Identification Of Lactobacillus Acidophilus Proteins by Using 2-D Gel Electrophoresis and MALDI- TOF Under the Influence of Sodium Fluoride

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***Abstract*—Proteomics, the large-scale study of proteins, is an effective approach for understanding the molecular mechanisms and physiological responses of probiotic bacteria under stress conditions. Although proteomic investigations of probiotics are relatively recent, they have become increasingly important for elucidating bacterial metabolism, adaptation, and survival. In the present study, the proteomic response of Lactobacillus acidophilus to sodium fluoride treatment was investigated using two-dimensional gel electrophoresis (2D-GE). Protein profiles of treated and untreated cells were analyzed with Image Master 2D Platinum 6.0 software. A total of 170 protein spots were clearly resolved and detected on the gels. Among these, 12 protein spots exhibited significant differential expression in response to sodium fluoride exposure. Five protein spots were found to be upregulated, whereas seven were downregulated when compared with the control. The differentially expressed protein spots were excised and subjected to matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for protein identification and functional characterization. The identified proteins were associated with diverse cellular processes, including metabolism, stress response, protein synthesis, and cellular adaptation. These findings provide insights into the molecular mechanisms employed by L. acidophilus to cope with fluoride-induced stress and enhance our understanding of the physiological adaptations of probiotic bacteria under adverse environmental conditions. This study demonstrates the utility of proteomic analysis in identifying stress-responsive proteins and contributes to the broader understanding of probiotic bacterial biology.**

***Index Terms*—Proteomics, Lactobacillus acidophilus, Sodium fluoride, 2D gel electrophoresis, MALDI-TOF MS, Differential protein expression, Stress response.**

I. INTRODUCTION

Fluoride is a non-metallic halogen and the thirteenth most abundant element in the Earth's crust. At appropriate concentrations, fluoride contributes to the maintenance of dental and skeletal health (Dehnen et al., 2021; Ullah et al., 2018). However, excessive fluoride intake, particularly through drinking water, has become a major public health concern worldwide and is associated with dental fluorosis, skeletal fluorosis, and other systemic disorders (Kumari et al., 2024). Elevated fluoride concentrations have also been reported to exert toxic effects on microorganisms by disrupting cellular metabolism, enzyme activity, and physiological processes (Shahab et al., 2017; Ullah et al., 2018).

Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits on the host (World Gastroenterology Organisation, 2023). Among probiotic bacteria, Lactobacillus acidophilus is one of the most extensively studied species and plays an important role in maintaining intestinal microbial balance and host health (Ferrari et al., 2024). Despite its recognized probiotic potential, little is known about the molecular mechanisms involved in its response to fluoride-induced stress.

Proteomics, the large-scale study of proteins expressed by a cell or organism, provides valuable insights into cellular functions, metabolic pathways, and stress adaptation mechanisms. Recent advances in proteomic technologies, including two-dimensional gel electrophoresis and mass spectrometry-based approaches, have facilitated the identification of

stress-responsive proteins and adaptive pathways in probiotic bacteria (Koirala et al., 2024; Muthuramalingam et al., 2023).

Therefore, the present study aimed to investigate the proteomic alterations in Lactobacillus acidophilus following sodium fluoride exposure using two-dimensional gel electrophoresis (2D-GE) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Identification of differentially expressed proteins may provide insights into the adaptive mechanisms employed by L. acidophilus under fluoride stress and enhance our understanding of probiotic stress physiology.

II. MATERIALS AND METHODS

2.1 Culture collection and maintenance:

The lyophilized probiotic strain Lactobacillus acidophilus (MTCC 10307) was obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The culture was transported under frozen conditions as per MTCC guidelines. The lyophilized cells were revived by rehydration in sterile 0.85% saline and subsequently cultured in de Man, Rogosa and Sharpe (MRS) medium under standard conditions suitable for Lactobacillus growth.

2.2 Chemicals:

Sodium fluoride was purchased from Bross Scientifics, Tirupati, Andhra Pradesh, India. All other chemicals used were of analytical grade.

2.3 Protein extraction and quantification:

The minimum inhibitory concentration (MIC) of sodium fluoride against L. acidophilus was determined using the microdilution method. For proteomic analysis, control and sodium fluoride-treated bacterial cells were harvested and subjected to protein extraction using sonication followed by centrifugation to obtain cell lysates. Protein concentration was estimated using the Bradford assay, One-dimensional gel electrophoresis (1D-GE) was performed.

2.4 Two-dimensional gel electrophoresis (2D-GE):

Protein samples were purified prior to analysis to remove salts and interfering substances, as 2D electrophoresis is highly sensitive to contaminants (Rabilloud et al., 2009). Proteins were separated based on isoelectric point and molecular weight using immobilized pH gradient (IPG) strips (pH 4–7, 24 cm) for isoelectric focusing (IEF) (Magdeldin et al., 2014). Samples were loaded onto the strip holder, and passive rehydration was performed overnight. IEF was carried out under optimized conditions until completion.

Following IEF, the strips were equilibrated using equilibration buffer containing iodoacetamide (IAA) and then subjected to second-dimensional separation by SDS-PAGE. After electrophoresis, gels were stained using Colloidal Coomassie Brilliant Blue and subsequently destained.

2.5 Image acquisition and analysis

Stained gels were scanned using a Typhoon Variable Mode Imager. Protein spot detection, matching, and quantitative analysis were performed using Image Master 2D Platinum 6.0 software. Differential expression of protein spots between control and treated samples was determined based on spot intensity and volume (Wongpia & Lomthaisong, 2010).

2.6 Protein identification

Differentially expressed protein spots were excised from the gel using a spot cutter and subjected to matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for protein identification.

2.7 Results

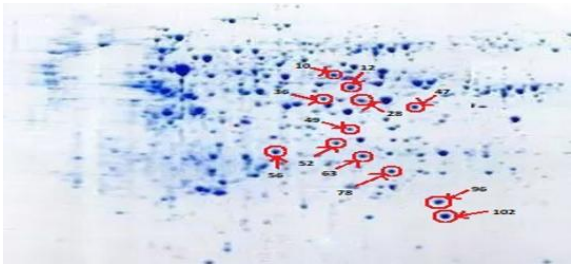
The 2D gel electrophoresis profiles of control and sodium fluoride-treated Lactobacillus acidophilus were presented in Figure 1. A total of 170 protein spots were resolved on the gels. Comparative analysis revealed 12 differentially expressed protein spots in response to sodium fluoride treatment. Among these, five spots were upregulated, while seven spots were downregulated.

Differentially expressed protein spots were excised and identified by MALDI-TOF mass spectrometry (Figures 2 and 3). The upregulated proteins included FeS biosynthesis domain-containing protein, Putative Trp repressor binding protein, 4-Oxalomesaconate hydratase, Gluconate kinase, and Hydratase. The downregulated proteins included Glyceraldehyde-3-phosphate dehydrogenase, Cyclopropane fatty acyl phospholipid synthase, NAD-dependent aldehyde dehydrogenase, Xylulose kinase, Glucan-1,6-alpha-

glucosidase, Alpha-glucosidase, and GTP-binding protein.

The identified proteins exhibited molecular weights ranging from 14.3 kDa to 68.5 kDa, with isoelectric points between 4.8 and 6.8. Protein score, sequence coverage, and amino acid length of the identified proteins were summarized in Table 1.

A. Control



B. Sodium fluoride treated

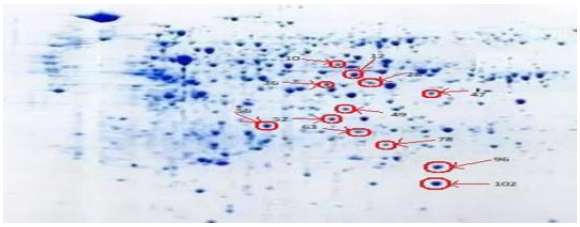
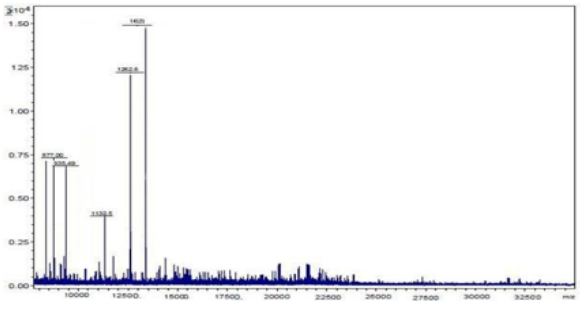


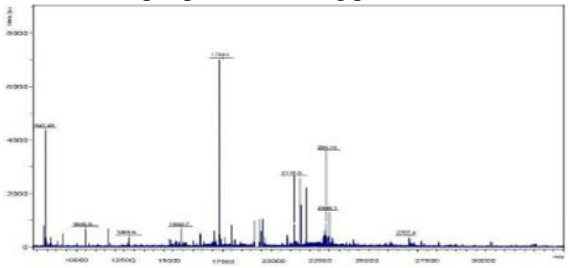
Figure 1: 2D gel analysis of differentially expressed proteins of L. acidophilus (pH 4 to 7)

MALDI TOF:

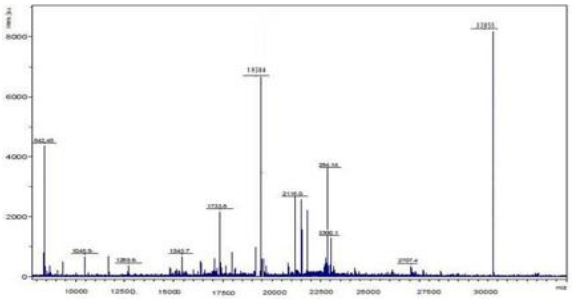
A. FeS Biosyn domain containing protein



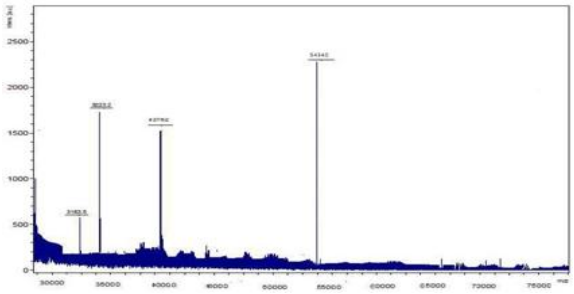
B. Putative trp repressor binding protein



C. Putative 4-oxalomesaconate hydratase protein



D. Gluconate kinase



E. Hydratase

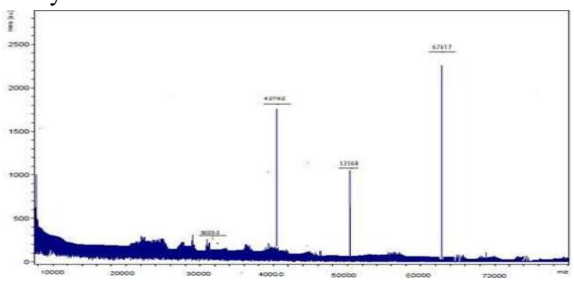
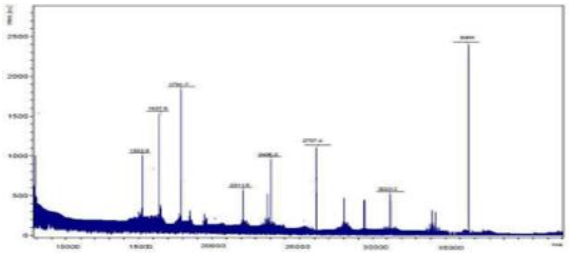
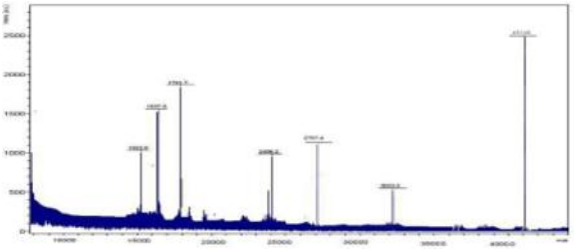


Figure-2: Up-regulated proteins

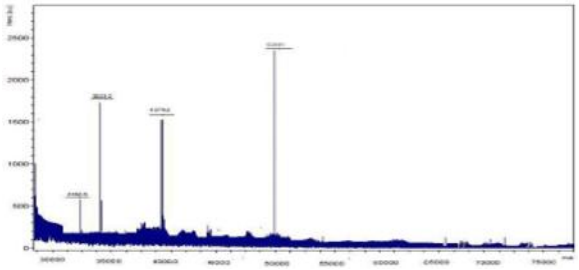
F. Glyceraldehyde 3 phosphate dehydrogenase



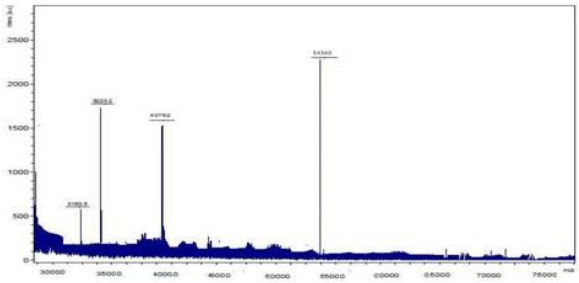
G. Cyclo propane fattyacyl phospholipid synthase



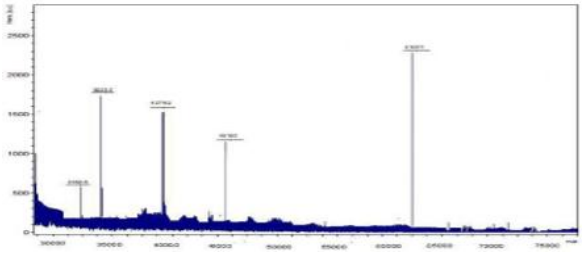
H. NAD dependent aldehyde dehydrogenase



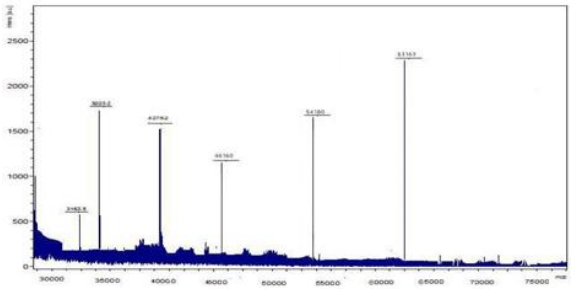
I. Xylulose kinase



J. Glucan 1, 6 alpha glucosidases



K. Alpha glucosidase



L. GTP Binding protein

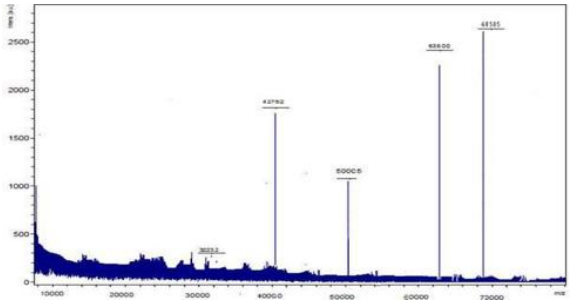


Figure-3: Down regulated proteins

Table: List of differentially expressed proteins (up regulated and down regulated) of Lactobacillus acidophilus identified by Mass Spectrometry by using peptide mass fingerprinting (PMF) analysis under Sodium Fluoride stress

S. No	Spot	Differentially Expressed Protein Name	Molecular weight	Calculated pI value	Protein Score	Number of Amino acids	Sequence coverage (%)
Upregulated proteins							
1	102	Fe Sbiosyn domain containing protein	14323.36	4.80	43	131	100
2	96	Putative trp repressor binding protein	17003.04	4.81	57	150	100
3	78	Putative 4-oxalomesaconate hydratase	33855.72	5.40	64	300	100
4	49	Gluconate kinase	54340.76	6.8	72	494	98
5	12	Hydratase	67617.50	5.16	47	591	98
Down regulated proteins							
6	63	Glyceraldehyde 3 phosphate dehydrogenase	36494.51	5.92	70	338	100
7	56	Cyclo propane fatty acyl phospholipid synthase	45145.29	5.44	68	393	98
8	52	NAD dependent aldehyde dehydrogenase	50391.10	5.14	82	460	98
9	47	Xylulose kinase	54340.76	6.8	76	494	98
10	36	Glucan- 1,6 alpha glucosidase	63009.58	4.8	32	539	100
11	28	Alpha glucosidase	63163.32	5.25	41	551	99
12	10	GTP binding protein	68505.53	5.29	49	825	52

III. CONCLUSION

Sodium fluoride exposure induced significant alterations in the proteomic profile of Lactobacillus acidophilus, as demonstrated by two-dimensional gel electrophoresis and MALDI-TOF mass spectrometry analysis. Differential expression of proteins indicates that fluoride stress affects key metabolic and cellular processes in L. acidophilus. The findings of this study provide insights into the stress response mechanisms of this probiotic bacterium under fluoride exposure and contribute to a better understanding of its adaptive physiological responses.

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