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EFFICACY OF *LACTOBACILLUS CASEI*, *LACTOBACILLUS PLANTARUM* AND *BIFIDOBACTERIUM BIFIDIUM* INDIVIDUALLY AND COLLECTIVELY AS CONSORTIA IN THE BIODEGRADATION OF AZO DYES; CARMOISINE AND TARTRAZINE

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ABSTRACT

This study explores the influence of various probiotic bacterial strains on the degradation of two common synthetic azo dyes, Carmoisine and Tartrazine. The experiment focuses on the impact *of Lactobacillus casei, Lactobacillus plantarum, Bifidobacterium bifidum,* and as consortium of these strains on the degradation of Carmoisine and Tartrazine. The investigation revealed differential responses in the degradation of these dyes Carmoisine and Tartrazine. The findings suggest that the probiotic strains studied hold the potential to effectively degrade Carmoisine but may have limited impact on Tartrazine degradation. Given the widespread usage of these dyes in various applications, these results could have implications for human health and environmental safety and contribute to minimizing food safety and environmental risks.

Keywords: Azo dyes, Biodegradation, Carmoisine, Lactic Acid Bacteria, Next-generation Probiotics, Tartrazine

INTRODUCTION

Azo dyes are a class of synthetic organic compounds widely used for colouring purposes in various industries, including textiles, cosmetics, food, and pharmaceuticals. Azo dyes dominate the food and textile industries, accounting for 60 to 70% of all dyes used. Azo dye colourants such as Tartrazine and Carmoisine are employed to enhance the visual appeal of food. These azo dyes have been found to be toxic and pose health risks to humans and the environment. (**Keshava *et al.*, 2023 Varghese & Ramamoorthy, 2023).** The concerns of use of azo dyes in food products due to potential health risks are serious. Intolerance to the dyes can cause itching, rashes, coughing, vomiting, and asthma attacks, and are potentially linked to child hyperactivity **(Ramos-Souza *et al.*, 2023; Arnold *et al.*, 2012)** and in some cases can also cause liver and kidney damage, and cancer **(Kamali *et al.*, 2023; Bafana *et al.*, 2011).**

While some countries have altogether banned their use in food commodities, some have stringent regulations on their use. In the European Union (EU), for instance, the use of certain azo dyes has been significantly restricted, particularly those known to metabolize into aromatic amines that have been associated with health risks **(Brookstein, 2009).** Among these azo dyes are Tartrazine and Carmoisine that are widely used for various industrial applications, food colouring agent being one of them. These dyes have been permitted by regulatory authorities in permissible limits that range from 100mgl^{-1} to 300mgl^{-1} in foods and beverages (Codex Alimentarius Commission and United States Food and Drug Administration). Both toxic and non-toxic claims have been reported on these dyes which further suggest risk assesment to fulfill the research gap **(Ramos-Souza *et al.*, 2023).** Furthermore, these azo dyes can also cause environmental toxicity **(Josephy *et al.*, 2023**). While explorative studies in pursuit of alternative colouring agents are underway **(Hussain *et al.*, 2023; Rajendran *et al.*, 2023).** Despite regulatory efforts to limit their usage in food products, these dyes remain prevalent, necessitating ongoing research into their safe degradation. An emerging area of interest is the application of microbial bioremediation to mitigate the toxic effects of azo dyes. Microorganisms are known to degrade azo bonds, transforming complex dye molecules into simpler, non-toxic by-products **(Çınar *et al.*, 2008)**. Within this context, probiotic bacteria, particularly strains of *Lactobacillus* and *Bifidobacterium*, have garnered attention for their dual role in promoting human health and biodegrading synthetic compounds. The probiotic strains *Lactobacillus casei*, *Lactobacillus plantarum*, and *Bifidobacterium bifidum* are of particular interest due to their ability to thrive in various environments, including the gastrointestinal tract and fermented food systems, making them suitable candidates for in situ and ex situ bioremediation.

Probiotics, commonly defined as live microorganisms that confer health benefits when administered in adequate amounts, play a vital role in maintaining the balance of gut microbiota. Beyond their well-documented benefits in gut health, recent studies have demonstrated that probiotics can also exhibit enzymatic activity that allows them to degrade harmful substances, including azo dyes **(Li *et al.*, 2023).** The enzymatic mechanisms involved, such as azoreductase activity, enable these microbes to cleave the azo bonds present in Carmoisine and Tartrazine, breaking them down into less harmful metabolites. *Lactobacillus casei* has been shown to possess robust metabolic pathways that can handle xenobiotic stress, making it a promising candidate for the degradation of synthetic dyes. Similarly, *Lactobacillus plantarum* exhibits a broad range of metabolic capabilities, including the breakdown of complex organic molecules, while *Bifidobacterium bifidum*, a key member of the gut microbiota, has demonstrated potential in reducing chemical stressors in food systems. The combined efficacy of these strains, both individually and as consortia, in degrading azo dyes, has yet to be fully explored. Therefore, this study aims to assess the biodegradation capacity of these probiotic strains—*Lactobacillus casei*, *Lactobacillus plantarum*, and *Bifidobacterium bifidum*—both individually and in consortia, with the goal of evaluating their potential as eco-friendly agents for the detoxification of Carmoisine and Tartrazine in food matrices.

MATERIAL AND METHODS

Culture and Chemicals

Tartrazine (GRM431-Himedia) and Carmoisine(RM 2038- Himedia) azo dyes were used in this study. A stock solution of 1ppm of tartrazine and carmoisine was prepared in distilled water. *Lactobacillus casei* (ATCC 12116) and *Lactobacillus plantarum* (ATCC 29521) and *Bifidobacterium bifidum* (ATCC 29521) were procured from National Collection of Industrial Microorganism (NCIM), National chemical Laboratory, Council of scientific and Industrial research (CSIR, INDIA) and Northern Regional Research Laboratory (NRRL), Agriculture Research services, United State Department of Agriculture (USDA) respectively. The cultures were further revived and identified using biochemical tests and were refrigerated for further use. Soybean Casein Digestive Medium (MH011- Himedia) and Lactobacillus MRS agar (M641- Himedia) were used in this study.

Degradation study of azo dyes with Lactobacillus strains

Lactobacillus casei (LC), *Lactobacillus plantarum* (LP), *Bifidobacterium bifidum* (BB), and a consortium (LC + LP + BB) were studied for their ability to degrade the azo dyes Tartrazine (TZ) and Carmoisine (CS). The cultures were revived in broth and incubated overnight at 37°C. Overnight cultures were standardized to a concentration of approximately 10^8 CFU/mL at the beginning of the experiment, verified by measuring the optical density at 600 nm, aiming for an OD value of approximately 0.6 to ensure equivalent starting cell density. For each experimental setup, 1 mL of the standardized culture of *Lactobacillus casei* was inoculated into 100 mL of Soybean Casein Digestive Medium (SCDM) containing 100 ppm of Tartrazine (LC + TZ) or 100 ppm of Carmoisine (LC + CS) at 37°C. Separate setups were established for *Lactobacillus plantarum* (LP + TZ and LP + CS), *Bifidobacterium bifidum* (BB + TZ and BB + CS), and the consortium (LC + LP + BB + TZ and LC + LP + BB + CS), with each strain or consortium being added to 100 mL of SCDM with 100 ppm of the respective dye. The effect of the test organisms (LC, LP, BB, and consortium) on the degradation of each dye (TZ and CS) was monitored for 72 hours at 37°C. Samples were taken at these intervals to evaluate the decolonization and degradation efficiencies of the dyes. The concentration of Tartrazine and Carmoisine was also measured to assess the extent of dye degradation over time.

Decolourization efficiency of Azo dyes by Lactobacillus strains

Decolurization in broth was measured using UV-vis spectrophotometer. 10ml broth was centrifuged at 5000rpm at 4° C for 5 minutes. The culture supernatant was collected and filtered with 0.45 µm syringe filter. The absorbance of filtered supernatant was read at λ_{max}, i.e. 480 nm and 520nm for tartrazine and carmoisine, respectively **(Alshehrei. 2023; Nazar *et al.*, 2015).** The decolourization efficiency (Rd) of *lactobacillus* strains was accordingly determined as follows:

$$Rd(\%) = \frac{C_{\mathrm{I}} - C_{\mathrm{t}}}{C_{\mathrm{I}}} \times 100$$

Where C_{I} and C_{t} were expressed as the Initial concentration and the concentration of dye at time *t.*

Quantification of Azo dyes using HPLC

The samples were centrifuged same as in previous step and the supernatant was filtered with 0.2 µm membrane filter. Azo dyes were separated using Agilent poroshell 120 EC- C18, 4.6× 150mm, 2.7 µm (45C) column in an Agilent 1260 infinity quaternary LC system, with Mobile phase A: 10Mm ortho- phosphoric acid (pH-7), and B: Methanol(pH-7) by gradient elution (Flow rate: 1.2 ml/ min). Standard stock solution of TZ and CS were prepared with both diluents (80:20) that were used as mobile phase, linearity curves were constructed. The dyes were detected with the Agilent 1290 Infinity Diode array detector (DAD) at λ_{max} of respective azo dyes and its concentration (C) in samples was calculated and expressed as:

$$C\ (PPM) = \frac{A_1}{A_2} \times \frac{\mathrm{W}}{\mathrm{V}} \times c$$

Where A_1 and A_2 were expressed as the sample area and standard area respectively, and W and V were the weight and volume of sample, and C was the concentration of standard.

RESULTS AND DISCUSSION

Effect of Tartrazine on test organisms

The addition of Tartrazine (T1) shows a variable effect on the growth of *Lactobacillus casei* (LC), *Lactobacillus plantarum* (LP), and *Bifidobacterium bifidum* (BB) across the time points 0h, 6h, 24h, and 30h. Log cfu/ml concentration of bacterial species in the presence of T1 and as control is given in Figure 1. For LC, T1 appears to slightly enhance growth at 6h and 24h, though this effect diminishes by 30h. LP shows a slight reduction in growth with T1 at 6h and 24h but experiences a marginal increase by 30h. In contrast, BB shows negligible differences in growth with or without T1, indicating a stable response. T1 seems to mildly stimulate the early growth of *Lactobacillus* species but has little to no effect on *Bifidobacterium bifidum*.

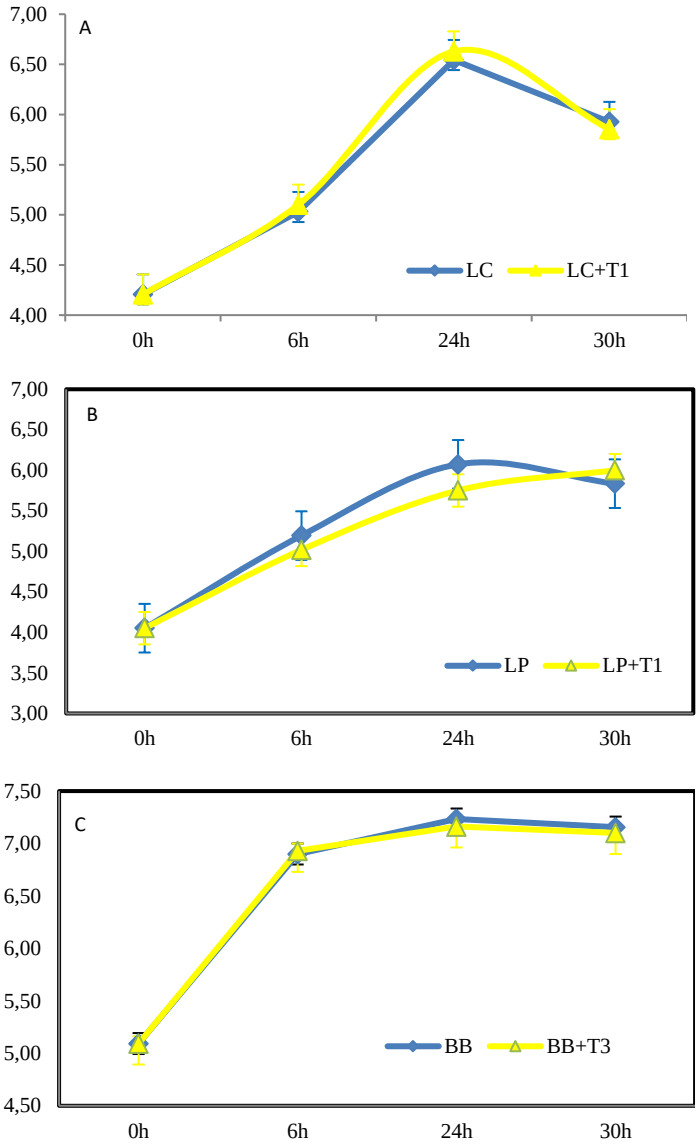

Figure 1 Growth Curve of LAB in Broth Medium at 37°C as Control and in the Presence of Tartrazine at 100 ppm (T1). Effect of Tartrazine on Log_{10} CFU/mL concentration of (A) *Lactobacillus casei*, (B) *Lactobacillus plantarum*, and (C) *Bifidobacterium bifidum.*

Effect of Carmoisine on test organisms

Log 10 concentration of LP in the presence of Carmoisine is given in Figure 2. For LC, Carmoisine (C1) slightly increases growth at 6h and 24h, with a more notable boost by 30h. LP demonstrates steady growth improvement with C1, especially at 30h, while BB experiences minimal yet consistent growth increments with C1 across all time points, with the most noticeable effect observed by 30h. Overall, C1 appears to support sustained growth across all three bacterial strains, with a more marked impact as time progresses.

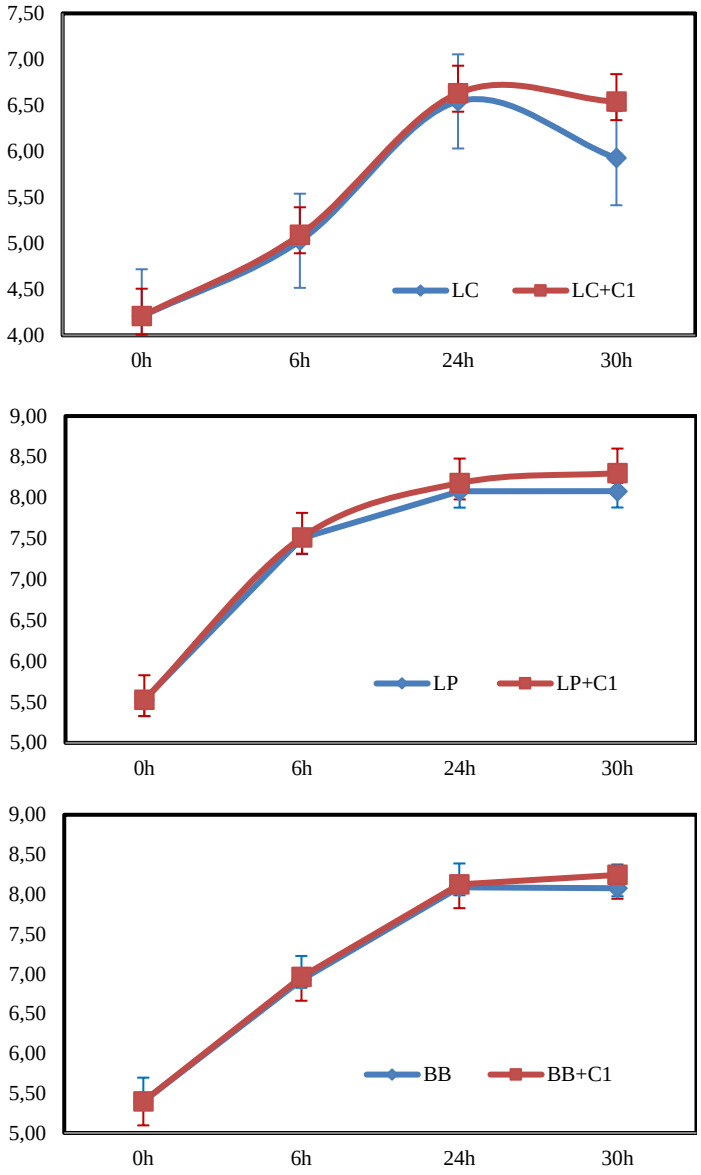

Figure 2 Growth Curve of LAB in Broth Medium at 37°C as Control and in the Presence of carmoisine at 100 ppm (T1). Effect of tartrazine on Log_{10} CFU/mL concentration of (A) *Lactobacillus casei*, (B) *Lactobacillus plantarum*, and (C) *Bifidobacterium bifidum.*

Decolourization study

A preliminary study aimed to investigate the effects of specific probiotic strains namely, *Lactobacillus casei*, *Lactobacillus plantarum* and *Bifidobacterium bifidum*, individually and as consortium on the decolourization of azo dyes Tartrazine and Carmoisine and vice-the-versa was conducted. While the results of the study discussing the effect of the dyes on the culture has been reported earlier **(Pathak *et al.*, 2023**). Here we discuss the results with reference to the effect of the cultures on the dyes over time. Tartrazine and Carmoisine are among the approved, regulated dyes widely in use in the food industry. Although in use these dyes have been reported to be toxic **(Amin *et al.*, 2023; Barciela *et al.*, 2023; Mongi *et al.*, 2011).**

The dyes, however, are in use as an additive, as colouring agents including in probiotic diets and supplement. The colour of these dyes is determined by the position of the azo group within its chemical structure, as well as the presence of other functional groups. In the case of Tartrazine, the lemon yellow colour is a result of the azo group being located between two aromatic rings, which contribute to its vibrant yellow shade (Figure 3A). Tartrazine dye is known for its stability across a wide pH range, making it suitable for various applications. On the other hand, carmoisine contains the same azo group, but it is positioned on the side chain, resulting in a crimson red colour (Figure 3B). When the chemical structure of these dyes is altered or degraded, it leads to the decolourization of the dye, indicating a change in its colour.

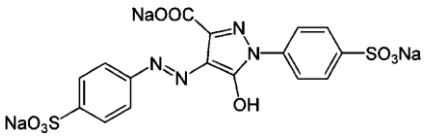

Figure3A Tartrazine

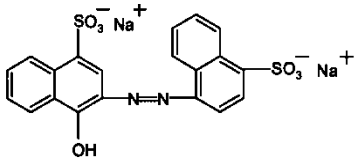

Figure3B Carmoisine

In our study *L. casei*, *L. plantarum* and *B.bifidum* were made to grow individually and as consortium in a broth medium (SCDM) containing the azo dyes Tartrazine and Carmoisine separately, the experimental design of which is as shown in Table 1. The test samples hereafter will be referred to as LC-C, LP-C, BB-C and Con-C, indicating *L.casei* in the presence of Carmoisine, *L. plantarum* in the presence of Carmoisine, *B. bifidum* in the presence of carmoisine and Consortium in the presence of Carmoisine, respectively. Similarly, LC-T, LP-T, BB-T and Con-T refers to the cultures in the order as mentioned earlier, but in the presence of Tartrazine.

Table 1 Experimental Design and Sample nomenclature

	Carmoisine			
	0 hours	**24 hours**	**48 hours**	**72 hours**
Control	C-0	C-24	C-48	C-72
L.casei	LC-C-0	LC-C-24	LC-C-48	LC-C-72
L.plantarum	LP-C-0	LP-C-24	LP-C-48	LP-C-72
B.bifidum	BB-C-0	BB-C-24	BB-C-48	BB-C-72
Consortia	Con-C-0	Con-C-24	Con-C-48	Con-C-72

	Tartrazine			
	0 hours	**24 hours**	**48 hours**	**72 hours**
Control	T-0	T-24	T-48	T-72
L.casei	LC-T-0	LC-T-24	LC-T-48	LC-T-72
L.plantarum	LP-T-0	LP-T-24	LP-T-48	LP-T-72
B.bifidum	BB-T-0	BB-T-24	BB-T-48	BB-T-72
Consortia	Con-T-0	Con-T-24	Con-T-48	Con-T-72

Legend: T – Tartrazine, C- carmoisine, LC- *L. Casei*, LP- *L.plantarum*, BB- *B. Bifidum*, Con- *Consortium*

Table 2 Absorbance values at λmax indicating dye concentration in cultures over time (upto 72h)

		Absorbance at λmax							
		Carmoisine (at 520)				**Tartrazine (at 480)**			
		***L.casei* (LC-C)**	***L.plantarum* (LP-C)**	***B.bifidum* (BB-C)**	**Consortia (Con-C)**	***L.casei* (LC-T)**	***L.plantarum* (LP-T)**	***B.bifidum* (BB-T)**	**Consortia (Con-T)**
Intervals	0h	3.94				3.99			
	24h	0.198	0.299	0.369	0.22	3.98	3.98	3.98	3.98
	48h	0.197	0.28	0.361	0.22	3.97	3.98	3.97	3.98
	72h	0.197	0.26	0.359	0.22	3.97	3.98	3.97	3.97

Effect of test organisms on Carmoisine

i. Physical observation and absorbance measurement of broth with test organisms and Carmoisine

Tests (Culture with dye) in broth that were initially crimson red in appearance due to Carmoisine, were physically observed to have been decolourised over time. The decolourisation was observed in all the tests containing Carmoisine dye that includes *L. casei*, *L. plantarum*, *B. bifidum* and the consortium of all the strains together in a time span of 24 hours of incubation. (Figure4). The absorbance of broth containing the test organisms and the dye carmoisine were

individually taken by Spectrophotometry at λ_{520} (wavelength) from the start of the experiment and successively after every 24h (24, 48, 72h) for 3 days. The results of the absorbance measurement are as shown in Table 2. Evidently, all the test organisms both individually and as consortium showed reduction in absorbance, indicative of degradation of dye within a span of 24h, whereafter the absorbance values remained stable till 72h.

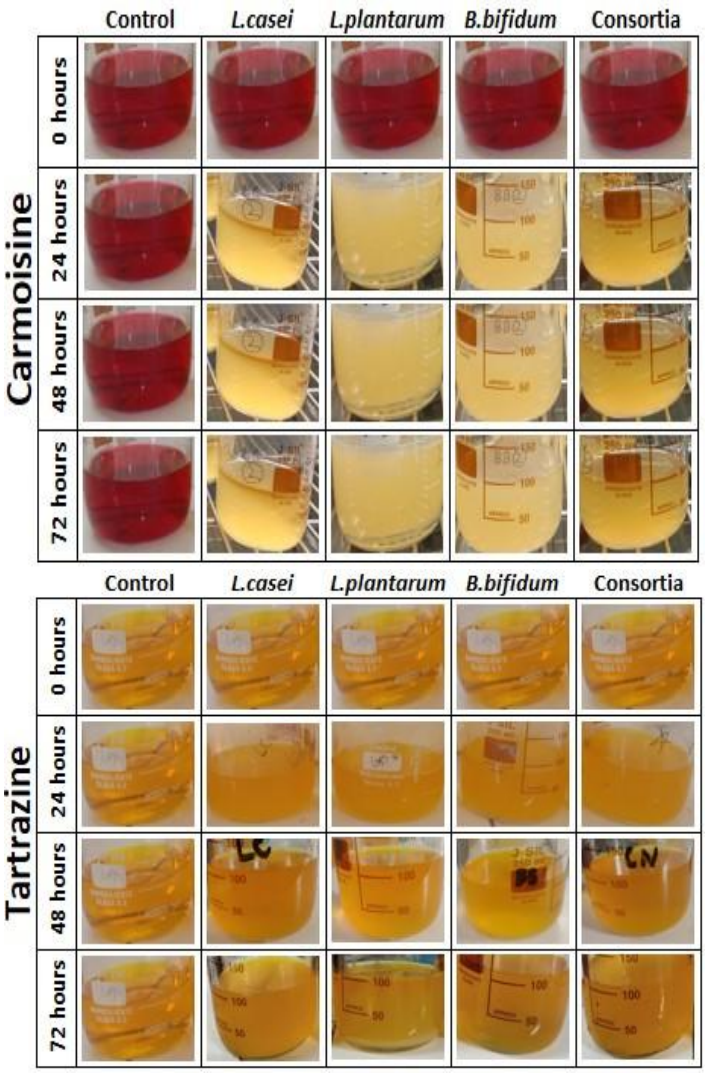

Figure 4 Physical Observation of Carmoisine and Tartrazine color change in Control and Tests (LC-C, LP-C, BB-C & Con-C, and, LC-T, LP-T, BB-T & Con-T)after 24, 48 and 72 hours of incubation.

Table 3 Area under curve and Carmoisine concentration (ppm) as standard and with test organisms over time

	Area Under Curve				**Cocentration(ppm)**			
	at 0h	at 24h	at 48 h	at 72 h	at 0 h	at 24h	at 48 h	at 72 h
Carmoisine as Control	**3788.752**	**3788.752**			**99.66**	**99.66**		
With *L.casei* (LC-C)		6.947	-	-		**0.18**	-	-
With *L.plantarum* (LP-C)		22.21	-	-		**0.65**	-	-
With *B.bifidum* (BB-C)		8.849	-	-		**0.23**	-	-
With Consortium (Con-C)		8.324	-	-		**0.22**	-	-

ii. Estimation of Carmoisine

Simultaneously the test samples were also subject to estimation of carmoisine by HPLC method at specified time intervals samples of 24, 48 and 72h. All the tests (LC-C, LP-C, BB-C and Con-C) were estimated for Carmoisine in parts per million (ppm) concentrations. The values estimated are shown in Table 3. The results clearly indicate that the concentrations of carmoisine (ppm) in all the tests were reduced close to nil overtime, within 24h. The reduction percentage was more than 99% with all the tests (Figure 7).

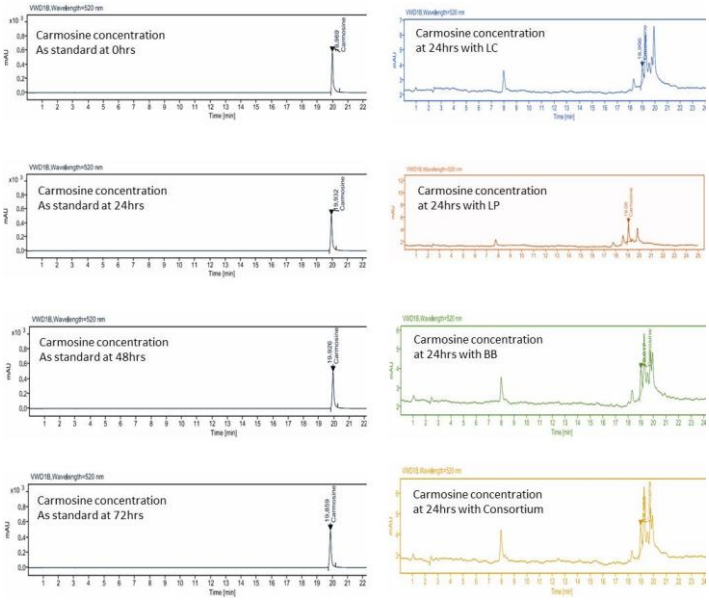

Figure 5 Chromatograms showing the concentration of Carmoisine in samples LC-C, LP-C, BB-C and Con-C at time intervals from 0, 24, 48 and 72 hours.

Effect of test organisms on Tartrazine

i. Physical observation and absorbance measurement of broth with test organisms and Tartrazine

Tests (Culture with dye) in broth that was initially lemon yellow in colour due to Tartrazine, were physically observed up to a period of 72h from the start of the experiment. It was observed that the colour of the remained unchanged till the last observation at 72h. No decolourisation was observed in all the tests containing Tartrazine that includes *L. casei*, *L. plantarum*, *B. bifidum* and the consortium of all the strains (Figure 4). Simultaneously, the absorbance measurements of broth containing the test organisms and the dye Tartrazine were individually taken by Spectrophotometry at λ_{480} (wavelength) from the start of the experiment and successively after every 24h (24, 48, 72h) for 3 days. The results of the absorbance measurement are as shown in Table 2. Evidently, all the test organisms both individually and as consortium showed no change in the absorbance values, indicative of consistency of dye concentration over a span of 72h.

ii. Estimation of Tartrazine

The test samples were also subject to estimation of Tartrazine by HPLC method at specified time intervals samples similar to that of physical observation and absorbance measurement (24, 48 and 72h). All the tests (LC-T, LP-T, BB-T and Con-T) were estimated for Tartrazine in parts per million (ppm) concentrations. The values estimated are shown in Figure 5. The results showed varied concentrations of Tartrazine (ppm) at different time intervals in each of the tests. The dye concentration in test with *L. casei* (LC-T) showed marginal to negligible reduction in concentration from initial concentration to 24, 48 and 72 h of study. After 72h the dye concentration reduction was 5.8%. In the case of tests with *L. plantarum* (LP-T), the reduction percentage was around 25%, but after 72h of incubation. Similar was the observation with *B. bifidum* (BB-T), wherein the reduction percentage of the dye concentration was 24% after 72h. However, it is notable that in the test with consortium of all the 3 strains (Con-T) the concentration of the dye reduction was to a percentage of 64.8% after 72h (Figure 7).

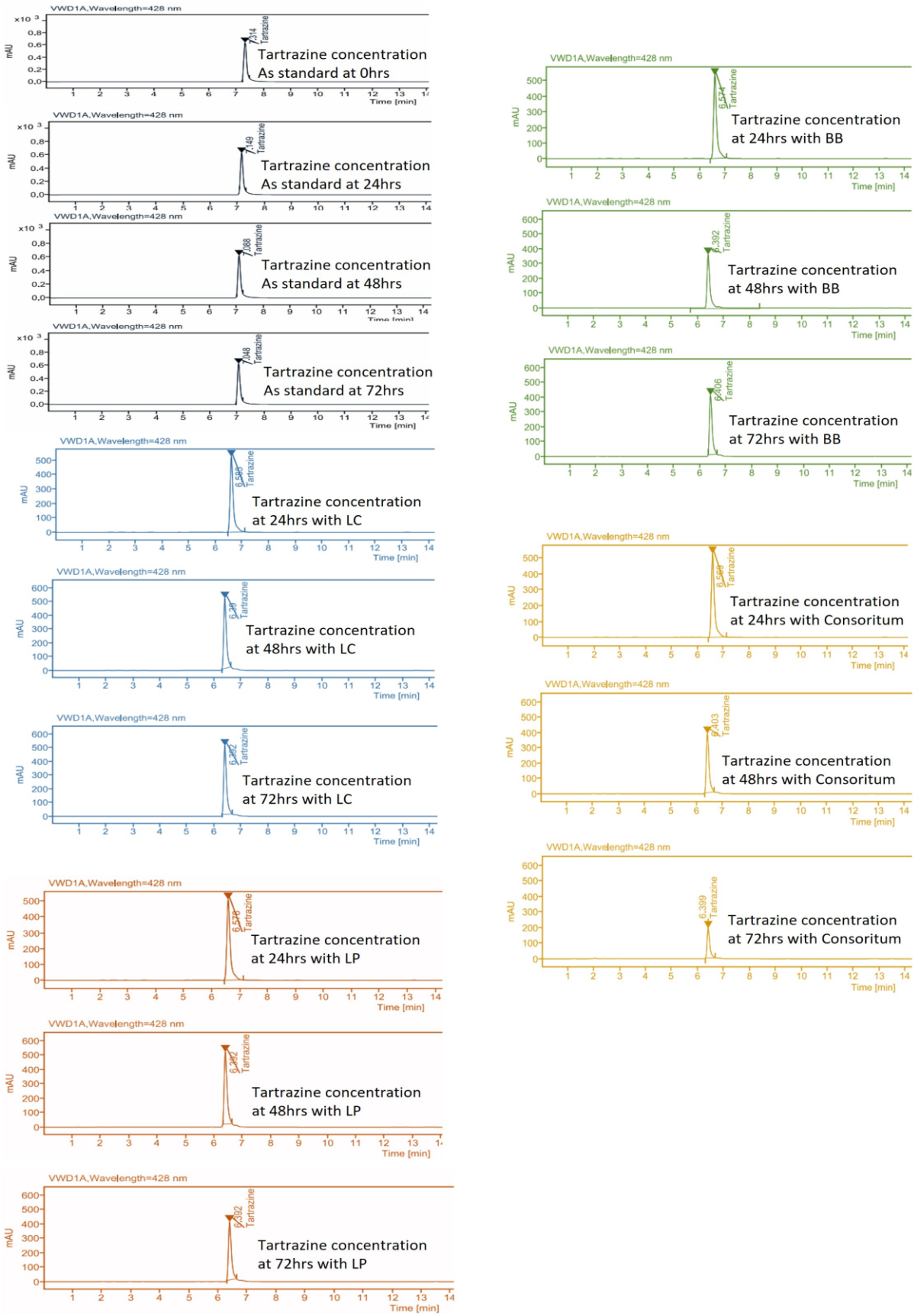

Figure 6 Chromatograms showing the concentration of Tartrazine in samples LC-T, LP-T, BB-T and Con-T at time intervals from 0, 24, 48 and 72 hours.

Azo dyes are derived from petroleum, composed of carbon, hydrogen, nitrogen, sodium, oxygen, and sulphur. These dyes are being drained into industries and get into environment. When they are released into the environment, either through wastewater or solid waste, they can undergo photo degradation and produce toxic by-products, such as aromatic amines, that can contaminate soil, water resources and ecosystem. Although regulations have been put in place in many countries to limit their use and regulate the disposal, detoxification of these dyes is a matter of concern. Both Tartrazine and carmoisine are also being used in food prducts including candies, soft drinks, flavored chips, sauces, ice craems, cakes, chewing gums, jams, jellies and fermented foods.

Table4 Area under curve and Tartrazine concentration (mg/kg) as standard and with test organisms over time

	Area Under Curve				Cocentration(mg/kg)			
	at 0h	at 24h	at 48h	at 72h	at 0 h	at 24h	at 48h	at 72h
Tartrazine as Control	**4655.916**	**4655.916**			**97.62**	**97.62**		
With *L.casei* (LC-C)		4449.141	3717.159	3699.207		**93.28**	**92.38**	**91.93**
With *L.plantarum* (LP-C)		4434.025	3648.113	2913.58		**92.97**	**90.66**	**72.41**
With *B.bifidum* (BB-C)		4500.469	3705.833	2951.216		**94.36**	**92.10**	**73.34**
With Consortium (Con-C)		4483.684	2919.225	1382.541		**94.01**	**72.55**	**34.36**

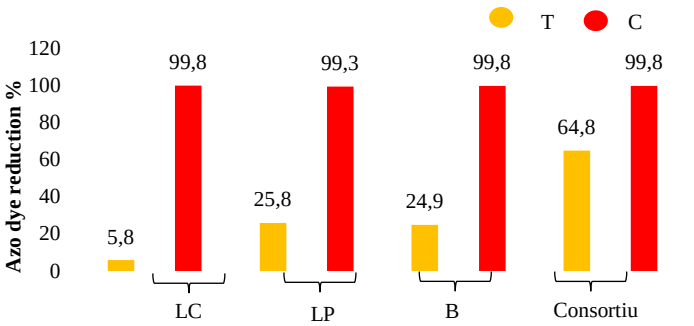

Figure 7 Reduction rate of Tartrazine (TZ) and Carmoisine (CS) concentration in test samples

Azo dyes, including tartrazine and carmoisine, are widely utilized in food products at levels regulated to a maximum of 100 mg/kg (100 ppm), as this concentration is deemed safe for human consumption. However, these dyes are resistant to degradation and may persist in the environment or the human gut, potentially impacting health and ecological balance. By choosing this concentration, our study simulates real-world exposure levels, making the findings more relevant to the actual conditions under which biodegradation would occur in the human gastrointestinal tract or natural environments. The ability of probiotics to degrade azo dyes at this concentration could offer a dual benefit of supporting gut health while also reducing dye persistence. The degradability of the azo dyes depends primarily on the chemical structure, characterized by two nitrogen atoms linking hydrocarbon groups. The toxicity of azo dyes is mainly due to this structure, which contains one or more azo (-N=N-) groups. During microbial degradation, bacteria utilize the azo dye as a source of carbon and nitrogen, and they produce enzymes called azo-reductases. Some aerobic strains utilize azo dyes as their exclusive source of carbon and nitrogen **(Coughlin *et al.*, 2002)**, while others specifically reduce the azo group through oxygen-tolerant azo reductases **(Ikram *et al.*, 2023).**Various microorganisms with the ability to decolourize a broad range of azo dyes include *Aspergillus versicolour* **(Taştan *et al.*, 2010)**, *Trametes versicolour* **(Aksu *et al.*, 2007),** *Pseudomonas putida K1*, *Serratia proteamaculans SL14* **(Mahmood *et al.*, 2014),** and *Phormidium spp*. **(Sadettin and Donmez, 2007).** However, since azo dyes are also commonly in use in the food industry as a colouring additive it would be of relevance to consider strains that are common in food and at the same time Generally Recognised as Safe (GRAS), and strains of Lactic Acid Bacteria therefore is an optimal candidate to be screened for potentials to detoxify these azo dyes. In this context, *Lactobacillus* and *Bifidobacterium* strains, those are not only safe but are also known to be probiotic were considered as subjects to be studied for their detoxifying potentials under *in-vitro* conditions. *L. casei*, *L. plantarum* and *B. bifidum* that are not only probiotic but have also been reported for various other beneficial properties such as high tolerance to acid and bile and capabity to adhere to intestinal surfaces, inhibitory effect on pathogens, resistant to antibiotics were ideal for the detoxification study. Effect of Tartrazine and Carmoisine on growth pattern revealed distinct effects on the growth of *L. casei*, *L. plantarum*, and *B. bifidum*. While T1 shows a mild enhancement of LC growth at 6h and 24h, this effect wanes by 30h, and LP experiences a slight reduction at earlier time points, with BB displaying stable growth regardless of Tartrazine. In contrast, Carmoisine consistently promotes growth across all time points, particularly enhancing LC and LP growth notably by 30h. BB also shows minimal but steady improvement with Carmoisine. Carmoisine demonstrates a more pronounced and sustained growth-supporting effect compared to Tartrazine which suggest them degradation**.**

The findings of the study indicate that Carmoisine dye was degraded within a span of 24h by all the three test organisms, individually and together as consortium. At the same time the cultures were less effective in degrading the dye tartrazine individually even after a time span of 72hours, although as consortia the test organisms did degrade the dye but not as effectively as in the case of carmoisine. It is of relevance to note that these dyes are also in use as additives in probiotic beverages and supplements, and the results answers to concerns relating to the effect of the dyes on the culture and vice-the-versa. In our earlier report Pathak *et al.* (2023), e had shown that the dyes had minimal effect on the probiotic strains with respect to their growth and proliferation. The result of the current study suggests that the probiotic strains could interfere and degrade the dye and therefore can be beneficial in reducing health risks due to the consumption of the dyes. This finding could also be of importance to the textile industry where azo dye consumption is common leading increased discharge to the environment. These strains have potential applications in the biodegradation of azo dyes, breaking them down into less harmful byproducts in effluents and thus offering environmental benefits by reducing toxicity. Studies have also shown that certain probiotic strains can suppress the formation and breakdown of nitrosamines, which are associated with cancer risk **(Sallan *et al.*, 2023)**. In this study, an unknown peak was observed near the retention time of Carmoisine, suggesting possible dye degradation.

Previous research by **Chen *et al***. (2009) demonstrated that reduction of azo dyes by *Lactobacillus fermentum* led to the formation of aniline compounds, which are considered genotoxic. These findings underscore the need for further investigation into the safety and implications of azo dye degradation by probiotic strains. Advanced techniques, such as mass spectrometry, should be employed to analyze the degradaded products and assess their potential health risks comprehensively. Degradation efficacy of *Bifidobacterium bifidum*, which inhabit human gut since after birth was reported for the first time. These findings suggest that usage of azo dyes in articles which are purposively consumed for human health benefits needs to be reconsidered after risk assesment using scientific approaches.

CONCLUSION

This study provides evidence that *L. casei*, *L. plantarum* and *B. bifidum* were able to degrade azo dyes Carmoisine and Tartrazine. While carmoisine was degraded both individually and as consortia of the test organisms, tartrazine could not be degraded as effectively. Probiotics that are GRAS (Generally Recognized as Safe), besides their other benefits to human health can also be beneficial in degradation of food additives such as the azo dyes, which otherwise possses health risk. At the same time these strains can also be suitable candidates for biodegradation of azo dyes into less harmful substances from the industrial effluent. Our results further encourage concentration-dependent studies to identify optimal conditions for effective dye biodegradation. This could enhance the understanding of probiotic interactions with food additives and their potential as natural biodegraders, contributing valuable insights for food safety, environmental sustainability, and the development of probiotic-based bioremediation strategies.

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