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# Changes in *Bacillus* Inoculated Fish Muscle Texture under Refrigerated Storage (5±1°C)

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**Abstract** *Bacillus tequilensis* FPTB 26 (Accession number: KF 574000) having strong antimicrobial activities against various indicators tested, viz. *Staphylococcus aurus* (ATCC 25923), *Enterococcus faecalis* (MTCC 2729) and *Vibrio cholera*, was used as a biopreservative for shelf life extension of Asian Sea Bass fillets at 5±1°C. The changes in *Bacillus* count at 5±1°C reveals no significant differences among the aerobic and vacuum packaging conditions. Application of *B. tequilensis* FPTB26 in combination with vacuum ($L_2$) showed higher hardness figures of 9167.22±22.17g when compared to its aerobic counter part ($L_1$) 4216.13±21.43 g. Application of vacuum appears to have a significant influence on texture adhesiveness as compared to aerobic control during 15 days of storage. Other texture profile parameters like springiness, cohesiveness, chewiness and resilience showed a positive impact of inoculation of *B. tequilensis* FPTB26 at 5±1°C both under vacuum and aerobic conditions. $L_2$ fillets exhibited maximum retention of color and flavor and were acceptable till the end of the storage period. Application of *Bacillius* seem to be very effective in preventing texture deterioration irrespective of aerobic or anaerobic packing compared to the respective controls.

**Keywords** *Bacillus tequilensis*, Biopreservation, Texture analysis of fish muscle, Sensory evaluation.

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## Introduction

The growing consumer demand for finding natural but effective preservation of food free of potential health risks has stimulated research in the field of biopreservation to find an attractive and alternative approach to chemical preservatives [1], that include biological antimicrobial compounds. The use of non-pathogenic microorganisms and / or their metabolites to improve microbiological safety and extend the shelf life of foods is defined as biopreservation [2].

*Bacillus* species produce a diverse array of antimicrobial compounds including cyclic peptides and bacteriocin which have a broad antimicrobial spectrum against various Gram positive and Gram negative spoilage and pathogenic bacteria. USFDA (United States Food and Drug Administration) certified the carbohydrase (amylase) and protease enzymes produced by *B. subtilis*, as GRAS in 1960. Luis-Villasenor et al. [3] reported that *B. tequilensis* YC5-2 inhibited the growth of *V. campbelli*, *V. vulnificus* and *V. parahaemolyticus*. The resistant properties of *Bacillus* spores raise the possibilities that they can be incorporated in a number of food products such as beverage, chocolate, baked cake and muffin as probiotic additives [4]. However, the study on the effect of application of *Bacillus* sp. as a biopreservative on the texture of fish fillet is scant. Such study is essential as texture is one of the most important parameters that determine the overall quality perception [5]. Soft flesh leads to reduced acceptability by the consumers [6]. Generally, double compression is used for texture profile analysis (TPA) by forming a plot of force-time curves [7].

Similarly, sensory acceptability of *Bacillus* sp. inoculated fillet is also important as sensory evaluation of food, according to Meilgaard et al. [8] is a scientific means of quantifying and interpreting the variations in food characteristics (odor, taste, tactile, appearance) by using human senses of sight, smell, taste, touch and hearing. The objective of the study is to determine the changes associated with isolated *Bacillus* inoculated fish muscle texture under 5±1°C storage temperature by correlating the instrumental textural parameters with respective sensory evaluation.

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## Materials and Methods

Bacterial strain

*B. tequilensis* FPTB26 (Accession no. KF574000) isolated from fish gut (*Oreochromis mossambicus*) was used in the present study as biopreservative as it expressed strong inhibition against *Staphylococcus aurus* (ATCC 25923), *Enterococcus faecalis* (MTCC 2729) and Vibrio cholera during soft agar overlaying technique.

Changes in microbial, textural and sensory properties of Asian sea bass (*Lates calcarifer*) fillets by cultures of *Bacillus* Strain

Fresh Asian Sea Bass (*Lates calcarifer*) with an average weight of 3–4 kg was purchased from the fish market in South Kolkata and were transferred to the laboratory in iced condition, decapitated and filleted by hand. Cubes were cut from the fillets such that the final weight of each piece was approximately 10 g (for microbiological) and 45 g with an average 2.6 ± 0.1 cm thickness (for texture analysis). The study was conducted as a completely randomized design with four treatments, two types of packaging (normal and vacuum packaging) and stored at 5±1°C temperature. There were five sampling intervals. The fillets were subjected in triplicate for microbial and sensory analyses at 3 days' interval starting from day 0 for *Bacillus* count by spread plating appropriate dilutions on Hi-Touch Selective *Bacillus* Count Media (Hi-media) [9] and results expressed as log cfu / g. The treatments were prepared aseptically maintaining good hygienic practices and stored at 5±1°C as follows:

No inoculation, under normal packaging condition ($K_1$), no inoculation, under vacuum packaging condition ($K_2$), FPTB26 treatment, under normal packaging condition ($L_1$), FPTB26 treatment, under vacuum packaging condition ($L_2$). For the cubes of treatment $L_1$ and $L_2$, 1 ml of the fresh concentrated culture of FPTB26 containing a population of $1 \times 10^7$ CFU/ml was added and distributed with a dropper onto the surface of the fillets. Control samples ($K_1$ and $K_2$) were prepared by adding 1 ml of sterile water to the fillets without any inoculation [10]. The fillets of treatment $K_2$ and $L_2$ of each set were vacuum packed by using INDVAC packaging machine as described by Chowdhury et al. [10].

TPA of fish fillets was performed at ambient temperature with TA-XT plus texture analyser (Stable Micro System, Surrey, UK) and a 50 kg load cell. The attributes evaluated were hardness, adhesiveness, chewiness, springiness, gumminess and cohesiveness. Each cube, weighing about 45 g with an average 2.6±0.1 cm thickness, was compressed vertically in two consecutive cycles of 50% compression, 5 seconds apart using a flat plunger (SMS-P / 75) and a heavy duty platform. This test was done according to the specifications used by Mao et al. [11]. The adopted test settings for this experiment were pre-test speed of 1.5 mm/second, test speed of 0.5 mm/second, post-test speed of 1.5 mm/second, strain at 50% compression, interval (time) of 5 seconds, Trigger type was auto (Force) and trigger force was 25

**Table 1.** Changes in *Bacillus* count and different textural parameters in *B. tequilensis* FPTB26 inoculated fillet at 5±1°C. Results are mean of three determinations with SD.

| Characteristics | | Days 0 | 3 | 6 |
|---|---|---|---|---|
| Log cfu/g of | $L_1$ | 3.82 ± 0.02 | 4.99 ± 0.03 | 5.17 ± 0.06 |
| *Bacillus* count | $L_2$ | 3.82 ± 0.01 | 4.81 ± 0.03 | 4.91 ± 0.08 |
| Texture | $K_1$ | 12909.86± 32.11 | 10092.24± 31.05 | 8206.44 ± 25.08 |
| Hardness | $L_1$ | 12909.86± 32.11 | 10256.14± 37.65 | 9850.38 ± 25.37 |
| (g) | $K_2$ | 12909.86± 32.11 | 12263.99± 22.32 | 11458.36± 25.65 |
| | $L_2$ | 12909.86± 32.11 | 12323.18± 27.67 | 11254.6 ± 28.65 |
| Texture | $K_1$ | −1467.13 ± 17.14 | −918.51 ± 12.11 | −274.77 ± 9.87 |
| Adhesiveness | $L_1$ | −1467.13 ± 17.14 | −1075.75 ± 7.46 | −857.14 ± 7.43 |
| (g/s) | $K_2$ | −1467.13 ± 17.14 | −1029.11 ± 9.56 | −1022.22 ± 8.45 |
| | $L_2$ | −1467.13 ± 17.14 | −1044.70 ± 4.34 | −946.46 ± 5.76 |
| Texture | $K_1$ | 1.17 ± 0.11 | 0.94 ± 0.09 | 0.76 ± 0.06 |
| Springiness | $L_1$ | 1.17 ± 0.11 | 0.96 ± 0.04 | 0.82 ± 0.06 |
| | $K_2$ | 1.17 ± 0.11 | 0.94 ± 0.07 | 0.78 ± 0.07 |
| | $L_2$ | 1.17 ± 0.11 | 0.98 ± 0.05 | 0.81 ± 0.07 |
| Texture | $K_1$ | 0.41 ± 0.02 | 0.39 ± 0.03 | 0.37 ± 0.04 |
| Cohesiveness | $L_1$ | 0.41 ± 0.02 | 0.43 ± 0.05 | 0.44 ± 0.04 |
| | $K_2$ | 0.41 ± 0.02 | 0.42 ± 0.03 | 0.42 ± 0.02 |
| | $L_2$ | 0.41 ± 0.02 | 0.41 ± 0.03 | 0.42 ± 0.05 |
| Texture | $K_1$ | 5293.04 ± 17.65 | 3935.97 ± 16.09 | 3036.38 ± 15.87 |
| Gumminess | $L_1$ | 5293.04 ± 17.65 | 4410.14 ± 15.65 | 4334.17 ± 17.17 |
| | $K_2$ | 5293.04 ± 17.65 | 5150.88 ± 19.65 | 4812.51 ± 17.43 |
| | $L_2$ | 5293.04 ± 17.65 | 5052.50 ± 18.44 | 4726.93 ± 18.33 |
| Texture | $K_1$ | 6192.86 ± 15.98 | 3699.82 ± 18.23 | 2307.65 ± 17.43 |
| Chewiness | $L_1$ | 6192.86 ± 15.98 | 4233.73 ± 17.87 | 3554.02 ± 18.54 |
| | $K_2$ | 6192.86 ± 15.98 | 4841.82 ± 16.65 | 3753.76 ± 14.76 |
| | $L_2$ | 6192.86 ± 15.98 | 4951.45 ± 18.32 | 3828.81 ± 15.44 |
| Texture | $K_1$ | 0.19 ± 0.05 | 0.18 ± 0.02 | 0.17 ± 0.03 |
| Resilience | $L_1$ | 0.19 ± 0.05 | 0.18 ± 0.05 | 0.19 ± 0.03 |
| | $K_2$ | 0.19 ± 0.05 | 0.19 ± 0.02 | 0.19 ± 0.06 |
| | $L_2$ | 0.19 ± 0.05 | 0.2 ± 0.03 | 0.2 ± 0.05 |

**Table 1.** Continued.

| Characteristics | | Days 9 | 12 | 15 |
|---|---|---|---|---|
| Log cfu/g of | $L_1$ | 5.45 ± 0.08 | 5.41 ± 0.04 | 5.30 ± 0.05 |
| *Bacillus* count | $L_2$ | 5.45 ± 0.08 | 5.42 ± 0.03 | 5.40 ± 0.05 |
| Texture | $K_1$ | 7349.36 ± 24.18 | 5647.98 ± 20.98 | 4922.69 ± 23.76 |
| Hardness | $L_1$ | 8719.4 ± 24.29 | 6618.12 ± 26.76 | 4216.13 ± 21.43 |
| (g) | $K_2$ | 11200.95± 37.54 | 10208.67± 21.54 | 10146.57± 19.76 |
| | $L_2$ | 10331.14± 24.87 | 9978.68 ± 24.33 | 9167.22 ± 22.17 |
| Texture | $K_1$ | −155.69 ± 7.17 | −185.36 ± 7.98 | −111.70 ± 4.23 |
| Adhesiveness | $L_1$ | −578.94 ± 8.12 | −308.68 ± 5.34 | −132.56 ± 6.36 |
| (g/s) | $K_2$ | −788.24 ± 9.23 | −554.12 ± 7.23 | −462.66 ± 7.45 |
| | $L_2$ | −394.63 ± 8.67 | −483.22 ± 7.58 | −355.82 ± 4.88 |
| Texture | $K_1$ | 0.43 ± 0.07 | 0.4 ± 0.04 | 0.37 ± 0.08 |
| Springiness | $L_1$ | 0.51 ± 0.03 | 0.5 ± 0.07 | 0.47 ± 0.06 |
| | $K_2$ | 0.48 ± 0.05 | 0.5 ± 0.03 | 0.45 ± 0.02 |
| | $L_2$ | 0.62 ± 0.04 | 0.57 ± 0.03 | 0.48 ± 0.06 |
| Texture | $K_1$ | 0.38 ± 0.03 | 0.34 ± 0.05 | 0.31 ± 0.05 |
| Cohesiveness | $L_1$ | 0.43 ± 0.03 | 0.41 ± 0.04 | 0.4 ± 0.04 |
| | $K_2$ | 0.43 ± 0.05 | 0.39 ± 0.06 | 0.37 ± 0.05 |
| | $L_2$ | 0.47 ± 0.04 | 0.43 ± 0.04 | 0.42 ± 0.02 |

**Table 1.** Continued.

| Characteristics | | Days 9 | 12 | 15 |
|---|---|---|---|---|
| Texture | $K_1$ | 2792.76 ± 16.76 | 1920.31 ± 13.43 | 1526.03 ± 14.23 |
| Gumminess | $L_1$ | 3749.34 ± 14.43 | 2713.43 ± 13.32 | 1686.45 ± 11.65 |
| | $K_2$ | 4816.41 ± 16.55 | 3981.38 ± 14.75 | 3754.23 ± 14.75 |
| | $L_2$ | 4855.64 ± 15.32 | 4290.83 ± 12.54 | 3850.23 ± 13.65 |
| Texture | $K_1$ | 1200.89 ± 16.65 | 768.13 ± 14.98 | 564.63 ± 15.36 |
| Chewiness | $L_1$ | 1912.16 ± 18.43 | 1356.71 ± 14.76 | 792.63 ± 18.32 |
| | $K_2$ | 2311.88 ± 17.87 | 1990.69 ± 15.95 | 1689.40 ± 16.43 |
| | $L_2$ | 3010.49 ± 15.31 | 2445.77 ± 17.65 | 1848.11 ± 13.76 |
| Texture | $K_1$ | 0.17 ± 0.05 | 0.15 ± 0.04 | 0.14 ± 0.04 |
| Resilience | $L_1$ | 0.19 ± 0.06 | 0.18 ± 0.03 | 0.18 ± 0.03 |
| | $K_2$ | 0.18 ± 0.06 | 0.18 ± 0.04 | 0.17 ± 0.05 |
| | $L_2$ | 0.2 ± 0.04 | 0.19 ± 0.06 | 0.18 ± 0.07 |

gm. Sensory evaluation was performed during storage as described by Chowdhury et al. [10].

## Results and Discussion

The changes in *Bacillus* count at 5±1°C. reveals no significant ($p > 0.05$) differences among the aerobic and vacuum packaging conditions suggesting a fairly uniform growth of *Bacillus* under both packaging conditions. The *Bacillus* count ranged from a 3.82 ± 0.02 log cfu/g to 5.30±0.05 log cfu/g for $L_1$ samples and 3.82 ± 0.01 log cfu/g to 5.40±0.05 log cfu/g for $L_2$ samples.

The changes in texture hardness value (g) at 5±1°C reveals a significant ($p < 0.05$) improvement in hardness for vacuum packed fillet ($K_2$) as compared to $K_1$ Application of *B. tequilensis* FPTB26 in combination with vacuum ($L_2$) showed higher hardness figures of 9167.22±22.17 g ($p < 0.05$) when compared to its aerobic counterpart ($L_1$)4216.13±21.43 g (Table 1). Application of vacuum appears to have a significant ($p < 0.05$) influence on texture adhesiveness as compared to aerobic control during 15 days of storage (Table 1). Other texture profile parameters like springiness (Table 1), cohesiveness (Table 1), gumminess (Table 1), chewiness (Table 1) and resilience (Table 1) showed a positive impact of inoculation of *B. tequilensis* FPTB26 at 5±1°C both under vacuum and aerobic conditions.

Thus, it may be concluded that, at 5±1°C application of the *Bacillus* strain both in aerobic and vacuum packaging condition may prove beneficial in improving the texture profile parameters. Olga [12] suggested that texture of fish flesh was influenced by many factors including postmortem pH decline, proteolysis, fat content, composition and its distribution in the fish muscle. Texture softening was mainly influenced by the autolysis and denaturation of muscle protein during the chilled and frozen storage [13]. Application of *Bacillus* strain probably inhibited the growth of spoilage bacteria and consequently reduce the denaturation of muscle protein which probably resulted in better figures of texture profile parameters. Liu et al. [13] reproted that bacterial growth and protein degradation affects water holding capacity and bacterial proteinases disintegrates collagen fibrils and contributes to texture softening.

Besides being considered as a good bio-preservative agent, the metabolic activity of *Bacillus* is known to produce substances responsible for typical spoilage change, produce TTX–an aminoperhydroquinazoline compound, making products eventually unfit for human consumption. These changes include formation of sour flavuor and off odor, slime formations and discolorations. According to Ghaly et al. [14] microbial spoilage of fish that leads to objectionable sensory rejection includes off-odor, flavor and taste ; formation of slime, visible pigmented and non-pigmented colonies ; discoloration and gas production, ultimately resulted the formation of pun-

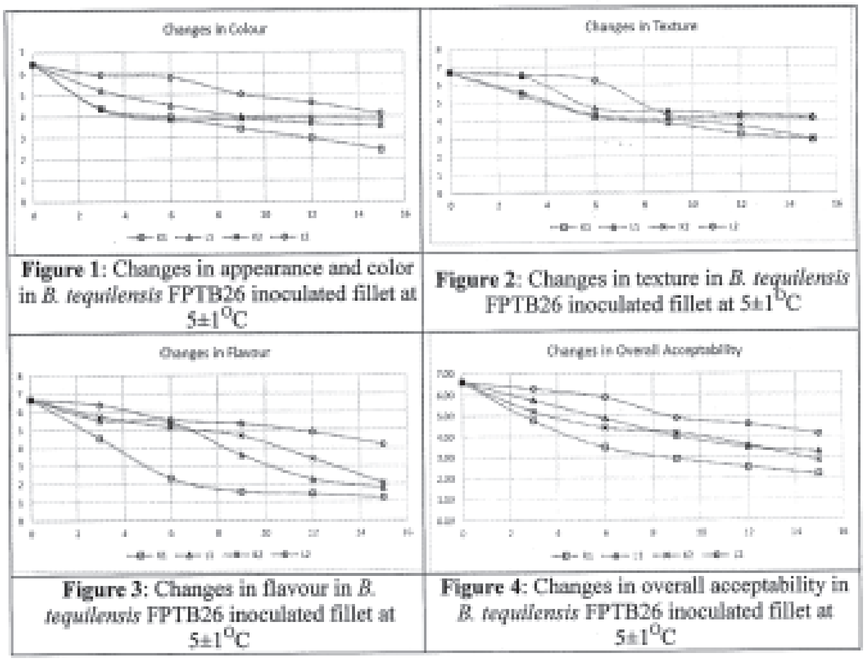


Figure 1: Changes in appearance and color in *B. tequilensis* FPTB26 inoculated fillet at 5±1°C

Figure 2: Changes in texture in *B. tequilensis* FPTB26 inoculated fillet at 5±1°C

Figure 3: Changes in flavour in *B. tequilensis* FPTB26 inoculated fillet at 5±1°C

Figure 4: Changes in overall acceptability in *B. tequilensis* FPTB26 inoculated fillet at 5±1°C

**Fig. 1.** Changes in appearance and color in *B. tequilensis* FPTB26 inoculated fillet at 5±1°C. **Fig. 2.** Changes in texture in *B. tequilensis* EPTB26 inoculated fillet at 5±1°C. **Fig. 3.** Changes in flavor in *B. tequilensis* FPTB26 inoculated fillet at 5±1°C. **Fig. 4.** Changes in overall acceptability in *B. tequilensis* EPTB26 inoculated fillet at 5±1°C.

gent, volatile odors resulting from the degradation of fish components by microbial action.

Observations of the values of color scores of Asian Sea Bass cubes, suggest a fairly uniform appearance for all treatments. *Bacillus* itself act as a spoiler at the end of 15th day of storage at 5±1°C for both the *Bacillus* inoculated treatments ($L_1$ and $L_2$). Vacuum seems to be effective in regards of color retention of the $L_2$ fillets as compared to their respective aerobic counterpart as well as controls. $L_2$ are still acceptable at the end of the storage period (Fig. 1). From the above findings it can be concluded that, *B. tequilensis* FPTB26 inoculated fillets, packed under vacuum exhibited maximum retention of color under 5±1°C storage temperatures.

Application of *Bacillus* seem to be very effective in preventing texture deterioration irrespective of aerobic or anaerobic packing at storage temperature 5±1°C compared to the respective controls (Fig. 2). The controls ($K_1$ and $K_2$) crossed their limit of acceptability leading to texture softening at the end of the storage period under both storage conditions. Whereas, none of the *Bacillus* inoculated treatments exhibited such texture deterioration.

At 5±1°C storage, the aerobic control ($K_1$) crossed the limit of acceptability of flavor (<4.0) after 3rd day of storage, whereas, the vacuum packed control ($K_2$) and $L_1$ crossed the limit (<4.0) after 9th and 6th day of storage respectively (Fig. 3). Chowdhury et al. [10] reported that the first changes associated with LAB spoilage in meat are cheesy, sour and acid flavor accompanied by odor and taste changes. On the contrary, the anaerobic vacuum packaged counterpart treatment $L_2$ did not cross the limit (<4.0) at all throughout their storage period. So, from the above result it can be inferred that, *B. tequilensis* FPTB26 in combination with vacuum seem to have positive effect in regards of flavor retention only at lower temperature such as, 5±1°C.

Although from the scorecard of overall acceptability it can be concluded that, *B. tequilensis* FPTB26 inoculated fillets packed under vacuum ($L_2$) are still acceptable till the end of the storage period at 5±1°C storage temperature (Fig. 4).

Thus it may be concluded that the isolate FPTB 26 *Bacillus tequilensis* (Accession no. KF574000) may be used as a potential biopreservative for Asian Sea-Bass fillet. The isolate in combination with vacuum and low temperature (5±1°C) extended the shelf life of Asian Sea-Bass fillets up to 15 days considering the result of sensory score. The changes in *Bacillus* count at 5±1°C reveals a fairly uniform growth of *Bacillus* under both packaging conditions. Application of vacuum was very effective over aerobic preservation at 5±1°C resulting in significant ($p < 0.05$) higher hardness values than uninoculated aerobic pack ($K_1$). Other texture profile parameters like springiness, cohesiveness, gumminess, chewiness and resilience showed a positive impact of inoculation of *B. tequilensis* FPTB26 at 5±1°C both under vacuum and aerobic conditions. So it can be summerised that, at 5±1°C application of the *Bacillus* strain may prove beneficial in improving the texture profile parameters especially in preventing texture deterioration irrespective of aerobic or anaerobic packing compared to the respective controls. Vacuum seem to be effective in regards of maximum retention of color and flavor in $L_2$ fillets as they are still acceptable till the end of the storage period.

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