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Impact of fructooligosaccharide supplementation on generation time and viable count of *Bacillus coagulans* IS2 in green tea and black tea infusion

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Abstract
This study investigated the impact of fructooligosaccharide supplementation and effect of polyphenols on viable count and generation time of *Bacillus coagulans* IS2 during a 24 hour fermentation period in different formulations of green tea and black tea. Results revealed that the addition of *B. coagulans* did not significantly alter the total polyphenols and antioxidant power of both types of tea. Initially the viable count of *B. coagulans* was 63.60% higher in black tea infusion in comparison to green tea infusion which can be attributed to the higher polyphenol content found in the green tea. However, the incorporation of fructooligosaccharide (5% w/v) reduced this disparity in viable count to 14.92%. Addition of fructooligosaccharide in green tea led to a decrease in the generation time of *B. coagulans* 23.42%. However, the incorporation of fructooligosaccharide in black tea infusion did not significantly alter the generation time of *B. coagulans*. Conversely, fructooligosaccharide did not significantly alter the generation time of *B. coagulans* in black tea which suggested that fructooligosaccharide was more effectively utilised by *B. coagulans* in green tea compared to black tea, compensating the inhibitory effects of polyphenols on growth of *B. coagulans*.

Keywords: *Bacillus coagulans* IS2, probiotics, prebiotics, synbiotics, tea, FOS

Introduction
Prebiotics are non-viable constituents of food that elicit a positive impact on the well-being of the host, which is connected to the modulation of the intestinal flora (Rugji & Dinçoğlu, 2022; FAO/WHO 2002) [39, 19]. Conversely, probiotics are living microorganisms that are administered in appropriate quantities to bestow health advantages (FAO/WHO 2002; Deshpande *et al*., 2022) [19, 13]. Synbiotics, on the other hand, are a combination of probiotics and prebiotics and are predominantly utilized because pure probiotics struggle to survive within the gastro-intestinal tract without their supply of prebiotic nutrients (Panesar *et al*., 2009) [34]. At present, an abundance of extensively characterized strains of *Lactobacilli* and *Bifidobacteria* have been developed for human application, with the objective of alleviating the possibility of gastrointestinal (GI) infections or providing remedial measures for such infections (Nagpal *et al*., 2012) [32]. Nevertheless, these cultures are unable to establish residence within the human gastrointestinal tract due to their susceptibility to an acidic gastric environment and intestinal bile salts (Del Campo *et al*., 2005) [12]. Within the diverse array of probiotic strains, *Bacillus coagulans*, a Gram-positive, catalase-positive, spore-forming, motile, facultative anaerobic rod, has garnered attention due to its resilience and robustness, particularly within the gastrointestinal milieu (Garrison., 2019; Majeed *et al*., 2021; Zhao *et al*., 2023) [12, 23, 49]. *Bacillus coagulans* can contribute to the establishment of an anaerobic and acidic gut milieu that is unfavorable to pathogens, thereby facilitating the proliferation of other beneficial probiotics (Cao *et al*., 2019) [10]. *B. coagulans* strains have the ability to metabolize free oxygen in the colon and stomach, thereby hindering redox processes as facultative anaerobic bacteria (Abhari *et al*., 2015) [2]. *Bacillus coagulans* Unique IS2, in particular, is a probiotic strain that is commercially available and has established clinical efficacy without causing harm (Ahire *et al*., 2020) [5]. It is also effective in the treatment of various conditions such as diarrhoea, constipation, IBS, IBD, hypercholesterolemia and depression (Adibpour *et al*., 2019b; Majeed *et al*., 2018b; Satti *et al*., 2023; Shinde *et al*., 2020; Sudha *et al*., 2012; Sudha *et al*., 2018) [4, 25-26-, 27, 40, 42, 44, 46, 45].

The therapeutic advantages of *Bacillus coagulans* may be attributed to its capability to produce antimicrobial substances like bacteriocins (Abdhul *et al*., 2015; Zhang *et al*., 2022) [1, 48], which prevent the growth of pathogens (Rugji *et al*., 2022) [39] and contribute to the balance of probiotic populations in the large intestine (Honda *et al*., 2011) [18]. Fructooligosaccharides (FOS), a subset of oligosaccharides, have emerged as prebiotic compounds that possess the ability to modulate the gut microbiota and enhance the viability of probiotic microorganisms (Roberfroid, 2007) [38]. Prebiotics are capable of reinforcing the following: (1) the production of short-chain fatty acids (SCFA) and lactic acid through fermentation; (2) the proliferation of probiotic bacteria, accompanied by an increase in the concentration of minerals such as calcium and magnesium within the colon; and (3) the immune response of the host, demonstrated by the production of IgA and the modulation of cytokines (Dable-Tupas *et al*., 2020) [11]. Various prebiotic compounds, including FOS, galactooligosaccharides, inulin, and polydextrose, have been documented to exhibit synergistic growth-promoting effects on *B. coagulans*, as reported by Majeed *et al*. (2019) [24]. Additionally, lesser explored prebiotics such as fibers derived from fenugreek, cranberry, sugarcane, green banana, and chitooligosaccharides have been observed to enhance the growth and survival of *B. coagulans* within the gastrointestinal tract (Majeed *et al*., 2018a; Majeed *et al*., 2018b; Shinde *et al*., 2019; Shinde *et al*., 2020a) [25-26, 27, 24, 43]. According to the International Scientific Association for Probiotics and Prebiotics (ISAPP), a synbiotic is presently defined as the combination of vital microorganisms and specific compounds that are utilized by the microorganisms present in the host. This results in a positive impact on the overall health of the host organism (Parhi *et al*., 2022; Swanson *et al*., 2020) [35, 47]. Consequently, the incorporation of suitable synbiotics within a single product is expected to yield an improved result compared to the individual effectiveness of probiotics or prebiotics alone (Bengmark, 2005) [8]. The most prevalent instance of a synbiotic is the combination of bacteria from the *Lactobacillus* or *Bifidobacterium* genus with fructooligosaccharides (FOS) (Markowiak *et al*., 2017) [30]. The utilization of prebiotics by probiotics as a source of energy enables them to extend their residence time in the gastrointestinal tract beyond their natural limitations (Gibson and Roberfroid, 1995; Dable-Tupas *et al*., 2020) [16, 11]. Furthermore, the synergistic effect aids in facilitating the appropriate establishment of probiotic dietary supplements in the large intestine, as well as promoting the growth of probiotics (Pandey *et al*., 2015) [33].

Green tea, scientifically known as *Camellia sinensis*, and black tea, specifically *Camellia sinensis var. assamica*, are widely consumed beverages worldwide due to their renowned antioxidant properties and positive impacts on health (Gramza *et al*., 2005; Sena *et al*., 2020) [17, 41]. Tea effectively acts as a carrier for *B. coagulans* Ganeden BC30, and the infusion treatments demonstrate high tolerance by the *B. coagulans* Ganeden BC30 spores (Poshadri *et al*., 2022; Polo *et al*., 2022) [37, 36]. Tea polyphenols, particularly catechins, possess distinguished antimicrobial and antioxidant qualities, which contribute positively to human well-being (Almajano *et al*., 2008) [7]. However, the effects of tea polyphenols on probiotic microorganisms, specifically *Bacillus coagulans*, remain relatively indeterminate. The comprehension of how different types of fructooligosaccharides (FOS) impact the growth and viability of *Bacillus coagulans* holds significant importance as it offers valuable insights into the creation of functional beverages that amalgamate the health benefits of both probiotics and tea. *B. coagulans* has been observed to maintain its viability when combined with various food products such as jelly candies (Miranda *et al*., 2020) [31], date paste (Marcial-Coba *et al*., 2019) [29], sweetener Nabat (Adibpour *et al*., 2019a) [3], orange juice, yogurt (Almada-Érix *et al*., 2021) [6], pasta (Fares *et al*., 2015) [14], and croissant (Blaiotta *et al*., 2023) [9]. In the present study, the objective is to enhance the current comprehension of the interaction between *Bacillus coagulans* and FOS by incorporating the Unique IS2 strain into both green tea and black tea. Furthermore, the aim is to compare the impacts of these teas on the viable count and generation time of *B. coagulans*, thereby illuminating their suitability as carriers for probiotics.

2. Materials and Methods

2.1 Bacterial strain and culture conditions

The spores-containing powder of *Bacillus coagulans* Unique IS2 (1.8×109 CFU/gram) was acquired from Unique Biotech Limited, Hyderabad, India. Glucose Yeast Extract Agar (GYEA) and de Man, Rogosa, and Sharpe (MRS) broth were procured from HiMedia, a supplier based in Mumbai, India. The bacterial strain was cultured in MRS broth at a temperature of 37 °C for duration of 24 hours. Following incubation, the bacterial cells were harvested through centrifugation and subsequently suspended in sterile saline. A 5% (w/v) proportion of this sterile saline solution was then added to flasks containing sterile infusions of Green tea and Black tea.

2.2 Preparation of tea infusion

Green tea and Black tea in powdered form were acquired from the local market. The purchase of 90% Fructooligosaccharide powder was made from TATA Chemicals Ltd, India, and the acquisition of sucrose was completed at HiMedia, Mumbai, India. The process of preparing Green tea infusion (GTI) and black tea infusion (BTI) involved steeping 1.5 grams of tea powder in 150 ml of sterile distilled water at 90 °C for 5 minutes, followed by filtration using Whatman filter paper.

2.3 Experimental design

Table 1: Formulations used in the experiment

Formulation		Composition				
		Green tea infusion (ml)	**Black tea infusion (ml)**	**Sucrose (%w/v)**	**FOS (%w/v)**	***Bacillus coagulans* (log CFU/ml)**
GTI formulations	N_1	50	-	-	-	3.20-3.25
	C_1	50	-	5	-	3.20-3.25
	T_1	50	-	5	5	3.20-3.25
BTI formulations	N_2	-	50	-	-	3.20-3.25
	C_2	-	50	5	-	3.20-3.25
	T_2	-	*50*	*5*	*5*	*3.20-3.25*

All six combinations were subjected to sterilization, followed by the introduction of *Bacillus coagulans* culture (5% w/v) at a temperature of 90 °C. The resulting mixture was then incubated in an orbital shaker incubator, operating at a speed of 130 r.p.m, for duration of 24 hours at a temperature of 40 °C. The formulations N1 and N2 were deemed as control groups for GTI and BTI, respectively, while the formulations C1 and C2, which contained sucrose, were regarded as control groups for GTI and BTI. For the subsequent analyses, samples were collected from all formulations at 0, 6, 12, 18, and 24 hours.

2.4 Determination of total polyphenols and antioxidant activity: The total polyphenol content (TPC) expressed as mg GAE (Gallic acid equivalent)/gram was analyzed for the tea formulations using the Folin-ciocalteu (Khanum *et al*., 2017) [21] method. The antioxidant activity of the formulations expressed as mg ascorbic acid equivalent (AAE)/gram was determined using FRAP (Ferric ion reducing antioxidant power) assay (Kiran *et al*., 2018) [22] using ascorbic acid as standard.

2.5 Viable count of *B. coagulans*: The quantification of *B. coagulans*' viable count was ascertained through serial dilution and subsequent plating on GYEA agar. Subsequently, the enumeration of colony-forming units was conducted following a 24-hour incubation period at a temperature of 40 °C (Majeed *et al*., 2016; Majeed *et al*., 2019) [28, 24].

2.6 Generation time of *B. coagulans*

The generation time of *B. coagulans* in the time interval of 6 - 12 hours (360 minutes) was calculated according to the following equation assuming linearity.

$$\text{Generation time(minutes)} = \frac{t}{n}$$

$$n = 3.33(\log N_t - \log N_0)$$

Where, t = time interval,
n = number of generations,
N_t = Number of bacteria at the end of selected time interval,
N_0 = Number of bacteria at the start of selected time interval

2.7 Statistical analysis

All the assays were conducted in independent triplicates. The results were statistically evaluated in one-way analysis of variance (ANOVA) followed by Tukey's test using Jamovi desktop statistical software (version 2.3.28, https://www.jamovi.org/), and statistical significance was determined at $p<0.05$.

3. Results

3.1 Total polyphenol content (TPC) and Ferric-reducing antioxidant power (FRAP)

The TPC and FRAP of all the formulations were analyzed before inoculation and at 0 hours and 24 hours after inoculation with *B. coagulans*.

Table 2: Total polyphenol content (TPC) (mg GAE/gram) of the formulations before inoculation with *B. coagulans* and at 0 hours and 24 hours after inoculation with *B. coagulans*.

	TPC (mg GAE/gram)		
Sample	**Before inoculation**	**0hr**	**24hr**
N_1 (GTI)	4.91 ± 0.02^{aA}	4.91 ± 0.02^{aA}	4.90 ± 0.01^{aA}
C_1 (GTI + sucrose)	4.9 ± 0.02^{aA}	4.91 ± 0.02^{aA}	4.91 ± 0.02^{aA}
T_1 (GTI + sucrose + FOS)	4.9 ± 0.01^{aA}	4.90 ± 0.01^{aA}	4.89 ± 0.02^{aA}
N_2 (BTI)	2.33 ± 0.01^{bA}	2.34 ± 0.01^{bA}	2.33 ± 0.02^{bA}
C_2 (BTI + sucrose)	2.34 ± 0.02^{bA}	2.33 ± 0.02^{bA}	2.32 ± 0.02^{bA}
T_2 (BTI + sucrose + FOS)	2.32 ± 0.02^{bA}	2.33 ± 0.02^{bA}	2.30 ± 0.01^{bA}

Values are presented as mean ± standard deviation (n = 3). ^{abc}Difference in lowercase letters within a column indicates significant difference at $p<0.05$ Tukey's test. ^{ABC}Difference in uppercase letters within a row indicates significant difference at $p<0.05$ by Tukeys' test.

Table 3: Ferric-reducing antioxidant power (FRAP) (mg AAE/gram) of the formulations before inoculation with *B. coagulans*, and at 0 hours and 24 hours after inoculation with *B. coagulans*.

	FRAP (mg AAE/gram)		
Sample	**Before inoculation**	**0hr**	**24hr**
N_1 (GTI)	8.36 ± 0.02^{aA}	8.34 ± 0.02^{aA}	8.33 ± 0.02^{aA}
C_1 (GTI + sucrose)	8.34 ± 0.01^{aA}	8.36 ± 0.01^{aA}	8.34 ± 0.02^{aA}
T_1 (GTI + sucrose + FOS)	8.35 ± 0.01^{aA}	8.34 ± 0.01^{aA}	8.33 ± 0.01^{aA}
N_2 (BTI)	3.60 ± 0.02^{bA}	3.59 ± 0.02^{bA}	3.58 ± 0.01^{bA}
C_2 (BTI + sucrose)	3.62 ± 0.02^{bA}	3.61 ± 0.02^{bA}	3.59 ± 0.02^{bA}
T_2 (BTI + sucrose + FOS)	3.61 ± 0.02^{bA}	3.61 ± 0.02^{bA}	3.60 ± 0.02^{bA}

Values are presented as mean ± standard deviation (n = 3). ^{abc}Difference in lowercase letters within a column indicates significant difference at $p<0.05$ by Tukey's test. ^{ABC}Difference in uppercase letters within a row indicates significant difference at $p<0.05$ by Tukey's test.

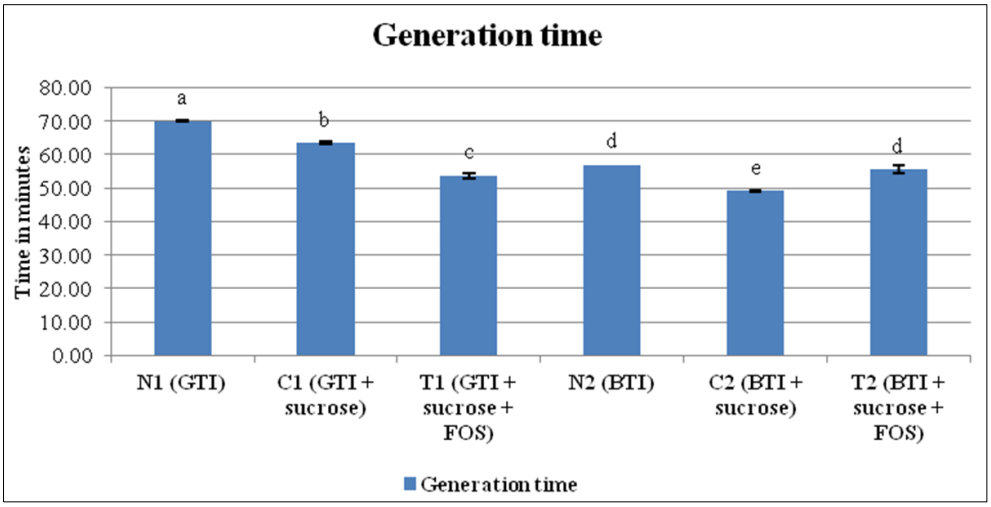
3.2 Viable count of *Bacillus coagulans* in tea

Table 4: Effect of FOS on viable count (log CFU/ml) of *B. coagulans* in different formulations over a growth period of 24 hours

	Viable count of B. coagulans (log CFU/ml)				
Variation	***0 hr***	***6 hr***	***12 hr***	***18 hr***	***24 hr***
N_1 (GTI)	3.22 ± 0.02^{a}	3.42 ± 0.01^{a}	4.96 ± 0.01^{a}	5.23 ± 0.03^{a}	5.22 ± 0.02^{a}
C_1 (GTI + sucrose)	3.23 ± 0.03^{a}	3.60 ± 0.02^{b}	5.30 ± 0.01^{b}	5.73 ± 0.04^{b}	5.71 ± 0.05^{b}
T_1 (GTI + sucrose + FOS)	3.21 ± 0.03^{a}	5.08 ± 0.07^{c}	7.09 ± 0.04^{c}	7.51 ± 0.04^{c}	7.77 ± 0.04^{c}
N_2 (BTI)	3.21 ± 0.03^{a}	6.07 ± 0.02^{d}	7.98 ± 0.01^{d}	8.45 ± 0.02^{d}	8.54 ± 0.03^{d}
C_2 (BTI + sucrose)	3.21 ± 0.04^{a}	5.92 ± 0.02^{e}	8.13 ± 0.01^{e}	8.56 ± 0.03^{e}	8.56 ± 0.03^{d}
T_2 (BTI + sucrose + FOS)	3.23 ± 0.03^{a}	6.59 ± 0.05^{f}	8.54 ± 0.01^{f}	9.02 ± 0.02^{f}	8.93 ± 0.04^{e}

Values with different lowercase letters indicate significant difference by Tukey's test ($p<0.05$)

3.3 Effect of FOS on generation time of *Bacillus coagulans*



Values with different lowercase letters indicate significant difference by Tukey's test ($p<0.05$)

Fig 1: Effect of FOS on generation time of *B. coagulans* in the interval of 6-12 hours (360 minutes)

3.4 Correlation tests

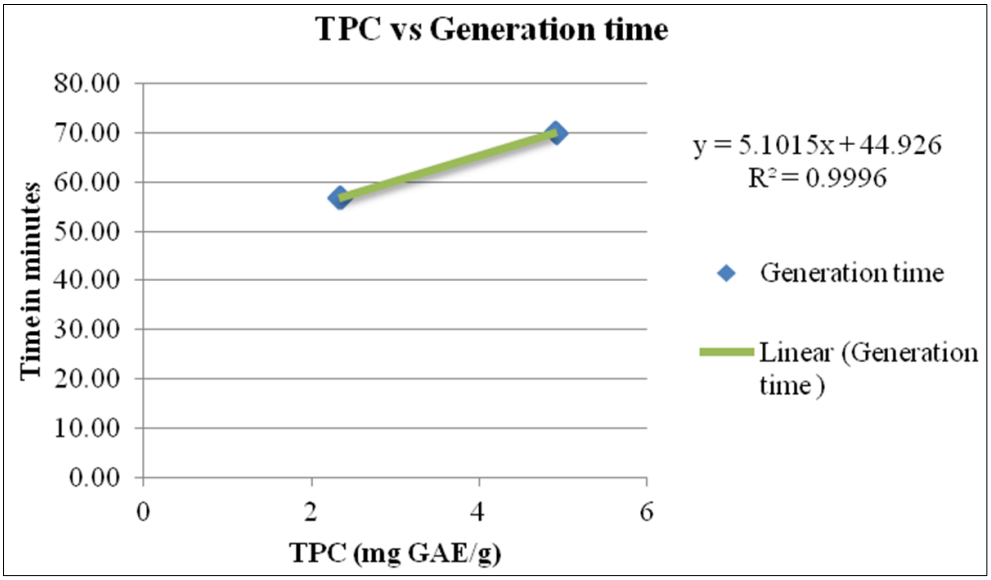


Fig 2: Correlation between generation time of *B. coagulans* and total polyphenol content (TPC) in tea

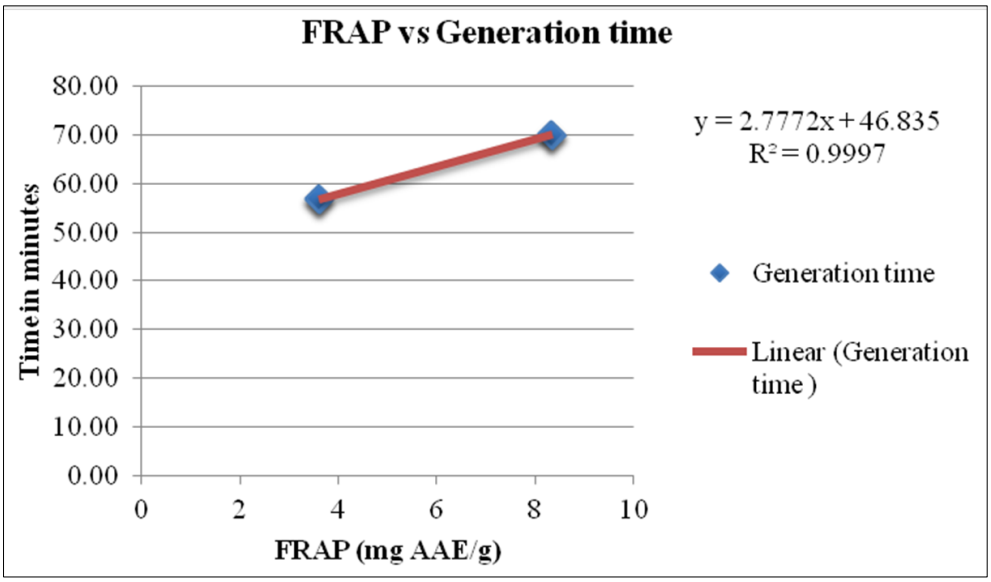


Fig 3: Correlation between generation time of *B. coagulans* and Ferric reducing antioxidant power (FRAP) in tea

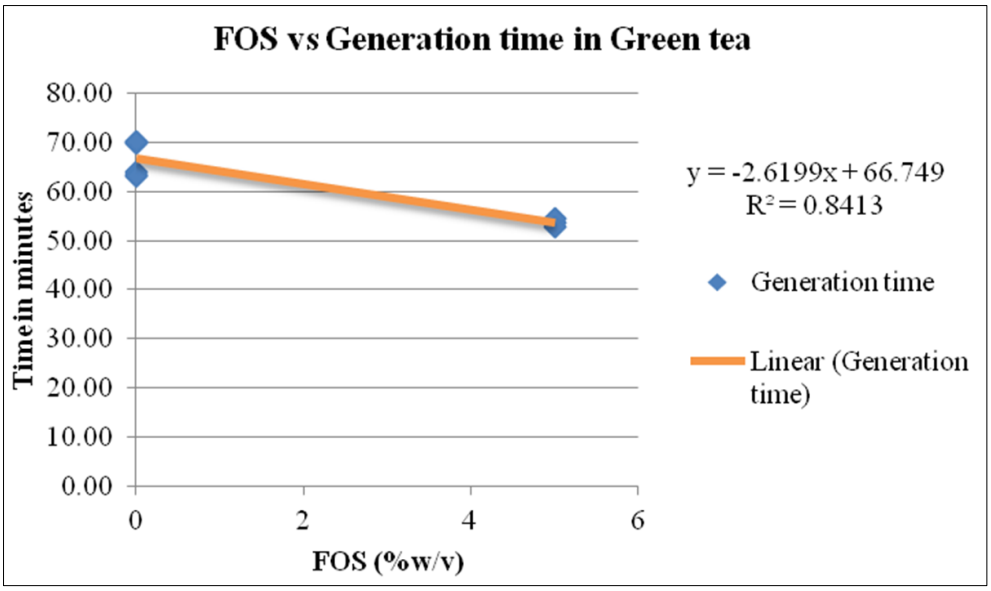


Fig 4: Correlation between generation time of *B. coagulans* and FOS in Green tea

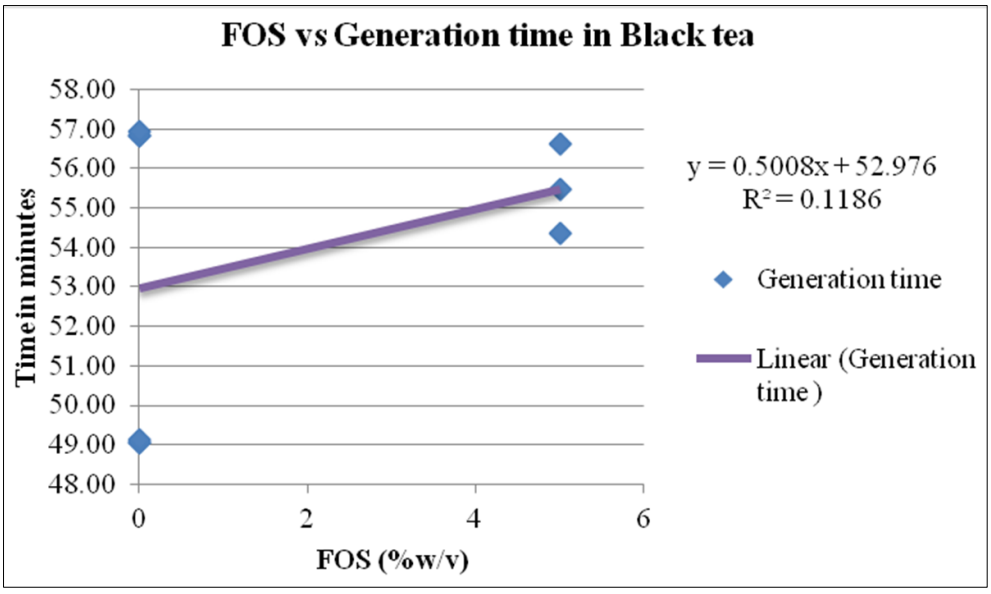


Fig 5: Correlation between generation time of *B. coagulans* and FOS in Black tea

4. Discussion

The data shows that the average total phenolic content (TPC) value of green tea formulations is 2.1 times higher (Table 1), and the average ferric reducing antioxidant power (FRAP) value is 2.3 times higher (Table 2) than that of black tea formulations. This finding aligns with the findings demonstrated by Kaur *et al*., (2015) [20] and Kiran *et al*., (2018) [22]. There was no statistically significant difference ($p>0.05$) in TPC and FRAP levels of the tea formulations containing *B. coagulans* after a fermentation period of 24 hours, in comparison to the samples without *B. coagulans*. The results of this experiment suggest that the probiotic *B. coagulans* IS2 did not impact the functional properties of both green and black tea, even after 24 hours of fermentation. This preservation of the antioxidant properties of tea is crucial, as observed by Majeed *et al*., (2019) [24].

At the initial point of time, there was no significant difference ($p>0.05$) in the viable count of *B. coagulans* IS2 across all the formulations, ranging from 3.21 ± 0.04 to 3.23 ± 0.03 log CFU/ml (Table 3). Utilizing green tea and black tea as the sole sources of nutrition, the count of viable *B. coagulans* after 24 hours of fermentation was 63.60% higher ($p<0.05$) in black tea infusion (N_2) compared to green tea infusion (N_1). Upon incorporation of sucrose, the difference reduced to 49.91% with C_2 (BTI + sucrose) exhibiting a higher count ($p<0.05$) than C_1 (GTI + sucrose). It should be noted that with the supplementation of FOS, the difference in viable count between black tea and green tea further decreased to 14.92% with T_2 (BTI + sucrose + FOS) showing a higher value ($p<0.05$) than T_1 (GTI + sucrose + FOS).

Comparing the viable count of *B. coagulans* after 24 hours of fermentation between green tea formulations C_1 (GTI + sucrose) and T_1 (GTI + sucrose + FOS) demonstrated that the addition of FOS alongside sucrose resulted in a 36.07% significant increase ($p<0.05$) in the viable count (table 3). However, comparing the same variable between black tea formulations C_2 (BTI + sucrose) and T_2 (BTI + sucrose + FOS) after 24 hours of fermentation resulted in a mere 4.32% increase in the viable count of *B. coagulans*, which is significant ($p<0.05$) but lower than what was observed in the case of green tea formulations (C_1 and T_1). In black tea formulations N_2 (BTI) and C_2 (BTI + sucrose), the addition of sucrose did not cause any significant change ($p>0.05$) in the viable count of *B. coagulans*, whereas in the case of green tea formulations N_1 (GTI) and C_1 (GTI + sucrose), the addition of sucrose resulted in a slight increase of 9.38% ($p<0.05$) in the viable count of *B. coagulans*.

Without both sucrose and FOS, the generation time of *B. coagulans* was 18.71% lower in N_2 (BTI) compared to N_1 (GTI) (Figure 1), which may be attributed to the higher content of total polyphenols in N_1. On the contrary, with both sucrose and FOS present in the formulations, the difference in generation time of *B. coagulans* decreased to 3.5% with black tea formulation T_2 (BTI + sucrose + FOS) higher compared to the green tea formulation T_1 (GTI + sucrose + FOS). This indicates the possible inhibitory effect of polyphenols on the growth of *B. coagulans*, resulting in a longer duration for *B. coagulans* to double.

In the case of formulations N_1 (GTI) and C_1 (GTI + sucrose) relating to green tea, the incorporation of sucrose led to a decrease in the generation time of *B. coagulans* by 9.28% ($p<0.05$). Besides, in T_1 (GTI + sucrose + FOS), the generation time of *B. coagulans* exhibited a 15.59% reduction ($p<0.05$) when compared to C_1 (GTI + sucrose), which can probably be attributed to the inclusion of FOS. In the presence of both sucrose and FOS, the generation time of *B. coagulans* in T_1 (GTI + sucrose + FOS) was found to be 23.42% lower than that in N_1 (GTI).

Regarding black tea, the introduction of sucrose led to a 13.7% reduction ($p<0.05$) in the generation time of *B. coagulans* when comparing N_2 (BTI) and C_2 (BTI + sucrose). However, when FOS was incorporated, the generation time increased by 13.03% ($p<0.05$) in T_2 (BTI + sucrose + FOS) in comparison to C_2 (BTI + sucrose). In consequence, there was no significant difference ($p>0.05$) in the generation time of *B. coagulans* between N_2 (BTI) and T_2 (BTI + sucrose + FOS).

Correlation tests revealed a highly positive correlation (R^2 = 0.99) between TPC and FRAP. Similar findings were reported by Kiran *et al*. (2018) [22], where a strong positive correlation was observed between the polyphenol content and antioxidant activity of both green tea and black tea. According to Almajano *et al*. (2008) [7], the higher antioxidant activity of green tea can be attributed to the presence of catechins such as Epi gallo catechin gallate (EPCG) and Epi gallo catechin (EPC). In the case of black tea, the antioxidant properties were attributed to thearubigins, phenolic acids, catechins, and theaflavins.

Interestingly, there was a highly positive correlation (R^2 = 0.9996) between the TPC and the generation time of *B. coagulans* (figure 2). Similarly, an equivalent correlation (R^2 = 0.9997) was observed between the generation time of *B. coagulans* and the FRAP of tea (figure 3). These results suggest that TPC and FRAP may have interfered with the growth of *B. coagulans*, potentially in combination with other negative factors, resulting in an increase in the generation time of *B. coagulans*.

The supplementation of FOS may have assisted in reducing the generation time of *B. coagulans* in green tea, as displayed by a highly negative correlation (R^2 = 0.8413) (figure 4). However, in the case of black tea, a negligible correlation was observed between the incorporation of FOS and the generation time of *B. coagulans* (R^2 = 0.1186) (figure 5). These findings suggest that *B. coagulans* may have utilized FOS more effectively in green tea formulations to counter the effects of polyphenols, resulting in a significant reduction in the generation time. However, no significant difference was observed in the generation time of *B. coagulans* in black tea with or without the presence of FOS and sucrose, which was supported by the negligible correlation observed between the supplementation with FOS and the generation time.

5. Conclusion

In conclusion, on the report of several researchers, there exists a strong positive correlation between the polyphenol content and antioxidant activity of tea. In particular, green tea demonstrates higher polyphenol and antioxidant values in comparison to black tea. This discrepancy in values may explain the higher viable count of *B. coagulans* found in black tea formulations as compared to green tea. Both green and black tea formulations exhibit a significant positive correlation between the total polyphenol content (TPC), ferric reducing antioxidant power (FRAP), and the generation time of *Bacillus coagulans* IS2. Interestingly, a highly negative correlation was observed between the incorporation of fructooligosaccharides (FOS) and the generation time of *B. coagulans* IS2 in tea infusions. The growth-promoting effect

of FOS on *B. coagulans* is particularly prominent in green tea, where the probiotic *Bacillus coagulans* may utilize FOS as a prebiotic substrate to compensate for the growth hindering effect of polyphenols, which may also explain the reduced generation time. However, the supplementation of FOS has a negligible effect on the generation time of *B. coagulans* in black tea. Therefore, the inclusion of FOS in tea infusions, especially in green tea, can impede the inhibitory effect of polyphenols and amplify the growth and viability of *Bacillus coagulans*. This finding holds important inferences for the development of functional beverages, as tea-based probiotic and synbiotic products can offer health benefits analogous with both synbiotics and tea antioxidants.

6. Acknowledgment

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7. Conflict of interests

The authors have no competing interests to declare that are relevant to the content of this article.

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