Recent Advances on Transcriptional Regulation for Nutraceutical Biofortification of Fermented Food using Probiotic Bacteria

Vanshika Chandel[1], Ashwani Mathur[1*]
[1*]Department of Biotechnology, Jaypee Institute of Information Technology, Sector-62, Noida
Email (First Author): vanshikachandel3@gmail.com;
Email (Corresponding Author): ashwani.mathur@mail.jiit.ac.in

Abstract

The widespread acceptance of nutritionally fortified foods containing nutraceuticals has emerged as a safe, suitable alternative for alleviating various diseases. Nutraceuticals, or compounds showing nutritional and therapeutic properties, are often the constituents of plant-based foods or an outcome of their fermentation. Food fermentation is primarily an outcome of biofortification of food ingredients using natural microflora or probiotic bacteria. Existing studies have reported that fermentation fortifies food ingredients with amino acids, vitamins, minerals, proteolytic enzymes, and other nutraceuticals, thereby improving the acceptability of fermented foods and often affecting shelf life. The growing understanding of different gut-associated axes necessitates a detailed understanding of the molecular regulation of probiotic bacteria during fermentation, vis-à-vis their impact on the food's modulated composition, enables the selection of catalogues of probiotic bacteria or their consortia for the development of fermented foods. Among probiotics, the genus *Lactobacillus* has gained particular attention for its ability to produce a diverse range of metabolites under different abiotic conditions, *viz.,* short-chain fatty acids, exopolysaccharides, vitamins, bioactive peptides, and carbohydrate-derived compounds. This review examines the molecular mechanism of probiotic bacteria, *Lactobacillus* sp., and understanding the molecular mechanism underlying food biofortification, a strategy that may be used in the future to develop designer or personalized food.

Keywords: Nutraceuticals, Probiotic, Molecular, Regulation, fermented, food

Introduction

Probiotics are microorganisms that are considered to be safe ('GRAS' according to the US Food and Drug Authority, USA), when administered in adequate quantity (~ 10^6 – 10^8 cfu/ml), conferring a catalog of health benefits [1]. These bacteria can be administered in different forms and through different routes [2]. Often, the fermentation of food by probiotic bacteria assists its biofortification with nutraceuticals, hydrolyses the protein and complex carbohydrates in food, enhancing their digestibility and shelf life [3]

The recent advancements in probiotics mediated fermentation may be categorized into two broad categories based on the nature of substrate, *viz.,* dairy food and non-dairy food. This categorization is important to segregate the uniqueness of probiotics and food combination, that are often based on the composition of food and affect the final composition, post fermentation. Table 1, enumerates some such dairy and non-dairy products, developed post fermentation using probiotics.

Table 1: Recent studies on probiotic fermentation of dairy and non-dairy foods

Probiotic organism	Substrate	Fortified Nutraceuticals	Fermented Food Properties	References
Lactobacillus sp., Bifidobacterium sp.	Sour Cream (Dairy)	Enriched in fatty acids	Sensory acceptance, improvement in texture	[4]
Lactobacillus plantarum	Sea Buckthorn (Non- Dairy)	Enhancement of phenolics and flavonoids	Anticancer properties, Antihypertensive property, Sensory acceptance	[5]
Lactobacillus casei, Lactobacillus plantarum	Cheddar cheese (Dairy)	Protein hydrolysate	Increase in DPPH activity	[6]
Lactobacillus plantarum, Lactobacillus delbrueckii	Tomato juice (Non-Dairy)	Decrease in pH	Improved antioxidant property	[7]

Molecular Regulation of Probiotics in Host

A unique association exists between probiotic bacteria and the host, especially human, responsible for associated health benefits. The extensive reports on indigenous colonization and the association of probiotic bacteria in the intestinal environment. Some of the key pathways of the hosts, modulated by the presence of probiotics, include mitogen-activated protein kinase, peroxisome proliferator activated receptor-gamma, heat shock proteins, and nuclear factor-kappaB, along with other pathways [8] Some of these properties are attributed to the interaction of probiotics or their metabolites on TLRs receptors on immune and epithelial cells.

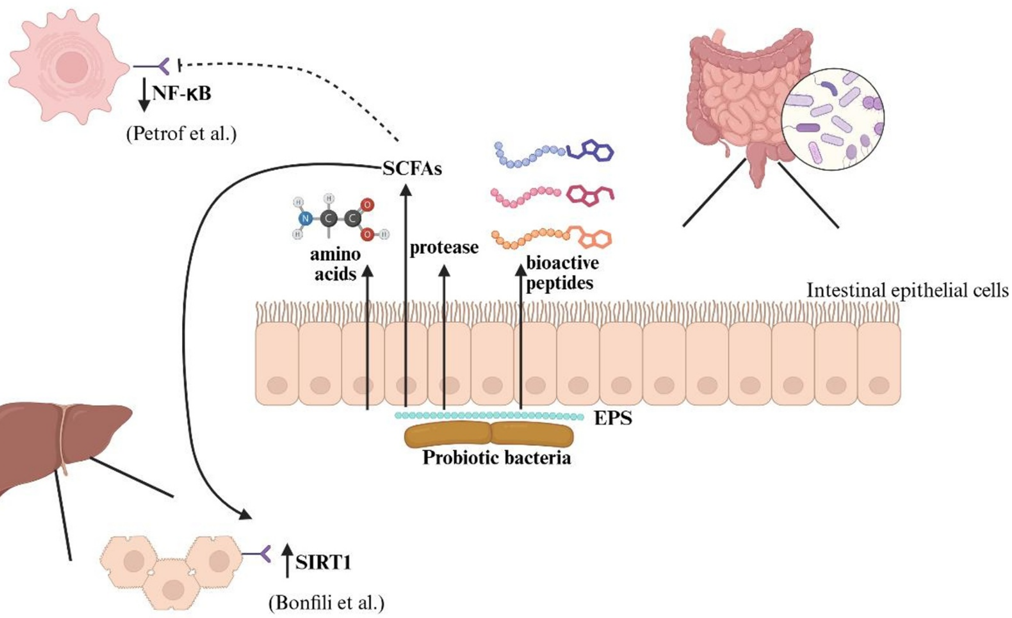

Figure 1: Image demonstrating the transcriptional regulation of probiotic bacteria towards various gut-related axis

Along with an understanding of host signalling pathways modulated by probiotics, it is important to explore the molecular regulations within probiotics that are significant in regulating the food fermentation and therapeutic enhancements due to biofortification. In one of the seminal studies with probiotic yogurt, meta transcriptome analysis revealed the activation of different pathways, the prominent one was protein hydrolysis [9]. In another study of fermented kimchi production, the transport function of *Lactobacillus plantarum* was affected by the acidic environment, indicating dynamic probiotic response to abiotic (pH) conditions (Jung & Lee, 2020).

The existing studies had shown via multiple transcriptomic investigations the dynamic changes during probiotic adaptation during fermentation. The coordinated interactions between host interactions with the abiotic parameters, during fermentation is controlled by tightly coordinated regulatory and metabolic networks. An exploratory study on evaluating the biofilm formation in obligate anaerobic probiotic bacteria, *Bifidobacterium bifidum* had shown a cascade of associations between the transcriptome and metabolome of more than 200 genes that were differentially expressed, proposing the multimodal associations between the omics analysis. These genes included stress-associated chaperons (e.g., groL, groS), quorum sensing components, and two-component regulatory systems that helps in the formation of bacterial biofilm ensuring more stable and resistance to environmental stress. Also, changes in the transcriptional profile were linked to the increased accumulation of metabolites such as butyric acid, amino acids, and vitamins, indication a functional coupling between regulatory gene expression and nutraceutical output [11]. Fermentation of different raw materials and food products by probiotics had shown profound associations between metabolism of nutrients through multimodal pathways, persistently linked with increased production of short-chain fatty acids (SCFA), such as propionate, lactate, acetate, propionate considered as important mediators of host-microbe interactions. A significant elevation of these metabolites has been seen in experimental fundamental models with lactic acid increasing more than tenfold along with detectable increase in acetate and propionate concentrations, further supporting the role of probiotics in maintaining gut homeostasis and metabolic balance [12].

The probiotics are also reported for regulating and altering microbial community structure and affecting metabolic flux towards SCFA, bioactive (antimicrobials, bioactive peptides, antioxidants) metabolites, along with the properties of fermented food, and conferring immunological control, epithelial integrity, and energy metabolism in the host, lending more credence to these conclusions [13].

The extension of transcriptome analysis to other genera in addition to *Lactobacillus* species has shown unique but complimentary functional capabilities. Studies of *Bifidobacterium* species at the genome level system levels demonstrate their crucial function in hot metabolic regulation and carbohydrate fermentation, especially through their effective use of complex substances and contribution to gut ecological balance [14]. Furthermore, new multi-omics research combining transcriptomics and metabolomics in probiotic-host interaction models has shown that the probiotic administration can alter host-associated metabolic pathways as well as microbial gene expression, minimizing pathogenic organisms and enhancing advantageous metabolites like amino acid intermediates and ascorbic acid derivatives [15].

Collectively, these studies highlight how transcriptomic regulation in probiotics is intrinsically linked to functional outputs, where metabolic production, stress resilience, and colonization efficiency are directly governed by adaptive gene expression under specific environmental or host-associated conditions. Designing targeted fermentation techniques and next-generation probiotic compositions with improved medicinal and nutraceutical potential is made possible by integrative understanding.

Table 2: Studies on transcriptional regulation of probiotics, associated with bacterial response

Probiotic	Fermented Food	Transcriptional Regulation	Outcome / Response	Reference
Lactiplantibacillus pentosus (LPG1)	Spanish style green table olives	RNA-Seq differential gene expression during brine fermentation. Downregulation of stress genes with elevation of clusters of genes involved in the production of carbohydrates, amino acids and exopolysaccharides.	Dynamic transcriptome adaptation during fermentation like changes in adhesion, EPS generation, and metabolism.	[16]
Lactiplantibacillus plantarum A6	Cereal-based fermented food (pearl millet matrix)	Differences in transcriptome between growth in food matrix and lab medium; upregulation of carbohydrate and	Indicates increased expression of nutrient-relevant pathways in food fermentation.	[17]

		vitamin biosynthesis related genes.		
Lactiplantibacillus pentosus (AP2-16)	Table olive matrix (olive oil-adapted)	Differential expression after adaptation to edible oils (affecting, host interaction, and robustness pathways) according to global transcriptomics.	Transcriptional rerouting that shows controlled functionally and robustness pertinent to fermentation performance.	[18]
Lactiplantibacillus plantarum (General LAB)	Kimchi fermentation	Transcriptome analysis under acidic conditions to identify genes for acid adaptation during kimchi fermentation	Characterized regulatory responses in transport and adaptation genes that influence LAB survival and metabolic output in fermented kimchi	[19]

Conclusion

Probiotic bacteria have been emerging as a source of catalogues of bioactive metabolites of nutritional and therapeutic importance. The diversity of fermented food, ius no longer limited to dairy based, but non-dairy products have also emerged as potential nutraceuticals in the global market. The composition of food or their ingredient, emerge every study as unique and innovative due to diversity of metabolic pathways, operative during fermentation conditions, often enhancing the palatability of food and enhancing their nutritive value along with therapeutic properties. Different dairy and non-dairy fermented foods are also emerging as an alternative vehicle for the delivery of probiotics. The elucidation of the molecular regulation of probiotic bacteria has been a breakthrough in development of novel food formulations. The existing studies highlights the role of abiotic environment and the compositions of food ingredients, pivotal in transcriptional responses of bacteria. Furthermore, the science can be better understood with detailed information on the whole genome profile of the bacteria. The detailed understanding of the metabolic pathways along with transcriptome analysis will be pivotal in developing a fermented food consortia which may be further extended towards designers and personalized food towards nutritional biofortification and therapeutic advancement.

Broadening this viewpoint, recent transcriptome studies unequivocally show that probiotic bacteria do not act as static inoculants in food matrices but rather dynamically rewire their gene expression in response to redox state, protein complexity, salt concentration, pH changes, and micronutrient availability. The formation of exopolysaccharides, stress tolerance, amino acid biosynthesis, central carbon metabolism, and vitamin biosynthesis all are regulated by this transcriptional flexibility, affecting the nutraceutical value of fermented foods. The study will pave the way for development of designer food at scalable quantity to meet the emerging global demand.

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