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Transcriptomic Analysis of Probiotic Oxidative Stress Resistance in Anti-inflammatory Pathways

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ABSTRACT

Oxidative stress caused by reactive oxygen species such as H_2O_2 and HOCl plays a central role in inflammation-related diseases. This study aimed to identify key genes and biological pathways that enable probiotics to tolerate oxidative stress, using transcriptomic analysis of *E. coli*, *L. plantarum*, and *L. reuteri* under exposure to H_2O_2 and HOCl. We retrieved three related probiotics datasets from the Gene Expression Omnibus (GEO) database and the Sequence Read Archive (SRA) databases, including *Lactobacillus plantarum* (GSE99096), *Lactobacillus reuteri* (GSE127961), and *Escherichia coli* (GSE144068). We used the CLC Genomics Workbench software to identify the differentially expressed genes (DEGs) and then applied STRING 11.5 to identify the interactions between the DEGs. The CytoHubba was used to determine the hub genes in the interactive networks. We assessed the Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway analysis of hub genes and evaluated the associated biological pathways. Among the identified hub genes, *GuaA* and *Tig* in *E. coli* were found to be uniquely involved in purine metabolism and ribosome assembly, highlighting novel targets for oxidative stress resistance. In addition, *ComEA* in *L. plantarum* and *UvrB*, *Mfd* and *GrpE* in *L. reuteri* represent diverse molecular strategies used by probiotics to cope with oxidative stress. These genes were associated with key pathways such as purine metabolism, mismatch repair, nucleotide excision repair and the pentose phosphate pathway. These critical genes and biological pathways can be used to improve the efficacy of probiotics in treating inflammatory diseases.

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Introduction

Reactive oxygen species (ROS) such as H_2O_2 and HOCl disrupt biomolecular stability and are key triggers of inflammation (Naji *et al*., 2025). Dysbiosis in the gut microbiota has been identified as a significant cause of oxidative stress in the body, which increases ROS generation and inflammatory processes (Ajam-Hosseini *et al*., 2024). Probiotics, which are live microorganisms that confer health benefits to the host (Heydari *et al*., 2022), have been shown to reduce oxidative stress and improve inflammatory diseases through various mechanisms such as metal ion chelating ability, antioxidant enzymes system, antioxidant metabolites and regulation of gut microbiota. Their ability to tolerate oxidative challenges, such as H_2O_2 and HOCl, critically determines their effectiveness in modulating inflammation (Pravda, 2020). While the stress response of inflammation-enriched enterobacteria, such as E. coli, is well-

characterized, studies on oxidative stress responses in beneficial probiotic bacteria remain limited in number and scope, often focusing on a few model strains, while broader mechanisms remain underexplored (Calderini *et al*., 2017). This research gap is mainly due to the historical focus of microbiology on pathogenic organisms with medical relevance, which has resulted in relatively fewer and more narrowly focused studies on oxidative stress responses in probiotic species. Several studies have explored oxidative stress responses in lactic acid bacteria beyond the commonly studied strains, including *Lactococcus lactis*, *Lactobacillus casei*, and *Lactobacillus rhamnosus*. These investigations have revealed conserved mechanisms involving purine metabolism, DNA repair, and protein folding that support survival under oxidative and other environmental stresses (Duwat *et al*., 2000; Broadbent *et al*., 2010; Zhang *et al*., 2020). These findings help establish a broader context for understanding how probiotics respond to oxidative damage.

Recent advancements in bioinformatics have introduced tools such as Metascape and single-cell RNA sequencing (scRNA-seq), which enable deeper analyses of oxidative stress-related gene networks in microbial systems and could be utilized in future probiotic studies (Hong *et al*., 2024).

Recent clinical trials have demonstrated that probiotic supplementation can significantly reduce oxidative stress and inflammation in patients with mild to moderate Alzheimer's disease, as evidenced by decreased serum levels of malondialdehyde and inflammatory cytokines (Akhgarjand *et al*., 2022). Similarly, a randomized controlled trial showed that daily consumption of synbiotic yogurt containing *Lactobacillus plantarum* and *Lactobacillus pentosus* improved antioxidant enzyme activities, including glutathione peroxidase and superoxide dismutase, in adults with metabolic syndrome (Zolghadrpour *et al*., 2024). These findings underscore the emerging role of probiotics in modulating oxidative stress responses in various inflammatory conditions.

To address this gap, we conducted a comparative transcriptomic analysis to investigate oxidative stress responses of three probiotic strains *E. coli Nissle* 1917, *L. plantarum*, and *L. reuteri*, under exposure to H_2O_2 and HOCl, using publicly available RNA-seq datasets and bioinformatics analysis. The analysis revealed the enrichment of key stress-related pathways such as nucleotide excision repair (NER), mismatch repair (MMR), homologous recombination (HR), purine metabolism, and the pentose phosphate pathway (PPP). These pathways are known to contribute to DNA damage repair and redox balance under stress conditions (Batty *et al*., 2000; Zhang *et al*., 2014; Christodoulou *et al*., 2018). Additionally, several stress-responsive hub genes were identified, including *GrpE*, which assists in protein folding, *Mfd*, involved in transcription-coupled DNA repair, and *guaA*, which participates in purine biosynthesis (López de Felipe *et al*., 2021; Duwat *et al*., 2000). These shared mechanisms across species provide a deeper understanding of how probiotics cope with oxidative damage and may inform future strategies to enhance their efficacy in managing inflammation and supporting gut health.

Materials and Methods

Data collection

We searched the sequence read archive (SRA) database (https://www.ncbi.nlm.nih.gov/sra), European nucleotide archive (ENA) database (https://www.ebi.ac.uk/ena) and gene expression omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) to identify appropriate datasets for our study. We selected datasets that met the following criteria: experimental technique (RNA-seq), organism (probiotic bacteria), groups (under oxidative stress treatment *vs*. control), and treatment duration (at least 30 minutes). Only datasets that included H_2O_2 or HOCl oxidative stress treatments were included. The selected datasets were downloaded and analyzed.

Screening of differentially expressed genes

We performed bioinformatics analysis of RNA-seq datasets using CLC Genomics Workbench version 20. Each dataset was analyzed individually, starting with a quality control assessment of the raw reads. The raw reads were then trimmed with default parameters (maximum number of ambiguities: 2 and quality scores limit: 0.05) and mapped to the reference genomes. Finally, the differentially expressed genes

(DEGs) were identified between the H_2O_2 or HOCl-treated group and control groups using parameters such as FDR *P*-value less than 0.05 and (|log fold-change (logFC)| greater than 1.0. All analyses were executed using CLC software.

Protein-protein interaction network analysis and hub gene identification

We used the Search Tool for the Retrieval of Interacting Genes (STRING) database (https://string-db.org) to extract the protein-protein interaction (PPI) network of DEGs for each. A confidence score of ≥ 0.4 was used as the analysis parameter. The PPI network was visualized using Cytoscape version 3.10. Hub genes were identified using the CytoHubba plug-in of Cytoscape with degree, closeness, and betweenness algorithms. The intersection of genes between the three algorithms was considered the hub genes.

Functional enrichment analysis of hub gene and related neighbor genes

We determined the neighbor genes associated with hub genes using the STRING database with a confidence score of ≥ 0.4 and a maximum number of interactors of <= 20. We performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis using STRING for the hub genes and their associated neighbor genes. Significant GO and KEGG terms were filtered based on FDR, with a *P*-value less than 0.05.

Results

Data collection

After searching the SRA, ENA, and GEO databases, three datasets were identified that met the specified criteria. The first dataset, GSE144068, was generated using the GPL26262 Illumina NextSeq 500 platform and included six samples of RNA sequencing from *Escherichia coli* Nissle 1917 before and after HOCl treatment (for 30 min). The second dataset, GSE99096, was generated using the GPL23496 Illumina HiSeq 2500 platform and included six samples of RNA sequencing from *L. plantarum* that were treated with H_2O_2 for 30 min compared to the control (before treatment). The third dataset, GSE127961, was generated using the GPL21222 Illumina HiSeq 2500 platform and included 12 samples of RNA sequencing from L. reuteri that were treated with both H2O2 and HOCl for 30 minutes, compared to the control (before treatment), as described in Table 1.

Table 1. The additional information of datasets.

Probiotics	Acc num*	Platform	Stress treat/ time**	Number of samples	Concent stress sub***
Escherichia coli Nissle 1917	GSE144068	GPL26262	HOCl/ 30 min	6 (3 cases & 3 control)	0.4 mM
Lactobacillus plantarum	GSE99096	GPL23496	H_2O_2/ 30 min	6 (3 cases & 3 control)	5 mM
Lactobacillus reuteri	GSE127961	GPL21222	H_2O_2/ 30 min	6 (3 cases & 3 control)	0.12 mM
			HOCl/ 30 min	6 (3 cases & 3 control)	1.25 mM

*= Accession number; **= Stress treatment/ time; ***= Concentration of stress-inducing substances

Screening of DEGs

The identified datasets were imported into the CLC Genomics Workbench software and analyzed separately. After performing all the necessary analysis steps, DEGs were obtained from each dataset (Table 2).

PPI network construction and hub gene screening

The PPI network of DEGs for each dataset was constructed using the STRING database and visualized in Cytoscape software. The *E. coli* dataset contained 458 nodes and 2417 edges (HOCl treatment), the *L. plantarum* dataset contained 455 nodes and 832 edges for H_2O_2 treatment (Fig. 1A and B), and the *L. reuteri* dataset contained 133 nodes and 257 edges for H_2O_2 treatment and 40 nodes and 41 edges for HOCl treatment (Fig. 2A and B).

Using the CytoHubba plugin with degree, closeness, and betweenness methods, hub genes were identified. For the *E. coli* dataset after HOCl treatment, *guaA* and *tig* were identified as the hub genes.

Only *comEA* was identified as the hub gene for the *L. plantarum* dataset after H_2O_2 treatment. For the *L. reuteri* dataset, *ComEC (Lreu_0645*) was identified as the hub gene for H_2O_2 treatment, and UvrB, Mfd, DeoC, and *GrpE* were identified as hub genes for HOCl treatment. These selected

hub genes for each probiotic could be considered representative genes against oxidative stress.

Functional enrichment analysis of hub genes and related neighbor genes

First, neighbor genes that were correlated with hub genes were identified using the STRING database (Fig. 3). Functional enrichment analysis of hub genes and their neighbor genes, including GO and KEGG analysis, was then performed using the same database and the most significantly enriched GO terms for each bacterial dataset were extracted (Fig. 4 and 5).

Table 2. The complete information of DEGs.

Probiotics	Stress treatment	DEGs count	Up- and down-regulated gene count
Escherichia coli Nissle 1917	HOCl	555	+300 and -255
Lactobacillus plantarum	H_2O_2	593	+230 and -363
Lactobacillus reuteri	H_2O_2	198	+82 and -11-
	HOCl	64	+40 and -24

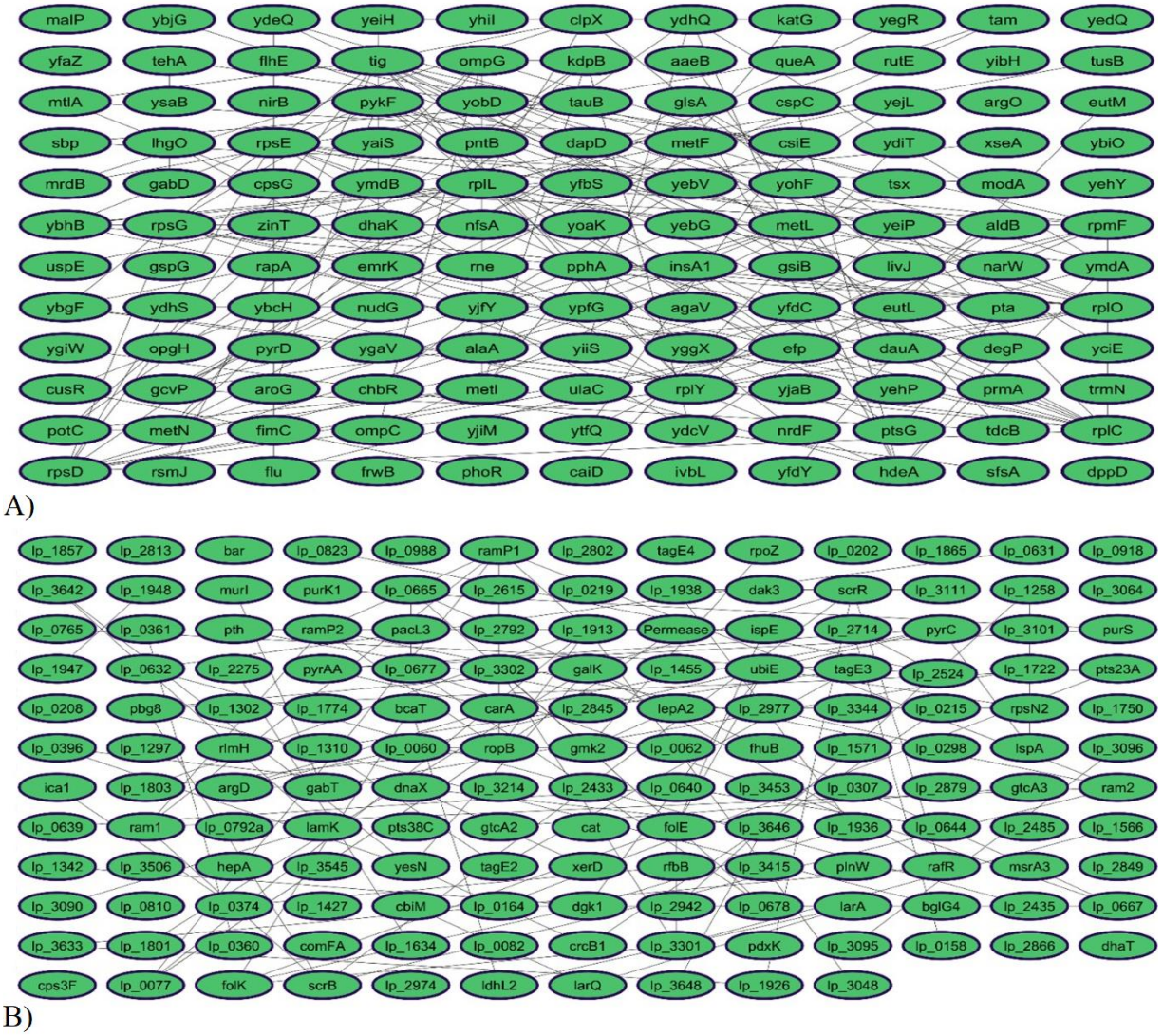

Fig. 1. PPI networks of DEGs in *E. coli* and *L. plantarum*: A) PPI network of DEGs in *E. coli*; B) PPI network of DEGs in *L. plantarum*. Each node represents a protein encoded by a DEG, and each edge represents a predicted functional or physical interaction based on the STRING database analysis.

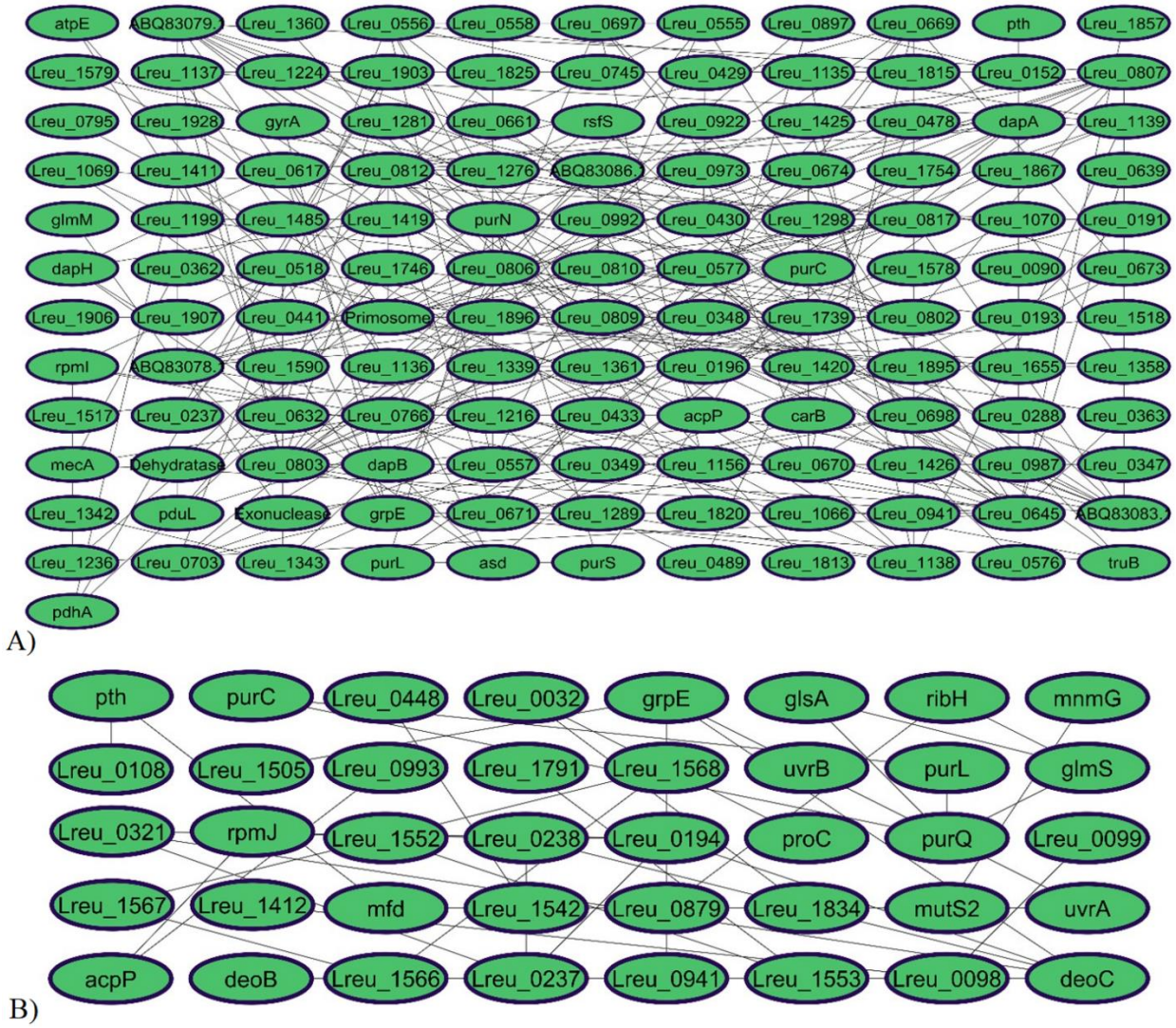

Fig. 2. PPI networks of DEGs in L. reuteri under H_2O_2 and HOCl stress: A) PPI network of DEGs in *L. reuteri* under H_2O_2 stress; B) PPI network of DEGs in *L. reuteri* under HOCl stress. Each node represents a protein encoded by a DEG, and each edge represents a predicted functional or physical interaction based on STRING database analysis with confidence scores ≥ 0.4.

The complete GO results for each bacterial dataset were investigated (Table 3).

KEGG analysis revealed that "ribosome" and "purine metabolism" were the most enriched pathways in the *E. coli*-HOCl dataset (Fig. 6A), while "DNA replication", "Mismatch repair" and "Homologous recombination" were the most significantly enriched pathways in the *L. plantarum*-H_2O_2 dataset (Fig. 6B). For the *L. reuteri*-H_2O_2 dataset, "Homologous recombination", "DNA replication" and "Mismatch repair" were the most enriched pathways (Fig. 6C) and for the *L. reuteri*-HOCl dataset, "RNA polymerase", "Nucleotide excision repair" and "Pentose phosphate pathway" were the most significantly enriched pathways (Fig. 6D).

Discussion

This study investigated transcriptomic adaptations of *E. coli Nissle* 1917, *L. plantarum*, and *L. reuteri* to oxidative stress induced by H_2O_2 and HOCl. Oxidative stress, a key driver of inflammation and dysbiosis (Pruchniak *et al*., 2016), challenges the survival of probiotics, although tolerance has been linked to anti-inflammatory effects (Heydari *et al*., 2022).

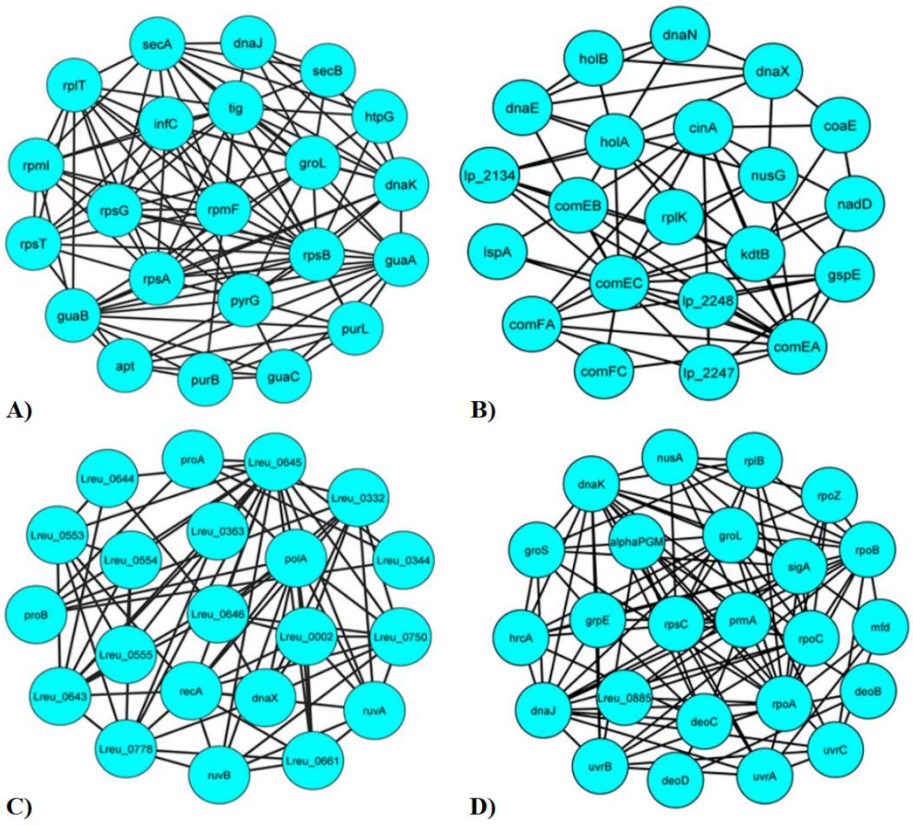

Fig. 3. Interaction networks of Hub genes and their first-degree interacting partners in oxidative stress response: A) Network showing hub gene *GrpE* in *E. coli* and its interacting partners; B) Network showing hub gene *Mfd* in *L. plantarum* and its interacting partners; C) Network showing hub genes *ComEA*, *UvrB*, and *DeoC* in *L. reuteri* and their interacting partners; D) Network showing hub gene *ComEC* (Lreu_0645) in *L. reuteri* and its interacting partners. All networks were generated using the STRING database (confidence score≥ 0.4). High-connectivity patterns highlight the central roles of these hub genes in stress-related functional modules such as protein folding, DNA repair, and purine metabolism.

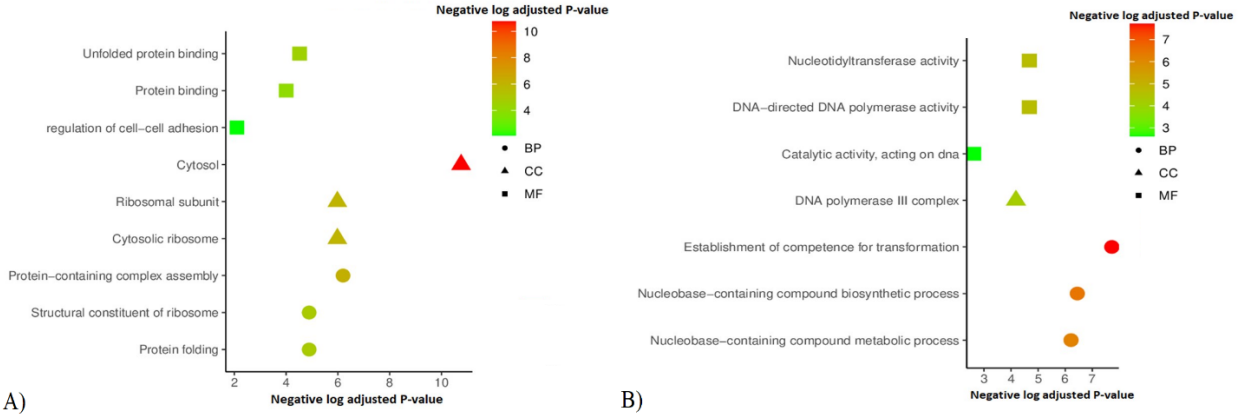

Fig. 4. GO enrichment analysis of DEGs in *E. coli* and *L. plantarum*: A) GO enrichment analysis of DEGs in *E. coli*, highlighting terms related to ribosome assembly and purine biosynthesis; B) GO enrichment analysis of DEGs in *L. plantarum*, showing enrichment in DNA replication and nucleobase biosynthetic processes. The enriched terms are grouped into biological process (BP), cellular component (CC), and molecular function (MF), reflecting species-specific responses to oxidative stress.

Enrichment of pathways such as purine metabolism, PPP, and NER indicates conserved and stressor-specific mechanisms in redox regulation and genome maintenance. These findings require further validation to confirm their functional and translational relevance. As mentioned below, several key biological pathways were identified as being enriched in the analyzed datasets, highlighting their potential role in the oxidative stress response of probiotics.

In *L. reuteri* under HOCl stress, the NER pathway was enriched, with *UvrB* identified as a hub gene, suggesting pathway activation in response to oxidative DNA damage. NER involvement in oxidative, acidic, and heat stress tolerance has similarly been observed in *E. coli*, *L. helveticus*, *S. mutans*, *L. lactis*, *T. thermophilus*, and *A. pasteurianus* (Truglio *et al*., 2006; Dhawale *et al*., 2021; Cappa *et al*., 2005; Hanna *et al*., 2001; Hartke *et al*., 1995; Kajfasz *et al*., 2011; Yamamoto *et al*., 1996; Zheng *et al*., 2015).

Homologous recombination (HR) was among the most enriched pathways in *L. plantarum* and *L. reuteri* under H_2O_2 stress, indicating its role in maintaining genome stability in response to oxidative damage. This mechanism, which facilitates error-free repair of double-strand breaks, has been associated with stress tolerance in lactic acid bacteria (Zhang *et al*., 2014).

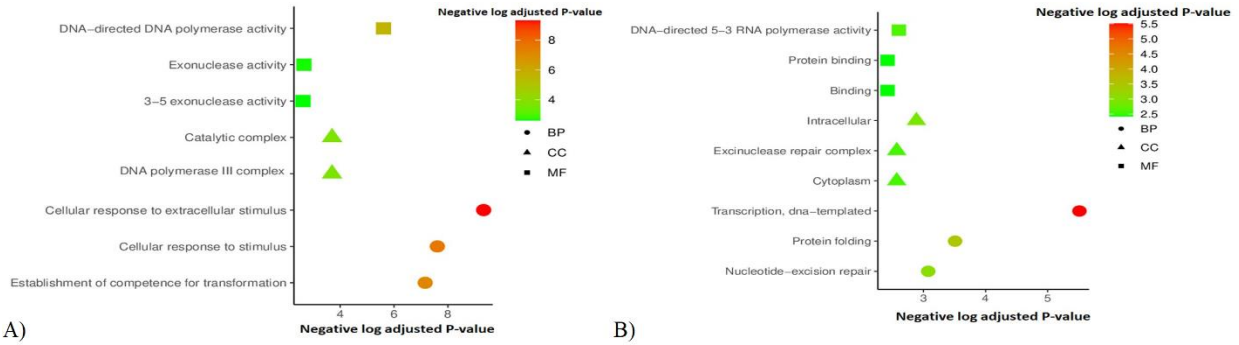

Fig. 5. GO enrichment analysis of DEGs in *L. reuteri* under oxidative stress: A) GO enrichment analysis of DEGs in *L. reuteri* under H_2O_2 stress showing significant biological processes such as DNA repair, response to stimulus, and cellular biosynthetic processes; B) GO enrichment analysis of DEGs in *L. reuteri* under HOCl stress highlighting similar biological processes along with key molecular functions including DNA polymerase activity and protein binding. These enriched terms support the activation of defense mechanisms related to genome stability and redox adaptation under oxidative stress. The enriched terms are grouped into biological process (BP), cellular component (CC), and molecular function (MF), reflecting species-specific oxidative stress responses.

The MMR was significantly enriched in *L. plantarum* and *L. reuteri* under H_2O_2 stress, suggesting its activation as part of the DNA damage response in these strains. As a conserved system that corrects base mispairing and maintains genome integrity, MMR has been implicated in resistance to oxidative stress in bacteria (Gu *et al*., 2020).

The PPP was enriched *in L. reuteri* under HOCl-induced oxidative stress, highlighting its potential role in cellular defense. This pathway contributes to oxidative stress resistance primarily by generating NADPH, which is essential for maintaining redox homeostasis and fueling antioxidant systems such as glutathione and thioredoxin. Previous studies have demonstrated that PPP activation enhances bacterial tolerance to ROS and supports survival under oxidative challenge (Christodoulou *et al*., 2018; Zhang *et al*., 2021). Purine metabolism was highly enriched in *E. coli* under HOCl stress, suggesting its contribution to maintaining energy homeostasis and nucleotide turnover during oxidative challenge. Previous studies have demonstrated that stress conditions such as bile and oxidative damage enhance purine nucleotide metabolism in probiotic strains like *L. salivarius* and *L. plantarum*, potentially increasing ATP availability and supporting bacterial survival (Lv *et al*., 2017). In addition to the enriched pathways, a set of specific hub genes (as listed below) was identified that may play critical roles in supporting probiotic survival under oxidative stress.

Table 3. The GO analysis for *E. coli Nissle* 1917-HOCl.

Groups	GO terms	Fdr P-value<0.05
A) *E. coli Nissle* 1917-HOCl		
Biological process	Protein-containing complex assembly	6.34×10^{-07}
	Protein folding	1.29×10^{-05}
	Cellular nitrogen compound biosynthetic process	1.29×10^{-05}
	Organonitrogen compound biosynthetic process	1.29×10^{-05}
	Translation	4.00×10^{-05}
	Purine ribonucleoside monophosphate biosynthetic process	5.54×10^{-05}
	Ribonucleoprotein complex assembly	5.54×10^{-05}
	Ribosome assembly	5.54×10^{-05}
	De novo protein folding	5.90×10^{-05}
	Ribonucleotide metabolic process	6.19×10^{-05}
Cellular component	Structural constituent of ribosome	3.00×10^{-05}
	Unfolded protein binding	9.92×10^{-05}
	Protein binding	0.0079
Molecular function	Cytosol	1.75×10^{-11}
	Cytosolic ribosome	1.05×10^{-06}
	Ribosomal subunit	1.05×10^{-06}
	Protein-containing complex	2.85×10^{-05}
	Cytosolic small ribosomal subunit	0.0002
	Cytosolic large ribosomal subunit	0.0094
B) *L. plantarum*-H_2O_2		
Biological process	Establishment of competence for transformation	1.90×10^{-08}
	Nucleobase-containing compound biosynthetic process	3.57×10^{-07}
	Nucleobase-containing compound metabolic process	6.09×10^{-07}
	Cellular nitrogen compound metabolic process	3.49×10^{-06}
	Cellular nitrogen compound biosynthetic process	1.18×10^{-05}
	DNA replication	1.93×10^{-05}
	Nitrogen compound metabolic process	5.94×10^{-05}
	Primary metabolic process	0.00087
	Cellular biosynthetic process	0.0012
	DNA metabolic process	0.0015
Cellular component	DNA-directed DNA polymerase activity	2.11×10^{-05}
	Nucleotidyltransferase activity	2.11×10^{-05}
	Catalytic activity, acting on DNA	0.0024
	3-5 exonuclease activity	0.0202
	Transferase activity, transferring phosphorus-containing groups	0.0202
Molecular function	DNA polymerase III complex	6.59E-05
C) *L. reuteri*-H_2O_2		
Biological process	Cellular response to extracellular stimulus	4.77×10^{-10}
	Cellular response to stimulus	2.49×10^{-08}
	Establishment of competence for transformation	7.03×10^{-08}
	DNA metabolic process	7.43×10^{-05}
	Heterocycle metabolic process	0.0002
	Organic cyclic compound metabolic process	0.00021
	DNA replication	0.00032
	DNA repair	0.00082
	Heterocycle biosynthetic process	0.0014
	Organic cyclic compound biosynthetic process	0.0015
Cellular component	DNA-directed DNA polymerase activity	2.47×10^{-06}
	Exonuclease activity	0.0022
	3-5 exonuclease activity	0.0024
	Catalytic activity, acting on dna	0.0029
	DNA binding	0.0194
	Transferase activity, transferring phosphorus-containing groups	0.022
Molecular function	DNA polymerase III complex	0.0002
	Catalytic complex	0.0002
D) *L. reuteri*-HOCl		
Biological process	Transcription, DNA-templated	3.08×10^{-06}
	Protein folding	0.00031
	Nucleotide-excision repair	0.00084
	Cellular macromolecule metabolic process	0.0011
	Cellular macromolecule biosynthetic process	0.0048
	Nucleobase-containing compound biosynthetic process	0.0058
	Nucleobase-containing compound metabolic process	0.0198
	Cellular process	0.0198
	Gene expression	0.031
	Cellular nitrogen compound metabolic process	0.0311
Cellular component	DNA-directed 5-3 RNA polymerase activity	0.0025
	Binding	0.0038
	Protein binding	0.0038
	Organic cyclic compound binding	0.0038
	Heterocyclic compound binding	0.0038
	Nucleic acid binding	0.0048
	Excinuclease abc activity	0.0048
	Unfolded protein binding	0.0061
	DNA binding	0.0092
Molecular function	Intracellular	0.0013
	Cytoplasm	0.0027
	Excinuclease repair complex	0.0027
	Cellular anatomical entity	0.0058

The *GrpE* gene was identified as a hub gene in *L. reuteri* following HOCl treatment and was upregulated, suggesting its role in protein quality control under oxidative stress. This heat shock protein facilitates protein folding and prevents aggregation during stress. Similar *GrpE* upregulation has been reported in *L. plantarum* WCFS1 under phenol-induced oxidative stress (López de Felipe *et al*., 2021), under ethanol stress (van Bokhorst-van de Veen *et al*., 2011), in *L. acidophilus* with polyphenols (Mazzeo *et al*., 2015), in *L. bulgaricus* under acid stress (Fernandez *et al*., 2008), and in *L. casei* (Heunis *et al*., 2014), indicating a conserved function in proteotoxic stress adaptation.

The *Mfd* gene was identified as a hub gene in *L. reuteri* under HOCl stress and was upregulated, suggesting its role in transcription-coupled DNA repair. *Mfd* encodes a factor that displaces stalled RNA polymerase at DNA lesions and recruits repair machinery (Deaconescu *et al*., 2006). Consistent with our findings, *Mfd* upregulation has been observed in *L. plantarum* WCFS1 under resveratrol-induced oxidative stress (López de Felipe *et al*., 2021), and *Bacillus subtilis* strains lacking *Mfd* show reduced survival under similar conditions (Martin *et al*., 2019), supporting its conserved role in oxidative stress adaptation.

The *GuaA* gene, which encodes guanosine monophosphate synthetase (GMPS), was identified as a hub gene in E. coli under HOCl stress, indicating a potential role in maintaining purine nucleotide synthesis during oxidative stress. While *GuaA* primarily contributes to GMP biosynthesis from IMP, its function becomes particularly critical under stress conditions when purine availability is limited. Our findings are consistent with previous studies demonstrating that mutations in *GuaA* reduce stress tolerance in *Lactococcus lactis* under oxidative (H_2O_2), heat, and acidic stress (Duwat *et al*., 2000; Rallu *et al*., 2000). Additionally, suppression of *GuaA* expression has been observed under acidic stress in *L. casei*, linked to the accumulation of (p)ppGpp and modulation of stress response pathways (Broadbent *et al*., 2010). These observations suggest that *GuaA* plays a broader role in stress adaptation by regulating purine metabolism and maintaining intracellular nucleotide pools.

The *UvrB* gene, a key component of the NER system, was identified as a hub gene in *L. reuteri* under HOCl stress and was upregulated, indicating its role in oxidative DNA damage repair. As a damage sensor, *UvrB* initiates NER by detecting nucleotide lesions (Crowley *et al*., 2006). Previous studies have reported *UvrB* upregulation in *L. plantarum* under salt stress (Li *et al*., 2019; Zhao *et al*., 2014), in *B. longum* under acidic stress (Jin *et al*., 2012), in *Enterobacter* under oxidative stress (Fei *et al*., 2020), and its involvement in antioxidant defense in *E. faecalis* (Arntzen *et al*., 2015), supporting its conserved role in stress tolerance.

Among the identified hub genes, *Competence protein (ComEA)* and *Trigger factor (Tig)* showed decreased expression under oxidative stress. *comEA* was downregulated in *L. plantarum* exposed to H_2O_2, while *tig* expression decreased in *E. coli* under HOCl stress.

ComEA encodes a membrane-bound DNA uptake protein essential for competence in bacteria such as *B. subtilis* and *S. pneumoniae* (Chen and Dubnau, 2004; Johnsborg *et al*., 2009), and *tig* encodes trigger factor, a ribosome-associated chaperone aiding in nascent protein folding (Bhandari and Houry, 2015; Saio *et al*., 2014). Although these genes are typically upregulated under environmental stress, their downregulation here may reflect strain- or condition-specific regulatory responses, emphasizing the complexity of probiotic adaptation mechanisms.

To interpret stimulus-specific probiotic responses, we compared the expression of hub genes under different oxidative stressors. In *L. reuteri* exposed to HOCl, *GrpE* and *Mfd* were significantly upregulated, reflecting enhanced protein folding and transcription-coupled repair activities. These findings align with reports of *GrpE* and *Mfd* induction in *L. plantarum*, *L. acidophilus*, and *B. subtilis* under similar stress conditions (Felipe *et al*., 2021; Mazzeo *et al*., 2015; Martin *et al*., 2019). Also, under HOCl, *UvrB* was upregulated in *L. reuteri*, supporting its role in nucleotide excision repair, as previously observed in lactic acid bacteria and Enterobacteriaceae under salt and acid stress (Li *et al*., 2019; Fei *et al*., 2020). Under H_2O_2 stress, *ComEA* was downregulated in *L. plantarum*, despite its established role in competence and genome repair (Chen and Dubnau, 2004).

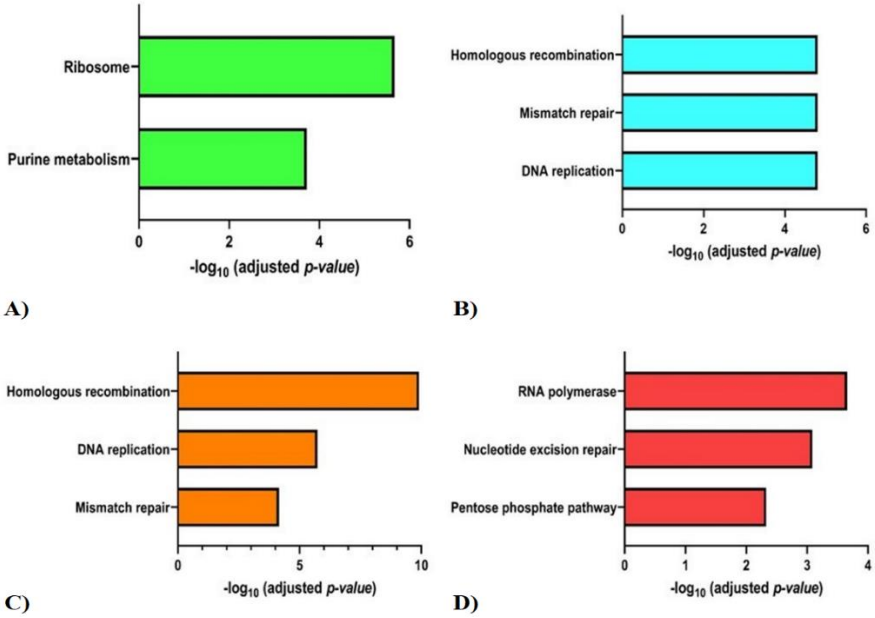

Fig. 6. KEGG pathway enrichment analysis of DEGs across probiotic strains: A) Enriched pathways in *E. coli* under HOCl stress, highlighting purine metabolism and ribosome function; B) Enriched pathways in *L. plantarum* under H_2O_2 stress, showing DNA replication and mismatch repair; C) Enriched pathways in *L. reuteri* under H_2O_2 stress, including homologous recombination and DNA replication; D) Enriched pathways in *L. reuteri* under HOCl stress, featuring RNA polymerase, nucleotide excision repair, and the pentose phosphate pathway.

In *E. coli*, *Tig* and *GuaA* were downregulated following HOCl exposure. *GuaA*, linked to purine biosynthesis and stress adaptation in *L. lactis* and *tig*, encoding a ribosome-associated chaperone, may be differentially regulated under HOCl stress (Duwat *et al*., 2000; Bhandari and Houry, 2015). These results demonstrate both conserved responses, such as DNA repair, and species- and stressor-specific transcriptional patterns.

A comparative analysis of H_2O_2 and HOCl responses revealed stimulus-specific patterns across the studied strains. While H_2O_2 in *L. plantarum* led to the downregulation of competence-related genes, such as ComEA, HOCl in L. reuteri triggered the strong upregulation of stress-response genes, including *GrpE*, *Mfd*, and *UvrB*. In *E. coli*, HOCl stress suppressed genes involved in protein folding (*Tig*) and metabolism (*GuaA*). These differences reflect the broader reactivity of HOCl with cellular proteins compared to the more DNA-targeted oxidative effects of H_2O_2.

Despite its insights, this study has limitations. For instance, experimental validations such as qPCR, gene overexpression, and gene knockout models are needed to confirm the roles of identified genes in oxidative stress resistance. Additionally, the RNA-seq datasets varied in oxidative stressor type and concentration (e.g., 5 mM H_2O_2 in *L. plantarum*, 0.12 and 1.25 mM H_2O_2 in *L. reuteri* and 0.4 mM HOCl in *E. coli*), as well as sequencing platforms (HiSeq vs. NextSeq), introducing heterogeneity that may impact interspecies comparisons. For example, DEG counts varied markedly between species, with 555 in *E. coli* and 64 in *L. reuteri*, likely reflecting both experimental factors and intrinsic differences between Gram-negative and Gram-positive bacteria. To reduce such variability, we applied standardized analysis thresholds (FDR < 0.05, |logFC| > 1.0) and focused on consistently regulated genes and pathways. Still, cross-dataset comparisons should be interpreted cautiously due to potential inter-study variability. Species-specific transcriptional dynamics and platform-related variability may have influenced expression outcomes. Thus, while our analysis highlights key stress-response candidates, experimental validation remains essential to confirm their biological significance.

Comparative metatranscriptomic analyses have shown that host-microbiota interactions vary in inflammatory conditions like IBD, highlighting the role of specific microbial taxa in modulating

immune responses (Priya *et al*., 2022). These insights suggest that integrating probiotic transcriptomic data with broader gut microbiota dynamics could enhance our understanding of inflammation. Similarly, changes in microbial composition and function have been linked to immune regulation and disease progression (Chen *et al*., 2024).

Conclusion

The efficacy of probiotics in the host's body relies on their ability to resist oxidative stress, such as H_2O_2 and HOCl. The results of this study, including the identification of key genes and pathways involved in probiotic resistance against oxidative stress, provide a broad perspective of how each probiotic responds to and copes with oxidative stress. These findings may provide a basis for improving probiotic efficacy by enhancing their survival and resistance to oxidative stress; however, further experimental studies are necessary to confirm their practical applicability and therapeutic relevance. Based on the key roles identified for genes such as *GrpE*, *Mfd*, and *GuaA* in oxidative stress resistance, these genes may serve as potential targets for future probiotic strain improvement. For instance, *GrpE* has been associated with enhanced protein folding capacity under stress, *Mfd* contributes to transcription-coupled DNA repair, and GuaA is involved in purine metabolism, which supports nucleotide synthesis during oxidative damage. Modulating the expression of these genes through overexpression or selection of naturally high-expressing strains could improve the survival and functionality of probiotics under inflammatory and oxidative environments. These insights may guide future research into improving probiotic stress tolerance; however, their therapeutic relevance remains to be experimentally validated. However, further experimental studies will be required to validate these findings and translate them into clinical practice.

Authors' Contributions

NA Study concept and design; **AH** Acquisition of data; **AH**, **FP**, and **NA** analysis and interpretation of data; **AH**, and **FP** Drafting of the manuscript; **FP**, and **NA** Critical revision of the manuscript for important intellectual content; **FP**, and **NA** Study supervision. All authors have made a significant contribution to this study and have approved the final manuscript.

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Availability of data and materials

All data produced and presented throughout the study are included in the manuscript.

Conflict of interests

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