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DECOLORIZATION OF FOOD DYES BY PROBIOTIC *LACTICASEIBACILLUS* RHAMNOSUS STRAINS

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Abstract

Synthetic food colors are widely used in processed foods; however, several azo dye-based colorants are persistent xenobiotic compounds with potential toxicological relevance. In the present study the ability of two human-origin potential probiotic *Lacticaseibacillus rhamnosus* strains, namely Lr 231 and Lr Fb to decolorize commercially available red, green and yellow food dyes under growing and non-growing conditions has been investigated. Decolorization was examined in MRS medium, phosphate buffer supplemented with glucose and/or yeast extract, and skimmed milk medium respectively, and residual color was measured by UV-visible spectrophotometry after incubation at 37°C. Both strains decolorized food dyes in liquid media. In MRS medium, food dyes with red and yellow dyes showed complete decolorization after 48 h, whereas green dye showed only partial removal. Both strains showed decolorization of red dye upto 94% after 48 h in phosphate buffer supplemented with glucose and yeast extract, while green and yellow dye showed limited decolorization with evidence of cell binding or spectral transformation. In skimmed milk, green dye decolorization reached 85% with Lr 231 and 89% with Lr Fb; yellow dye decolorization was greater with Lr 231 (81%) than with Lr Fb (66%). Chilli powder and turmeric did not inhibit the growth of either strain. These findings implicate that probiotic *L. rhamnosus* strains can decolorize selected synthetic food colorants, however detailed work need to verify the biotransformation to less toxic compound.

Keywords: Probiotics, *Lacticaseibacillus rhamnosus*, food colors, decolorization, skimmed milk, spices

1. Introduction

Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [1]. Lactic acid bacteria (LAB), particularly species belonging to the genus *Lactobacillus*, are widely used as fermentation cultures, dietary supplements and biological control agents because of their long history of use in fermented foods and their generally recognized safety status [2,3]. Probiotic activity is strain specific and is commonly assessed through gastrointestinal stress tolerance, cell surface characteristics, antimicrobial activity and safety-related properties [4,5]. Probiotics exhibits wide array of potential such as antimicrobial activity, anticancerous activity, antioxidative, anti-inflammatory, may assist in management of obesity and diabetics, alleviating anxiety and depression via influencing neurotransmitter synthesis and inflammation [6,7,8].

In addition to their above potential probiotic functions, LAB may play important role in detoxification of dietary and environmental contaminants. Azo dyes and synthetic food colorants contain chromophoric groups that enhance chemical stability and make them relatively resistant to degradation. Although several intestinal microorganisms are known to reduce or transform azo compounds, the ability of probiotic lactobacilli to remove food-grade colorants remains less well characterized [9,10]. This property could represent an additional functional attribute for strains intended for food or gut-associated applications.

The present investigation evaluated the decolorization of food dyes by probiotic *L. rhamnosus* strains isolated from human faeces. The study was focused on dye decolorization ability of Lr 231 and Lr Fb by growing and non-growing cells, in skimmed milk, and the effect of commonly consumed spices on probiotic growth.

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2. Materials and methods

2.1 bacterial strains

Lacticaseibacillus rhamnosus 231 and *Lacticaseibacillus rhamnosus* Fb, previously isolated from human faeces on De Man, Rogosa and Sharpe (MRS) medium, were used in this study [11,12].

2.2 food dyes and spices

Commercially available red, green and yellow food dyes (Blue Star, Ahmedabad) were purchased from the local market. A 1% stock solution of each dye was prepared in distilled water. Four chilli powder samples and two turmeric samples were also purchased locally. Chilli powder and turmeric stock solutions were prepared at concentrations of 5% and 1%, respectively.

2.3 preparation of lactobacilli cell suspension

Both the strains Lr 231 and Lr Fb were grown in MRS medium at 37°C for 24 h from glycerol stock of culture preserved in -20°C. Cells were harvested by centrifugation at 5000 rpm for 15 min at 4°C, washed twice with phosphate-buffered saline (PBS; 0.1 M, pH 7.0, containing 0.85% NaCl), and resuspended in phosphate buffer (0.1 M, pH 7.0) to obtain a cell suspension with an A_{600} of 1.

2.4 decolorization by growing cultures

Decolorization of food dyes by growing cultures was evaluated in 5 ml of MRS medium containing 20-100 µL of stock solution containing 1% food dye. Tubes were inoculated with cell suspensions of strains Lr 231 and Lr FB and incubated at 37°C for 48 h. Samples were collected at 24 h intervals and centrifuged, and the supernatants for residual dye present were analyzed using single beam UV-visible spectrophotometry (Spectrophotometer 2373, EI, India). Percent decolorization was calculated at the maximum absorbance wavelength (λ max) of each color as previously described [9].

2.5 decolorization by non-growing cultures

Washed cells from 24 h cultures were suspended in phosphate buffer and evaluated under four conditions: (i) phosphate buffer alone, (ii) phosphate buffer with 0.2% glucose, (iii) phosphate buffer with 0.05% yeast extract, and (iv) phosphate buffer containing both glucose and yeast extract. In all four sets of tubes food dyes 20-100 µL from the 1% stock solution were added and tubes were incubated at 37°C for 24 and 48 h. Residual dye was measured spectrophotometrically after centrifugation [9].

2.6 decolorization on mrs agar and in skimmed milk

Decolorization of food dye by Lactobacilli in skimmed milk media

The dye decolorization was evaluated in the 10% skimmed milk. Cell suspension (OD600 = 1) was inoculated in skim milk medium having 20 - 100 µl food dyes (1% stock) and incubated at 37°C. After 24 h of incubation 1 ml sample was harvested from all the tubes and cells were pelleted by centrifugation (7000 rpm, 10 mins, 4°C) and supernatants were analyzed for residual food dye by scanning in UV-Visible range using spectrophotometer

2.7 influence of spices on growth

The effect of chilli powder and turmeric on Lr 231 nd Lr FB was evaluated on MRS agar and in MRS broth containing different concentrations of spice stock solutions. Inoculated plates and broth tubes were incubated at 37^0C for 48 h, and growth was assessed by colony development and optical density measurement at A_{600}.

3. Results

3.1 decolorization of food dyes by probiotic lactobacillus strains

Both *L. rhamnosus* strains exhibited decolorization in liquid media. In MRS medium, decolorization generally decreased with increasing dye concentration. Lr 231

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showed a gradual reduction in decolorization at higher concentration of food dye, whereas strain Lr Fb maintained a relatively consistent decolorization pattern across the tested concentrations. Red and yellow dyes were completely decolorized at 37°C after 48 h, while green dye intensity was reduced however was not completely decolorized, (Fig. 1)

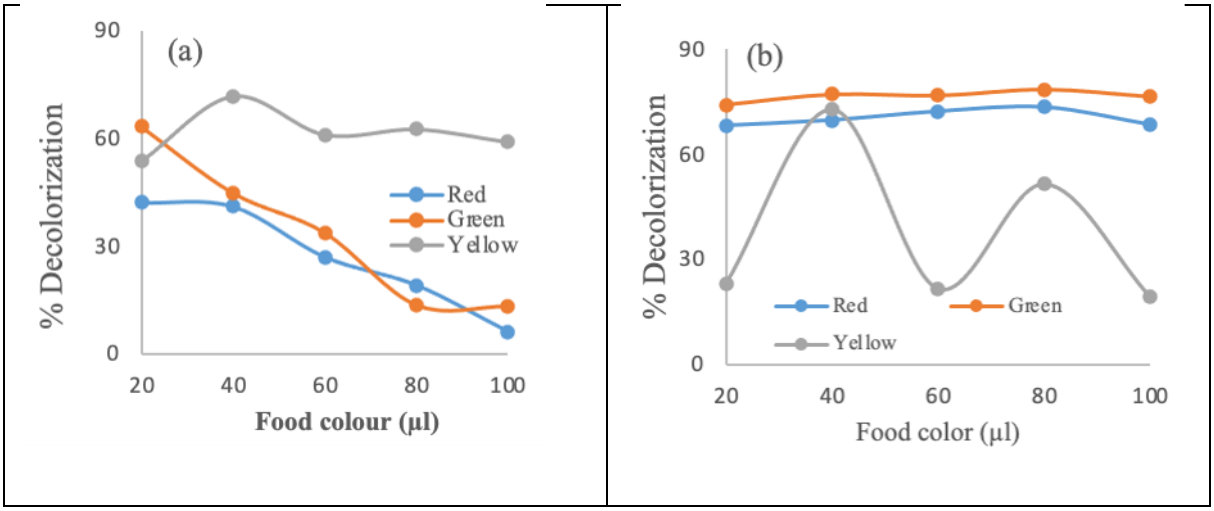


Fig. 1. Percent decolorization of commercially available red, green and yellow food colors by *L. rhamnosus* Lr 231 (a) and *L. rhamnosus* Fb (b)

3.2 decolorization by non-growing cultures

Lactobacillus strains cultivated in complex MRS medium demonstrated a broad capacity to decolorize diverse food dyes, with the notable exception of green. To elucidate the underlying mechanisms, decolorization potential of Lr 231 and Lr Fb was evaluated in a phosphate buffer system lacking additional nutrients, as well as phosphate buffer supplemented with individual or combined carbon (glucose) and nitrogen (yeast extract) sources. The extent of decolorization depended heavily on the specific dye and the presence of nutritional supplements (Fig 2 A & B). Red dye decolorization reached 94% after a 48-h incubation in the presence of both glucose and yeast extract. Conversely, green dye showed no decolorization with 48 h; however, cell-binding occurred, which did not increase with prolonged incubation. No visible decolorization of yellow dye was observed at 24 h or 48 h.

Effect of different concentration of food dyes on decolorization by two strains were evaluated (Fig 3A & B). Red dye consistently exhibited the highest decolorization efficiency by Lr 231 and Lr Fb across all tested concentrations. For the green dye, cell-binding capacity decreased at higher dye concentrations. Notably, spectrophotometric analysis of the yellow and green dye treatments revealed the emergence of novel peaks, indicating structural biotransformation despite the absence of visible decolorization.

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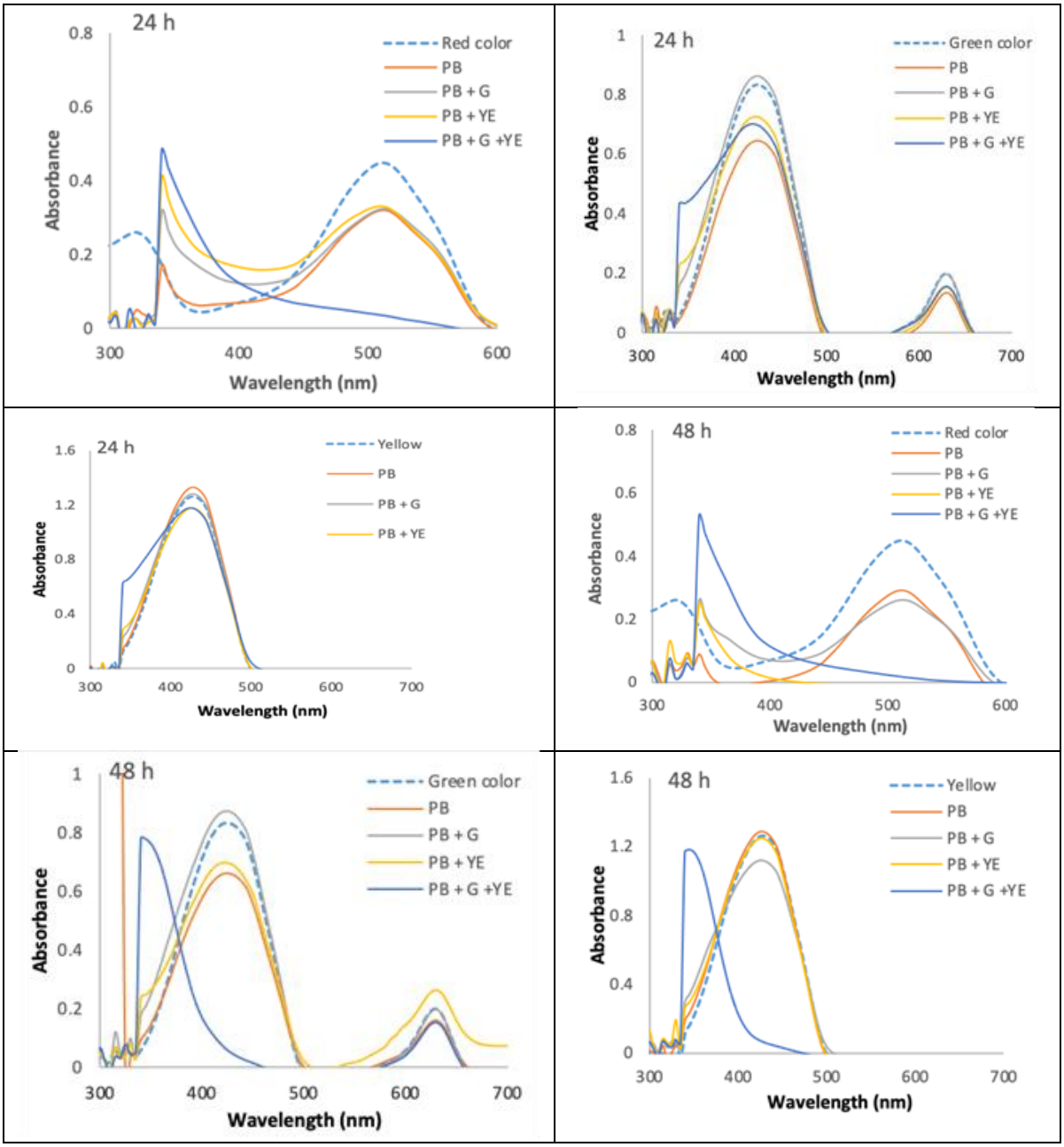


Fig. 2A Absorption spectra of Red, Green and Yellow after 24 h and 48 h incubation with *Lr 231* in Phosphate buffer (PB), Phosphate buffer + 0.2% glucose (PB+G), Phosphate buffer + 0.05% yeast extract (PB+YE), Phosphate buffer + glucose+ yeast extract (PB+G+YE), dotted line indicates food colour control without bacterial cells

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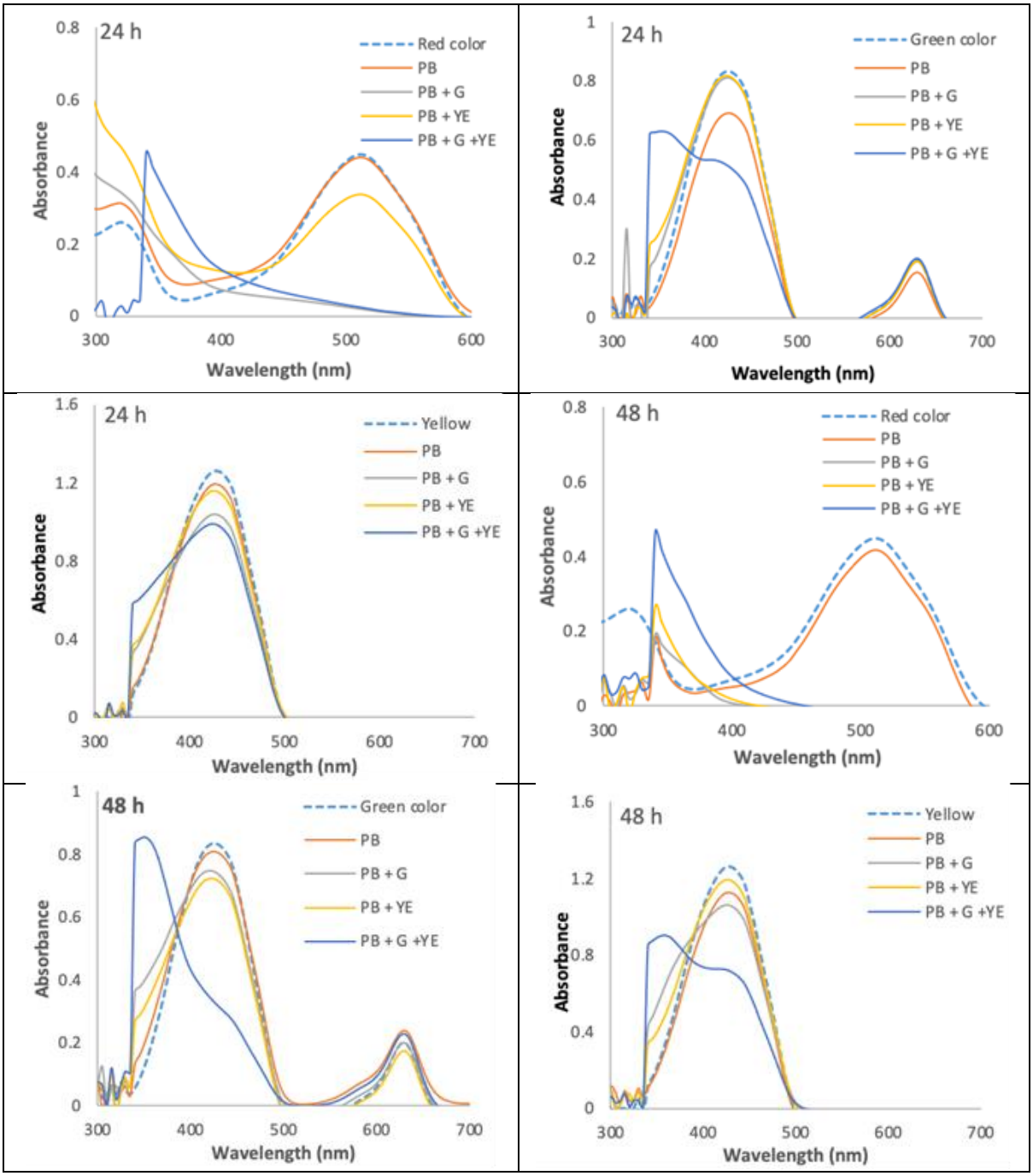


Fig. 2B Absorption spectra of Red, Green and Yellow after 24 h and 48 h incubation with Lr Fb in Phosphate buffer (PB), Phosphate buffer + 0.2% glucose (PB+G), Phosphate buffer + 0.05% yeast extract (PB+YE), Phosphate buffer + glucose+ yeast extract (PB+G+YE), dotted line indicates food colour control without bacterial cells

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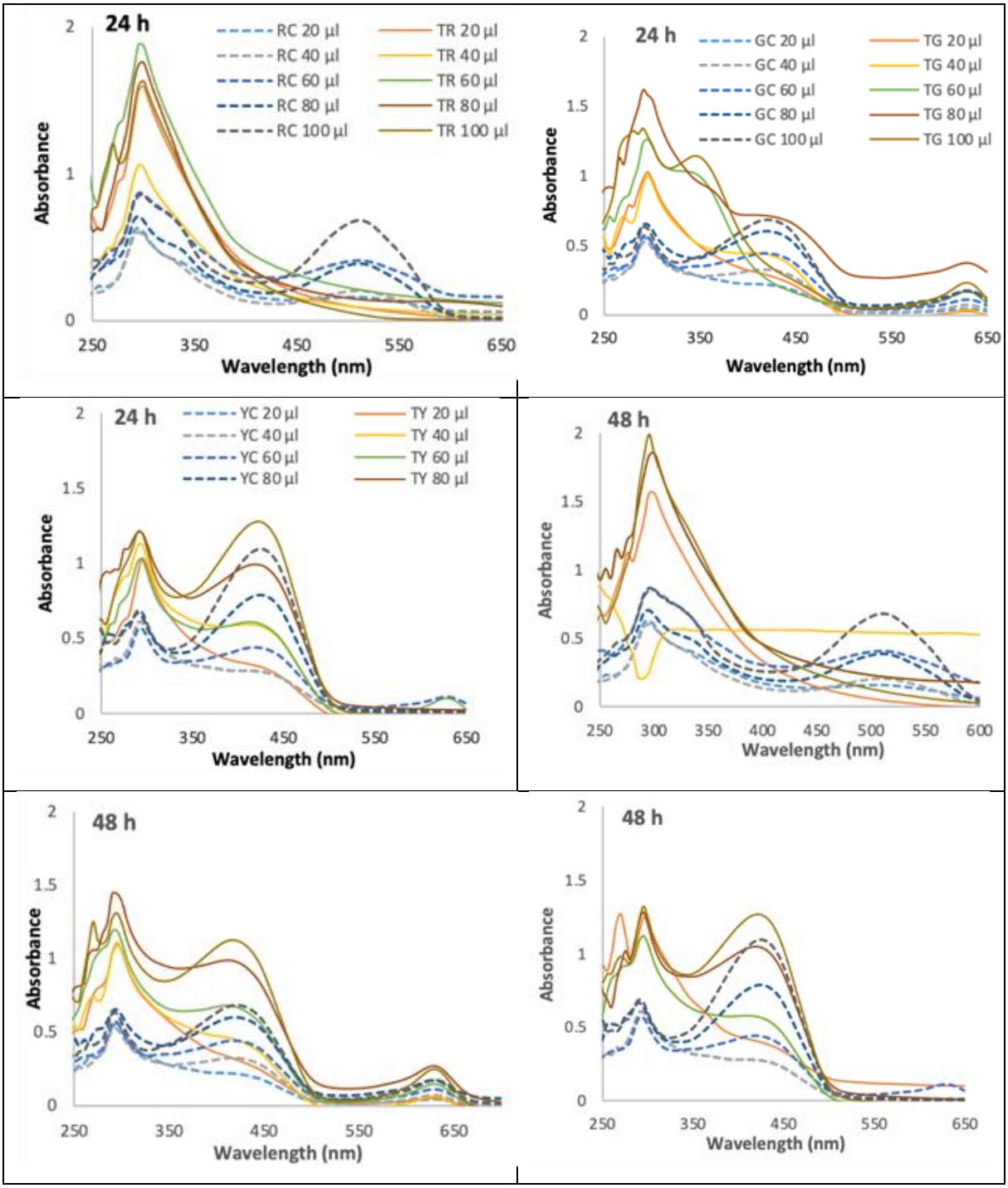


Fig. 3A Absorption spectra of different concentration of Red, Green and Yellow colours (20, 40, 60, 80,100 µl) after 24 h and 48 h incubation with strain *Lr 231*Phosphate buffer + glucose+ yeast extract (PB+G+YE), dotted line indicates food colour control without bacterial cells

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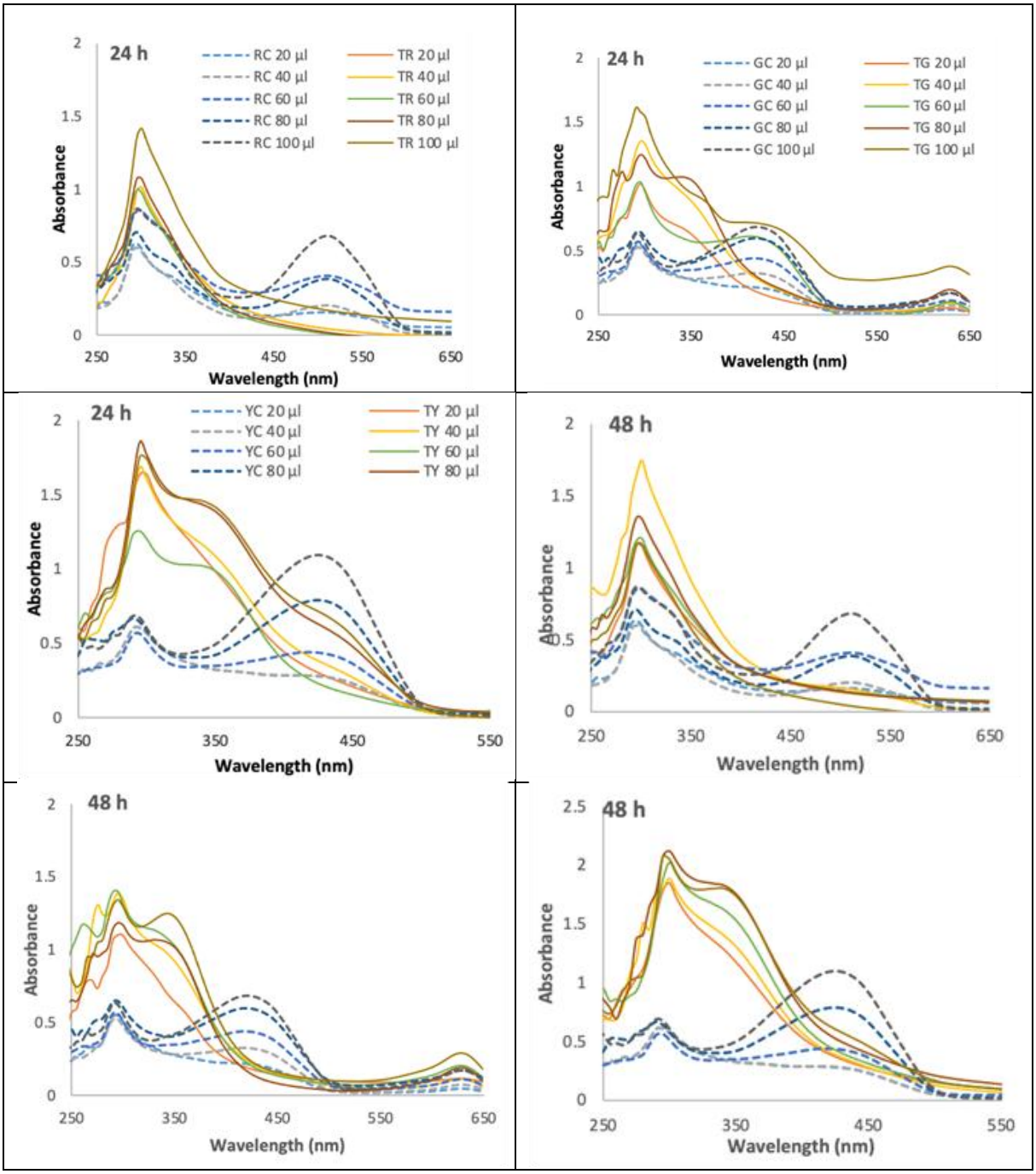


Fig. 3B Absorption spectra of different concentration of red, green and yellow colors (20, 40, 60, 80,100 µl) after 24 h and 48 h incubation with strain Lr Fb in Phosphate buffer + glucose+ yeast extract (PB+G+YE), dotted line indicates food color control without bacterial cells

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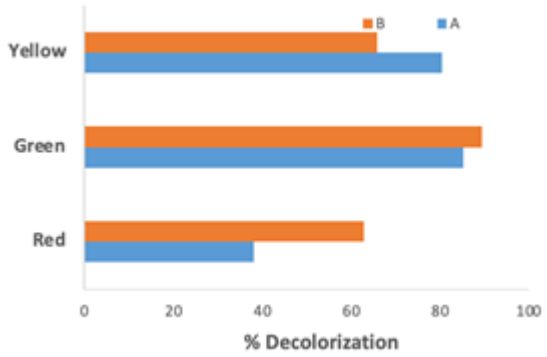


Fig. 4 Decolorization (%) of food colors by *Lactobacillus* strains (A) Lr 231 and (B) Lr Fb in skimmed milk medium after 24 h incubation at 37°C

3.3 decolorization in skimmed milk

Skimmed milk supported marked decolorization of the tested food dyes. Lr 231 and Lr FB showed 85% and 89% decolorization of green dye with cells grown in skimmed after 24 h respectively. Lr 231 and Lr Fb showed upto 62% and 40% decolorization of red dyes respectively. Decolorization of yellow dye by Lr 231 and Lr Fb reached 81% and 66% respectively.

3.4 influence of spices on growth

Chilli powder and turmeric did not inhibit either Lactobacillus strain. Growth was observed on MRS agar supplemented with 5% chilli powder and 1% turmeric, and broth assays similarly indicated that the tested spice concentrations did not adversely affect cell growth.

Collectively, the results show that growing cells in MRS medium completely decolorized red and yellow dye after 48 h, while green dye showed partial decolorization. Non-growing cells showed the highest red color removal in phosphate buffer supplemented with glucose and yeast extract. Skimmed milk supported substantial decolorization of green and yellow colors, and the tested spices did not inhibit Lactobacillus growth.

4. Discussion

The findings demonstrate that human-origin probiotic Lactobacillus strains can remove or transform commercially available food colors under selected culture conditions. The extent of decolorization depended on the dye specific, concentration, incubation period and medium composition. This pattern is consistent with previous reports showing that microbial dye reduction is strongly influenced by nutrient availability, electron donors and the chemical structure of the dye [10,13, 14].

Growing cultures in MRS medium efficiently decolorized red and yellow colors, whereas green color was more resistant and appeared to interact with cells through binding or partial transformation. Non-growing cell assays confirmed that carbon and nitrogen sources were important for enhanced decolorization, with red color removal being most efficient in phosphate buffer supplemented with glucose and yeast extract. Yeast extract may have supplied growth factors and reducing equivalents required for dye transformation by fastidious lactobacilli strains used in the study [15-17].

Interestingly, while the yellow dye showed no visible color removal and the green dye remained visibly stable, spectrophotometric analysis revealed the emergence of novel absorption peaks for both treatments. In microbial biotransformation, the appearance of new peaks in the UV-visible spectrum strongly indicates the cleavage or modification of the parent dye molecules, resulting in the formation of colorless metabolic intermediates or aromatic amines [18-20]. Therefore, the lack of visible decolorization for the yellow dye does not imply metabolic inactivity; rather, it suggests an incomplete or alternative biotransformation pathway that alters the molecular chromophore without eliminating spectrophotometric absorbance. Future studies should focus on characterizing these metabolites

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using LC-MS or FTIR to fully elucidate the degradation pathways involved.

Skimmed milk served as a favorable medium for food dye decolorization. The higher removal of green color in skimmed milk than in buffer systems suggests that milk nutrients may support bacterial metabolic activity or color-binding interactions. It needs further investigation to find out reasons for variation exhibited by strains for decolorization of same dye in different media.

The ability of the strains to remain viable in the presence of chilli powder and turmeric is also relevant, because probiotic cultures intended for food applications may be exposed to spices, additives and other dietary compounds.

Overall, decolorization of food colors may be considered an additional functional property of probiotic *L. rhamnosus* strains, in addition to the previously reported antimutagenic, antimicrobial and probiotic attributes of human-origin lactobacilli [11-13, 21, 22]. Further studies are focused on identify the chemical nature of the transformation products and determine whether decolorization occurs through degradation, adsorption or a combination of mechanisms.

5. Conclusion

The study indicates that probiotic strains Lr 231 and Lr Fb can decolorize commercially available red, green and yellow food colors, although the efficiency varies with color type and growth condition. MRS medium and skimmed milk supported effective decolorization, while glucose and yeast extract were important for decolorization by non-growing cells. Chilli powder and turmeric did not inhibit probiotic growth. These attributes suggest that the tested strains may have potential for food-related applications in which removal or transformation of synthetic food colorants is desirable. More detailed studies are warranted to explore biotransformation of dyes by lactobacilli strains.

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