of Tamil Nadu. Samples collected were stored appropriately in cool, dry places, away from light, or in the fridge at 4^0C. A loopful of the serially diluted sample was streaked onto MRS agar (de Man, Rogosa, Sharpe media) plates and incubated at 37^0C. After 48 hours of incubation, the colonies obtained, which could be differentiated by their morphology (size, shape, and appearance), were subcultured to obtain pure cultures of the selected colonies [17,18]. Gram staining was performed on the isolated pure cultures. Biochemical tests for identification of the isolated bacteria were carried out for *Lactobacillus*. Selected colonies were inoculated in MRS broth and incubated overnight under anaerobic conditions at 37^0C.

2.2. Molecular Identification and 16S rRNA Gene Sequencing

Genomic DNA was isolated from overnight cultures using a commercial DNA Extraction Kit (QIAamp DNA Mini Kit) following the manufacturer's instructions. The 16S rRNA gene was amplified by universal primers 27F (5′-AGAGTTTGATYMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACT-3′) [19]. DNA was amplified using a Wee32® Thermal Cycler. The PCR amplicon was bead-purified and further subjected to Sanger Sequencing. Bidirectional DNA sequencing of the PCR amplicon was carried out with forward and reverse primers using BigDye™ Terminator v3.1 Cycle sequencing kit on Applied Biosystems™ 3730xl DNA Analyzer. Sequences obtained were evaluated by BLAST search against the NCBI GenBank database for species-level identification.

2.3. Treatment of *Lactobacillus* with Allura Red

Allura red AC (FD&C Red No. 40) was purchased from Sigma-Aldrich (CAS Number 25956-17-6). A 10 mM stock solution of Allura Red (molecular weight, 496.2 g/mol) was prepared by dissolving 0.049 gm of the dye in 10 ml of sterile distilled water. The stock solution was mixed thoroughly until complete dissolution. The dye solution was filter-sterilized using a 0.22 µm membrane filter. Varying concentrations (15.6, 31.5, 62.5, 125, 250, 500, 1000, and 1500 µM) of Allura Red, which is physiologically relevant for *in vitro* exposure, were prepared for the study by diluting the stock solution [7]. *Lactobacillus* cultures were divided into control and treatment groups. Varying concentrations of prepared dilutions of Allura Red were added to the treatment groups. The control group received no dye. All the tubes were incubated at 37°C for 24 hours.

2.4. Growth Curve Analysis

To evaluate the effect of Allura Red on bacterial growth, *Lactobacillus* cultures were exposed to different concentrations of the dye (15.6, 31.5, 62.5, 125, 250, 500, 1000, and 1500 µM) in MRS broth and incubated under anaerobic conditions at 37°C for 24 hours. Bacterial growth was monitored spectrophotometrically by measuring the optical density at 600nm (OD600) at predetermined time intervals (0, 2, 4, 6, 8, 12, and 24 hours). Growth curves were constructed by plotting OD600 values against incubation time.

2.5. Viability Assessment by Serial Dilution

Following 24 hours of exposure to different concentrations of Allura Red, bacterial cultures were serially diluted in sterile phosphate-buffered saline. Appropriate dilutions were spread onto MRS agar plates and incubated under anaerobic conditions at 37°C for 24 hours. After exposure, the colony density and morphology were recorded.

2.6. RNA Isolation, Complementary DNA (cDNA) Synthesis, and Gene Expression

Total RNA was extracted from bacterial cultures using the HiMedia DNA/RNA isolation kit according to the manufacturer's protocol. Briefly, bacterial pellets were harvested by centrifugation at 5,000 × g for 10 minutes and resuspended in the provided lysis buffer to ensure complete cell disruption. The lysates were transferred to silica-based spin columns, and bound nucleic acids were subjected to successive

washing steps with kit-supplied wash buffers to remove proteins, salts, and other contaminants. To obtain RNA specifically, on-column DNase I digestion was performed as recommended by the manufacturer in order to remove residual genomic DNA. After additional washing, pure RNA was eluted in RNase-free water. The concentration of RNA was measured by NanoDrop (DeNovix DS-11). The RNA samples were stored at −80 °C until use. Complementary DNA was synthesized from 1 µg of total RNA using a Hi-cDNA Synthesis Kit (HiMedia, India) with random hexamer: Oligo(dT) mix primers, following the supplier's protocol. The samples were stored at −20 °C until use.

The cDNA was used for RT-PCR in the same conditions described above, for primer validation, in technical triplicate. Relative expression of target genes, including bile salt hydrolase (*bshA* and *bshB*), antibiotic resistance gene (*vanX*), superoxide dismutase (*sodA*), and stress response gene (*dnaK*), was evaluated using SYBR Green-based qualitative RT-PCR. Specific primers were designed from published sequences and purchased from Barcode Biosciences Pvt. Ltd (Table 1). The specificity of the primer pairs was independently evaluated using the NCBI BLASTn tool against publicly available nucleotide sequences, and the primer sequences showed correspondence with *Lactobacillus acidophilus* sequences. Amplification was performed under the following conditions: initial denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 45 seconds, annealing at 55°C–63°C (gene-specific) for 30 seconds, and extension at 72°C for 30 seconds. Melting curve analysis confirmed primer specificity; 16S rRNA was used as the reference gene.

2.7. Statistical Analysis

Relative fold changes in gene expression were calculated using the 2^−ΔΔCt method. All experiments were performed in technical triplicates, and data were expressed as mean ± standard deviation. Statistical analysis was carried out using GraphPad Prism 9.0 (GraphPad Software, USA), with significance determined at $p < 0.05$.

3. RESULTS

3.1. Isolation and Preliminary Identification of *Lactobacillus sp.*

Curd samples were serially diluted and plated on MRS agar to selectively isolate lactic acid bacteria. After incubation at 37^{0}C for 24 hours, under anaerobic conditions, colonies with small, round, opaque, and creamy white morphology were observed. The purification of these colonies was done by repeated streaking on MRS agar (Fig. 1). The isolates were Gram-positive, rod-shaped bacteria with biochemical profiles showing catalase-negative and oxidase-negative reactions that were in line with the genus *Lactobacillus*.

3.2. Molecular Identification and 16S rRNA Gene Sequencing

The pure culture was successfully used to extract high-quality genomic DNA. Agarose gel electrophoresis was used to determine the integrity of the extracted DNA and showed a single and distinct band of high molecular weight. The amplification of the 16S rRNA gene with universal bacterial primers (27F and 1492R) by PCR yielded one amplicon of about 1500 bp, which was observed as a distinct band on a 1.2% agarose gel that was stained with ethidium bromide

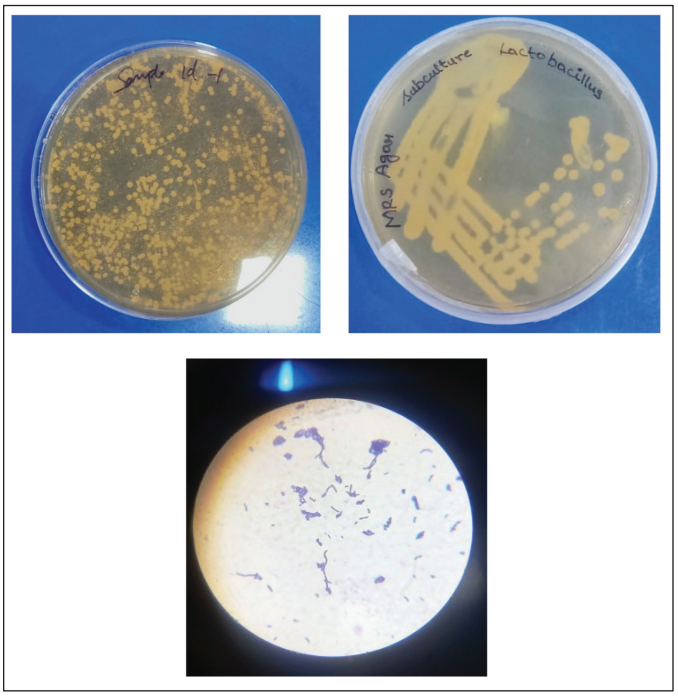

Figure 1. Isolation and preliminary identification of *Lactobacillus*.

Table 1. List of primers used for qualitative RT-PCR analysis.

Gene	Function	Primer sequence (5′→3′)	References
bshA	Bile salt hydrolase A—resisting bile toxicity	F:AAAGTCGACGAAAAGGGGCTTGGTA	[32]
		R:AAGAATTCCATCAGGTTGTTTAC	
bshB	Bile salt hydrolase B—functional redundancy	F:AGGATCCAGTTAGTTCCATCAGAATA	[32]
		R:TATAAGCTTGGTATGGCCGGACTCAAC	
vanX	Vancomycin resistance gene	F:GCGTTAAAGGGGTGATAAGC	[33]
		R:GTCCGTGCAACGGTTAAAAG	
sodA	Superoxide dismutase—oxidative stress response	F:TCCCCGGGAAAGGTTGATTCCTATGAC	[34]
		R:GCTCTAGATCAGGCGTTTGTATCGGG	
dnaK	Chaperone protein (Heat shock resistance)	F:ATGGTGGGTCAACTCGGATT	[35]
		R:AAAGTGGCGTAACATCGAGC	
16S rRNA	Housekeeping gene	F:AGAGTTTGATYMTGGCTCAG	[19]
		R:TACGGYTACCTTGTTACGACT	

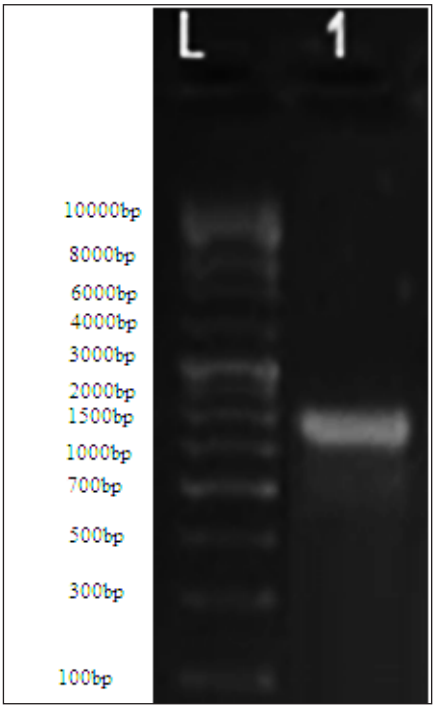

Figure 2. Agarose gel showing single 1500 bp of 16*s* amplicon.

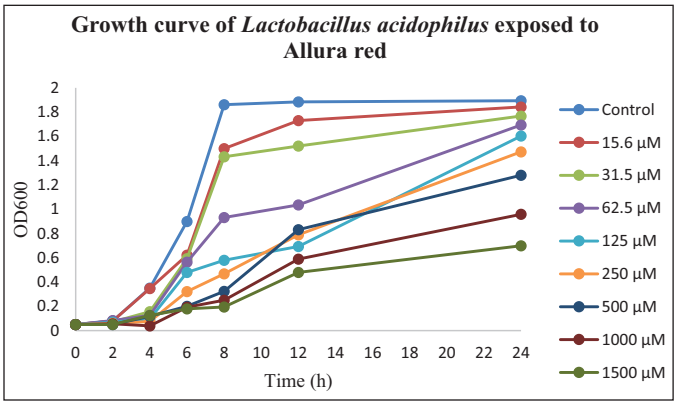

Figure 3. Growth curve of *Lactobacillus acidophilus* exposed to different concentrations of Allura Red.

(Fig. 2). This proved the effective amplification of the desired region. The PCR product was purified, and Sanger sequencing was done in forward and reverse directions. The molecular identity of the isolate was confirmed by BLAST analysis of the consensus sequence against the NCBI GenBank database, with the highest similarity being *L. acidophilus*.

3.3. Effect of Allura Red on Growth and Viability of *Lactobacillus*

The growth curve analysis shows concentration-dependent effects of Allura Red on *Lactobacillus* growth. The lower concentrations showed growth curves similar to the control, while the higher concentrations showed decreased OD values and a delay in the exponential phase. This inhibitory effect was more noticeable at concentrations of 1,000 and 1500 µM (Fig. 3). A further decrease in

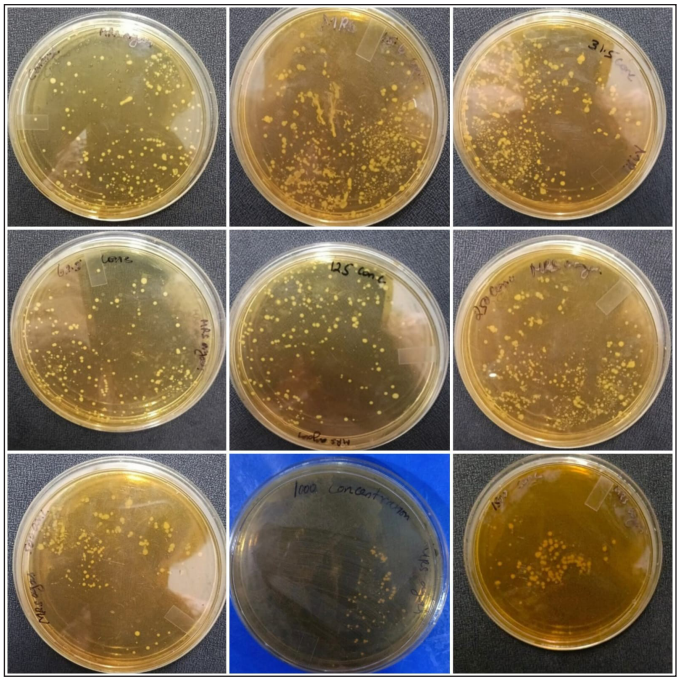

Figure 4. Plate based assay (Serial dilution) of *Lactobacillus* exposed to different concentrations (Control, 15.6, 31.5, 62.5,125, 250, 500, 1000, 1500 µM).

Table 2. Serial dilution.

Allura red concentration (µM)	Colony density	Colony morphology	Interpretation
Control	++++	Large, smooth circular, creamy white opaque colonies with convex elevation	Normal viability
15.6	++++	Similar to control	No major stress
31.5	++++	Slightly reduced size	Mild stress adaptation
62.5	+++	Moderate colonies	Early stress response
125	+++	Slight reduction in size	Moderate stress
250	+++	Compact colonies	Adaptive response visible
500	++	Reduced colony number	Growth inhibition begins
1000	+	Small sparse colonies	Significant stress
1500	+	Tiny stressed colonies	Severe inhibitory effect

Colony Density Scale:
++++ = Heavy growth
+++ = Moderate growth
++ = Reduced growth

bacterial viability as a function of the increasing concentration of the dye was also shown by serial dilution. The analysis of colony density and morphology showed a severe inhibitory effect with increasing dye concentrations, suggesting that higher concentrations of Allura Red AC had a negative impact on the survival of the bacteria (Fig. 4 and Table 2).

3.4. Qualitative RT-PCR Profiling of Selected *Lactobacillus* Genes

The transcriptional expressions of five functional genes *bshA* and *bshB* (bile salt hydrolases), *vanX* (vancomycin resistance), *sodA* (superoxide dismutase), and *dnaK* (heat shock protein) of *Lactobacillus* were investigated following the exposure of genes to Allura Red at various concentrations (15.6 µM to 1500 µM). Relative expression values were normalized to the 16S rRNA housekeeping gene, and fold changes were calculated using the 2^–ΔΔCt method relative to the untreated control (Table 3). All the amplification curves exhibited stable threshold cycle (Ct) values in the technical replicates, confirming primer specificity and comparable amplification efficiency (Fig. 5).

3.5. Gene Expression of Selected *Lactobacillus* Genes

Both *bshA* and *bshB* exhibited dose-dependent reduction in ΔCt values with increasing dye concentration, indicating transcriptional upregulation. The *bshA* gene exhibited marked upregulation at all tested concentrations, with the highest increase in the fold change (51.3-fold) at 250 µM. The fold change was between 1.3 to 51-fold, indicating that the degree of induction was strongly dose-dependent in the mid-concentration range, after which the induction became saturated. Similarly, *bshB* was expressed significantly at 250 µM (16.1-fold), beyond which the expression decreased at the higher and lower concentrations. The magnitude of the response suggests an adaptive process that upregulates bile salt hydrolase activity, potentially in order to protect membrane integrity under chemical stress [20].

Table 3. Relative expression levels and regression statistics of target genes under different concentrations.

Gene	Fold change range	Maximum expression (fold)	Concentration at maximum expression (µM)	*p*-value
bshA	1.3–51.3	51.3	250	0.018
bshB	0.86–16.1	16.1	250	0.032
vanX	0.61–14.16	14.16	31.5	0.036
sodA	8.86–150.33	150.33	250	0.009
dnaK	0.67–34.82	34.82	250	0.021

The *vanX* transcript was detectable in all dye-treated samples, with ΔCt values always below the control. The greatest reduction was at 500 µM; elevated concentrations caused only insignificant extra reduction in the ΔCt values. The *vanX* gene clearly showed a concentration-dependent increase in transcriptional activity. The maximum expression was at 31.25 µM (or 14.16-fold), and total fold changes were between 0.6- and 14-fold.

Exposure to Allura Red caused a considerable upregulation of the oxidative stress-related *sodA* gene by a reduction in the ΔCt values from lower to higher concentrations. Expression of the *sodA* gene was highly elevated across treatments, with a maximum of 150-fold increase at 250 µM. The gene response suggests that gene upregulation with increasing concentrations reflects a strong oxidative stress response, consistent with the generation of reactive oxygen species (ROS) induced by synthetic dye exposure [21].

The *dnaK* transcript reflected the pattern seen in *sodA*. ΔCt values decreased from untreated controls to the highest dye concentration. The *dnaK* gene, a heat-shock chaperone, was also induced with fold changes of 0.67 to 34.8-fold. It was most upregulated at 250 µM, like *bshA* and *sodA*. The pattern suggests activation of the system for molecular chaperones in support of protein refolding and cellular defense against dye-induced proteotoxic stress [22,23].

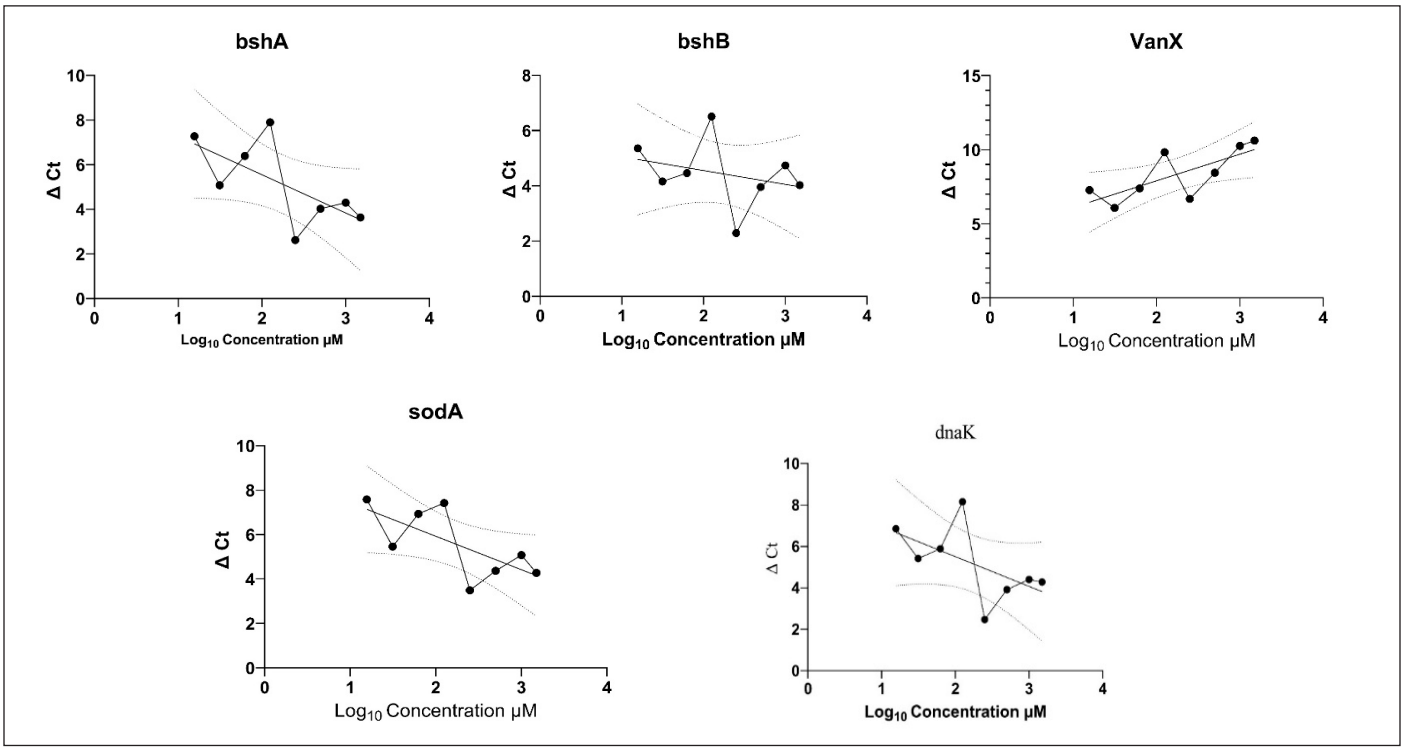

Figure 5. RT-PCR ΔCt values of target genes across different concentrations of Allura Red.

The 16S rRNA housekeeping gene exhibited negligible variation among all treatment conditions with no identifiable trend, thereby validating its appropriateness as an internal reference for the computations of relative expression. Overall, decreasing Ct values across increasing dye concentrations for *bshA*, *bshB*, *vanX*, *sodA*, and *dnaK* indicate dose-dependent gene upregulation in *Lactobacillus* upon exposure to Allura Red. These results suggest that the bacteria activate stress-responsive and protective mechanisms under chemical stress induced by the synthetic dye.

4. DISCUSSION

Lactobacillus acidophilus is a small, slender, non-motile, gram-positive bacterium that is anaerobic and catalase- and oxidase-negative; they are predominantly found in the human oral cavity, vagina, and gastrointestinal tract. It exhibits stronger tolerance to both acid and bile salts than many other probiotic bacteria. These characteristics enable it to survive and proliferate in the demanding conditions of the gastrointestinal tract [24]. Additionally, it is also present in a variety of fermented foods, such as fermented milk and yoghurt; owing to its potent probiotic properties, *L. acidophilus* is widely employed in commercial dairy production. Studies have demonstrated that *L. acidophilus* offers a range of probiotic benefits to humans, including lowering cholesterol, boosting immunity, aiding in lactose digestion, and acting as a barrier against infections [25].

The present study provides a comprehensive look into the molecular responses of *L. acidophilus* when exposed to the synthetic dye, Allura Red. The study primarily focused on the growth curve, viability analyses, and transcriptional regulation of stress- and resistance-associated genes (*bshA*, *bshB*, *sodA,* and *dnaK*). The upregulation of these genes across varying concentrations of the dye reflects the activation of complex protective mechanisms that help in safeguarding cellular integrity under chemical stress. The growth curve and viability analyses gave context to the transcriptional responses that were observed in *Lactobacillus*. Moderate concentrations of Allura Red AC altered the expression of stress-related genes without affecting bacterial viability. However, the reduced growth and lower viable cell count observed at higher concentrations indicate that the growth suppression seen at 1000 and 1500 µM may be due to poor cellular fitness rather than an adaptive response. These findings highlight the importance of considering bacterial physiological status when assessing the effects of food dye exposure on transcriptional changes.

The significant increase in the expression of *bshA* and *bshB* genes following exposure to Allura Red suggests that these genes may contribute to cellular adaptation to membrane stress induced by xenobiotic compounds. Detoxification of bile salts is done by *Lactobacillus* species with the help of bile salt hydrolases (BSHs) and also helps them to survive stress and to colonize the gut. Previous studies have demonstrated that BSH-positive strains of *L. acidophilus* exhibit greater tolerance to bile stress and improved cell surface adaptations compared to BSH-negative strains, highlighting a stress-adaptive advantage [26]. Additionally, the structural insights into the substrate specificity of BSH enzymes from *Lactobacillus* species support their potential multifunctionality in response to environmental stressors [27]. Similar studies have shown that higher BSH expression is associated with greater bile tolerance and improved survival under chemical and antibiotic stress. The cross-protective mechanism of bile salt and antibiotic resistance is commonly mediated by multifunctional enzymes like the BSHs that also degrade β-lactam antibiotics [28]. The high dose-dependent activation at 250 µM concentration shows that Allura Red induces membrane-associated stress, leading to an adaptive response of hydrolases that stabilizes the membrane.

A crucial stage in peptidoglycan alteration linked to vancomycin resistance is the hydrolysis of the D-Ala–D-Ala dipeptide by the D,D-dipeptidase encoded by the *vanX* gene. Environmental stressors such as dyes and heavy metals have been demonstrated to co-induce antibiotic resistance determinants through global regulatory stress networks, despite being generally associated with antibiotic pressure. Given that *Lactobacillus* species with enzymes that provide both bile and antibiotic tolerance have been shown to exhibit bifunctional stress resistance patterns, this response may represent an indirect protective strategy. The upregulated expression of *vanX* is likely part of a common cellular defense mechanism against xenobiotic stress rather than a specific response to antibiotic exposure. The high induction of *sodA* relative to dye concentrations (maximum of 150-fold) suggests that exposure to Allura Red induces oxidative stress through the generation of ROS. Superoxide dismutase is an important component of the antioxidant defence system in lactic acid bacteria, where it contributes to the detoxification of superoxide radicals and protection against oxidative damage [29]. Increased *SodA* expression enhances the detoxification of superoxide radicals, thereby protecting cellular macromolecules and maintaining redox homeostasis. Comparable increases in expression of *sodA* have been reported in lactic acid bacteria under exposure to oxidants and toxic compounds, highlighting its critical role in stress tolerance and probiotic survival.

The upregulation of *dnaK* further supports the activation of a general stress response system. Its role in reducing proteotoxic stress is highlighted by the notable increase in *dnaK* transcription following Allura Red treatment. Heat shock chaperone *dnaK* helps refold denatured proteins and preserve proteostasis in challenging circumstances. The presence of *sodA* as a co-expressed gene suggests that it acts in a coordinated response to oxidative and proteotoxic stress and has been described in a number of *Lactobacillus* strains in response to bile or environmental stresses. As a key component of bacterial stress-management systems, elevated *dnaK* expression in *Lactobacillus* species has been associated with enhanced resistance to heat and chemical stressors [30]. The simultaneous upregulation of *dnaK, sodA* and *bshA* suggests coordinated activation of cellular defense and repair pathways.

The coordinated modulation of *bshA*, *bshB*, *vanX*, *sodA* and *dnaK* in *L. acidophilus* indicates an integrated stress response strategy that improves survival under xenobiotic pressure. These findings are consistent with the ecological adaptability of *Lactobacillus* species, which are well adapted to survive under conditions of bile stress, oxidative stress, and exposure to antimicrobial agents. Furthermore, as previously observed in probiotic *Lactobacillus* models, stress-induced overexpression of BSH-related genes may change bile acid pools and cholesterol metabolism, influencing host metabolic consequences [31].

5. CONCLUSION

Overall, exposure to the synthetic food dye Allura Red induces significant changes in the expression of selected stress-responsive genes in *L. acidophilus*, indicating a complex cellular response to chemical stress. The coordinated modulation of genes associated with detoxification (*bshA* and *bshB*), antibiotic resistance (*vanX*), oxidative response (*sodA*), and protein homeostasis (*dnaK*) demonstrates that the bacteria employ a multilayered strategy to maintain cellular homeostasis. The upregulation of bile salt hydrolase genes suggests membrane-associated defense mechanisms that help mitigate the disruptive effects of xenobiotic compounds, whereas increased expression of *sodA* suggests an enhanced antioxidant response to ROS produced by dye exposure. Induction of *dnaK* also emphasizes the need

for protein refolding and stabilization as part of the stress response mechanism. The upregulation of *vanX* indicates the activation of cross-protective resistance pathways that may overlap with antibiotic defense mechanisms, emphasizing broader environmental resilience. Collectively, these molecular activities highlight the adaptive capacity of *L. acidophilus*, which may improve its survival in chemically challenging conditions, such as the gut environment exposed to food additives. These findings raise concerns that exposure to synthetic dyes may alter bacterial gene expression, thereby affecting probiotic activity and stress response dynamics.

6. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be authors as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All the data are available with the authors and shall be provided upon request.

11. PUBLISHER'S NOTE

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12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI) tools for writing and editing of the manuscript, and no images were manipulated using AI.

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