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# Tapioca Starch Modulates Cellular Events in Oral Probiotic *Streptococcus salivarius* Strains

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## Abstract

Considering the implications of microbiota in health, scientists are in search of microbiota-oriented strategies for the effective prevention and/or treatment of a wide variety of serious diseases. A microbiota comprises diverse microorganisms with either probiotic or pathogenic properties. The fermentation of prebiotic carbohydrates by probiotic bacteria can affect host metabolism. Therefore, understanding the prebiotic-mediated metabolic modulations in probiotics is crucial to develop functional foods for the improvement of disturbed microbiota. Studies have emphasized the importance of prebiotics in probiotic therapies for mucosal diseases and highlighted the need for extensive research on oral bacteria. In the present study, the cellular events have been studied in batch cultures of probiotic *Streptococcus salivarius* exposed to the natural prebiotic, tapioca starch (TS). TS modulated the keystone metabolic events in *Streptococcus salivarius* in a dose-dependent manner. Besides increasing the live cell counts and altering the colony morphologies, TS affected the protein metabolism in terms of cellular expression and conformational changes in protein secondary structures. After treatment with TS, the nucleic acid synthesis increased and B-DNA was more than A- and Z-DNA, together with the diminished fatty acids and increased polysaccharide synthesis. The study results can be considered for the assessment of functional foods and probiotics in oral health.

**Keywords** Bacterial metabolism · Probiotics · Prebiotics · *Streptococcus salivarius* · Oral microbiota · Tapioca starch

## Introduction

The effect of nutrition on human health has triggered the interest of consumers, researchers, and the functional food industry [1]. In this respect, the pharmaceutical and food industry encourages the combined use of probiotics and prebiotics because of the synbiotic relationship between prebiotics and probiotics to protect and improve consumer health [1]. Most contextual studies have focused on the treatment or prevention of gastrointestinal diseases [1]. Similar to the intestine, the human oral cavity is a complex area composed of various structures and colonized by a broad spectrum of microorganisms [2]. These microorganisms form complex communities that are balanced in the oral cavity of a healthy individual [3]. The composition of these microbial populations is hypervariable, depending on the region of the oral cavity, age, and diet of the individual [1]. Disturbed oral microbiota triggers the production of various metabolites leading to the development of oral diseases; however, this negative functionality can be reversed by appropriate nutritional strategies [4]. Basic and clinical research studies have revealed that probiotics can successfully prevent oral diseases, such as halitosis (bad breath), tooth decay, periodontitis, and gingivitis [5, 6]. Probiotic lactobacilli may not only alter the protein composition of salivary glands but also affect the oral ecology by preventing the adherence of other bacteria [7]. People are consuming lactobacilli in the form of pills or pastilles to reduce gingivitis [8]. Moreover, some studies have evaluated the potential of probiotics in controlling tooth decay [5, 9]. Zhang et al. (2018) argued that the dental caries rates and *Streptococcus mutans* (*S. mutans*) concentrations are lower in the *Lactobacillus rhamnosus*-treated groups with respect to untreated children [10]. Another study has shown that the short-term consumption of cheese containing two probiotic lactobacilli may reduce oral cariogenic microbial flora in young adults [11].

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Although different strains of probiotic bacteria are effective in the prevention of acute upper respiratory tract infection, only *Streptococcus salivarius* K12 (*S. salivarius* K12) strain has been found useful in the prevention of pharyngotonsillitis [12]. This strain also has a good safety profile and colonizes permanently within the upper respiratory tract [12]. The probiotic *Streptococcus salivarius* M18 (*S. salivarius* M18) strain improves disturbed oral microbiota by producing dextranase and urease enzymes that reduce the dental plaque deposition and acidification, respectively. Important cariogenic bacteria are targeted by bacteriocins produced by *S. salivarius* M18 [13]. In a study involving 100 children, the *S. salivarius* M18 strain was reported to be safe and effective in reducing tooth decay and plaque formation [13]. *S. salivarius* M18 strain was also found to prevent new tooth decays in children [14]. Full details of the probiotic mechanisms of these strains are still emerging, and there is a need for additional studies to be conducted [15]. Recent studies have demonstrated a close relationship between the oral microbiota and some intestinal diseases [16]. The bacterial genera of stool and the oral cavity were found to be identical in more than 45% of the subjects analyzed in the Human Microbiome Project [17]. The periodontitis-causing oral bacteria *Porphyromonas gingivalis* (*P. gingivalis*) can even invade the intestine and cause colon dysbiosis, a well-known phenomenon associated with immune dysfunction and common metabolic diseases [18, 19]. Apart from *P. gingivalis*, other oral bacteria are also able to translocate to the gut [19]. Studies have also emphasized the importance of prebiotics in probiotic therapies and highlighted the need for a coordinated approach for the treatment of oral and intestinal diseases [19]. Further studies to investigate the link between oral dysbiosis and systemic diseases, including gut inflammation, cancer, atherosclerosis, diabetes, and obesity, are warranted [19].

Although the health effects of cassava-derived starch are not thoroughly explored, a limited number of studies have investigated these effects in intestinal probiotic bacteria [20]. The prebiotic properties of tapioca starch (TS) are reported in intestinal probiotic bacteria only [21]. Considering the lack of knowledge about the prebiotic effect of TS on intestinal bacteria and the interplay between TS and oral bacteria, the present study aimed to elucidate TS-associated modulations in cellular events in the oral probiotic *S. salivarius* M18 and *S. salivarius* K12 strains. The cellular events are presented through changes in live cell counts and colony morphologies and molecular alterations in proteins, polysaccharides, and nucleic and fatty acids. Our study can help the health-care companies to optimize and manufacture the functional probiotic products, particularly for the maintenance and protection of oral microbiota.

## Materials and Methods

Commercial TS and inulin powders extracted from the roots of the cassava (*Manihot esculenta*) and chicory plants (*Cichorium intybus*), respectively, were used as natural prebiotic polysaccharides. Please refer to the supplementary information of this manuscript for all experimental details.

### Bacterial Strains and Aerobic Culture Conditions

The *S. salivarius* M18 strain was grown on tryptic soy agar (TSA) and tryptic soy broth (TSB) media, whereas the *S. salivarius* K12 strain was grown on nutrient agar (NA) and nutrient broth (NB) media. The *Lactobacillus plantarum* B-1846 strain (*L. plantarum* B-1846) was grown on de MAN, ROGOSA, and SHARPE (MRS) agar and broth media. All the consumables used were heat-sterilized (autoclaved at 121 °C for 15 min) unless otherwise indicated. The bacteria were cultivated at 37 °C under aerobic conditions (80% air, 20% media) in an orbital shaker at 150 rpm for 18 h.

### Culturing the Bacteria with TS and Inulin in a Low-Oxygen Environment

The bacteria were inoculated onto media containing different concentrations of TS and inulin, as detailed in Supplementary Table S1. Inulin was included as another source of prebiotic polysaccharides to compare it with TS. The details of the experiments can be found in the supplementary information of this manuscript.

### Determination of Colony-Forming Units (CFUs)

Samples, 100 uI each, were serially diluted and incubated on the corresponding solid media in triplicate. Following incubation (37 °C for 24 h), the CFUs were calculated.

### Colony Morphology Assay

The colonies grown on solid media supplemented with Congo red and Coomassie blue were visualized under a fluorescence microscope in the bright-field mode (Olympus BX53, JP). The details of the experiments can be found in the supplementary information of this manuscript.

### Isolation and Separation of Proteins

The bacterial pellets were collected from the solid media and lysed for protein isolation, and the proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The experimental details of protein isolation and separation can be found in the supplementary information of this manuscript.

### Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) Spectroscopy Measurements

The bacterial colonies grown for 24 h were scraped from solid media and immediately analyzed by a Frontier FTIR spectrometer (PerkinElmer, US) equipped with a universal ATR Miracle accessory. The details of the experiments can be found in the supplementary information of this manuscript.

### Statistical Analysis

The bacteria were categorized into cells only (CO) and TS-supplemented (1.0–5.0%) groups. Statistical analysis was performed to compare the band intensities in the TS-supplemented (1.0–5.0%) groups with the band intensities in the CO bacterial groups. The Dunnett method (confidence level, 95%) was used as a part of the two-way analysis of variance (ANOVA) in GraphPad Prism 6.01 (GraphPad, USA). However, the total protein (%) expression data were statistically analyzed using one-way ANOVA. The degrees of significance were denoted as less than or equal to $P < 0.05$ *, $P < 0.01$ **, $P < 0.001$ ***, and $P < 0.0001$ ****. The results were expressed as means ± standard error of the mean.

## Results

### TS Modulates Live Cell Counts

Supplementary Table S2 demonstrates the live cell counts in terms of CFUs for the CO and TS-supplemented groups of *S. salivarius* M18 cells at 24 and 48 h. At 24 h, a tenfold increase was found in the CFUs of 1.0% and 2.5% TS groups as compared with the CFUs in the CO group. This increase even reached up to a hundredfold for the 5.0% TS group (Table S2). At 48 h, a roughly threefold increase in the CFUs was observed for all the TS-supplemented groups as compared with the CO group. The CFUs of the *S. salivarius* M18 strain at 48 h were considerably higher than the corresponding values at 24 h for all the groups (Table S2).

Similarly, for *S. salivarius* K12, a twofold increase in the CFUs was observed in 1.0% and 2.5% TS-supplemented groups with respect to the CO group at 24 h. The CFUs of the 5.0% TS-supplemented group were almost five times higher than those of the CO group (Table S3). However, the CFUs of the TS-supplemented groups were almost equal to those of the CO group at 48 h. When CFUs were compared between 24 and 48 h, the CO group demonstrated a twofold increase in the CFUs at 48 h, whereas a very small decrease was observed in the 1.0% and 2.5% TS-supplemented groups at 48 h. However, an approximately threefold decrease was calculated for the 5.0% TS-supplemented group at 48 h with respect to 24 h (Table S3).

The comparative CFU experiments were also conducted with both the strains of bacteria supplemented with identical doses of inulin. Inulin was chosen as it is the most widely studied natural prebiotic polysaccharide and extensively used in many commercially available probiotic supplements [22]. In terms of the CFUs, TS was found to be more effective than inulin under the present experimental conditions (Tables S2–S3).

### TS Modulates Colony Morphology

Figure 1 shows the effect of TS on single colony morphologies of *S. salivarius* M18. The colonies were grown on TSA, and Congo red [23] and Coomassie blue [24] dyes were used to track the morphological changes. The single colonies of the *S. salivarius* M18 CO group were found to have a bigger and rounder shape and more well-defined margins than those of the TS-supplemented groups. The roundness of the colonies was found to disappear with increasing TS concentrations gradually. TS dose-dependently enhanced the numbers of red-colored carbohydrate-based formations inside the stained colonies. A similar phenomenon, except the disappearance of roundness, was also observed in *S. salivarius* K12 (Fig. S1). The black and opaque colonies of another probiotic bacterium, namely *L. plantarum* B-1846, did not demonstrate these morphological alterations, except for size reduction (Fig. S2).

### TS Modulates Cellular Metabolism

The TS-associated modulations of cellular events in *S. salivarius* M18 and *S. salivarius* K12 were elucidated at a molecular level using the principal component analysis (PCA) model, which was developed using a large dataset obtained by ATR-FTIR spectroscopy. The PCA model was developed on the fingerprint (1800–1000 $cm^{-1}$) spectral region that represents molecular vibrations emerging from different cellular molecules' divergent functional groups. The modulations in these molecular vibrations affect the conformation and functionality of keystone biomolecules.

Figure 2 represents the scores, loadings, and variance plots of the PCA model for *S. salivarius* M18. According to the scores plot (Fig. 2a), the untreated bacterial group (CO, control) and 1.0% TS-supplemented bacterial group were segregated on the positive side (positive scores), whereas the 2.5% and 5.0% TS-supplemented groups were mainly clustered on the negative side (negative scores) along PC1. The scores of growth medium without bacteria (only media/OM) group and growth medium without bacteria but supplemented with TS (M + TS) group were also evaluated. However, the OM and M + TS groups were found to be clustered together and far away from the CO and TS-supplemented groups. In all data analyses, PC1 was considered because it represents the largest (77%) proportion of data standing behind discrimination.

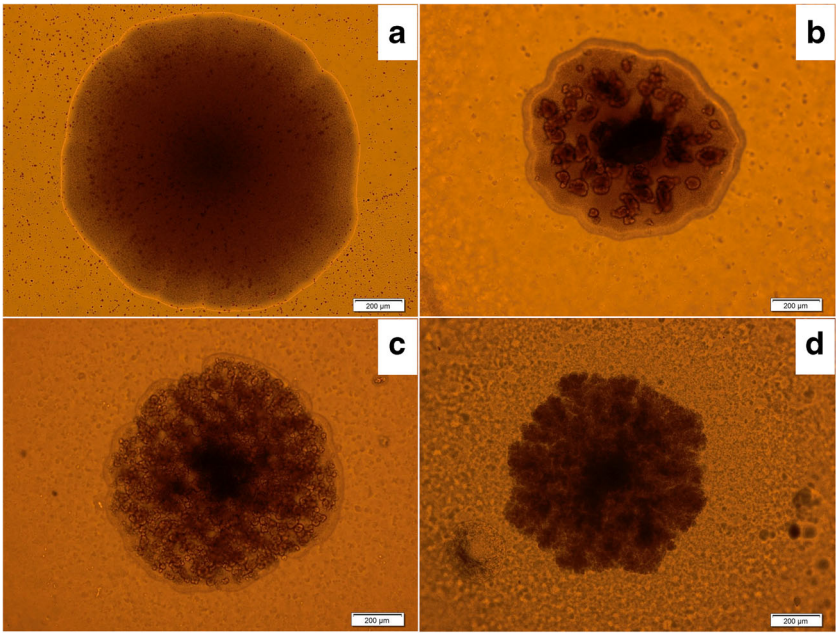


**Fig. 1** Tapioca starch modulates the colony morphologies in *Streptococcus salivarius* M18 visualized by a fluorescence microscope in the bright-field mode. **a** Cells only, **b** 1.0%, **c** 2.5%, and **d** 5.0% TS-supplemented groups grown on tryptic soy agar supplemented with Congo red and Coomassie blue dyes. Scale bar, 200 μm

PC2 (20%) was also examined but not considered in this study because the obtained variables of PC2 were too small to be recognized as a real variation. The PC1 loadings plot indicates that the discrimination seen in the scores plot emerged from variations in positive discriminators (Fig. 2b). Biologically, each of these discriminators represents a specific molecular and/or cellular event [25–28]. An explained variance plot is provided to show the magnitude of involved PCs that is PC1 (77%) and the indistinguishable patterns of calibrated and validated variances (Fig. 2c).

A different PCA pattern was obtained for *S. salivarius* K12 (Fig. S3), in which the OM and M + TS groups were located on the negative side (negative scores), whereas all other groups including CO were found on the positive side (positive scores) along PC1 (91%) (Fig. S3a). Similarly, the positive discriminators were most effective in the discrimination of groups, as shown in the loadings plot (Fig. S3b). The discrimination power of PC1 (91%) and identical validation and calibration variances can be seen in the variance plot (Fig. S3c). According to the findings, TS supplementation at all the studied concentrations induced vibrational stretching in the various functional groups of cellular molecules in both bacterial strains [27–32].

The loadings plots of both bacterial strains demonstrate important clues in terms of positive discriminators about quantifiable modulations occurring in protein, nucleic acid, fatty acid, and carbohydrate metabolism. These molecular alterations that are associated with specific metabolic events can be quantified using proper data-processing tools to highlight the influence of TS on the metabolism of probiotic bacteria. Accordingly, the only significant metabolic modulations were quantified using the second-derivative infrared spectra, given that resolving the important sub-bands from the raw infrared spectra was impossible (Fig. 3).

## TS Modulates Protein Metabolism

Initially, the secondary protein conformations were analyzed by considering the sub-bands of the amide I band (1700–1600 $cm^{-1}$) from the second-derivative spectra (Fig. 4). The analysis revealed the major protein conformations of *S. salivarius* M18 (Fig. 4a), in which the only significantly altered ones were quantified in terms of their absolute intensities (Fig. 4b). It was found that the TS supplementation enhances the triple α-helical, α-helical, and β-turn secondary conformations and reduces the randomly coiled/unordered structures in a dose-dependent manner. Moreover, the reduced β-sheet and increased aggregated β-sheet conformations were observed, albeit insignificantly (Fig. 4b). The similarly modulated protein structures were also quantified in *S. salivarius* K12 (Fig. S4a, b).

## TS Modulates Protein Expression

SDS-PAGE protein profiling was performed to elicit the TS-associated modulations in protein expression profiles (Fig. 5, Fig. S5). The analysis revealed distinct alterations in the overall expression profile of proteins. TS significantly enhanced the total protein expression in *S. salivarius* M18 in a dose-dependent manner (Fig. 5a, b). However, a decrease in total protein expression was detected in *S. salivarius* K12 (Fig. S5a). For comparison, inulin was also found to be an effective modulator of protein expression in *S. salivarius* K12 (Fig. S5b).

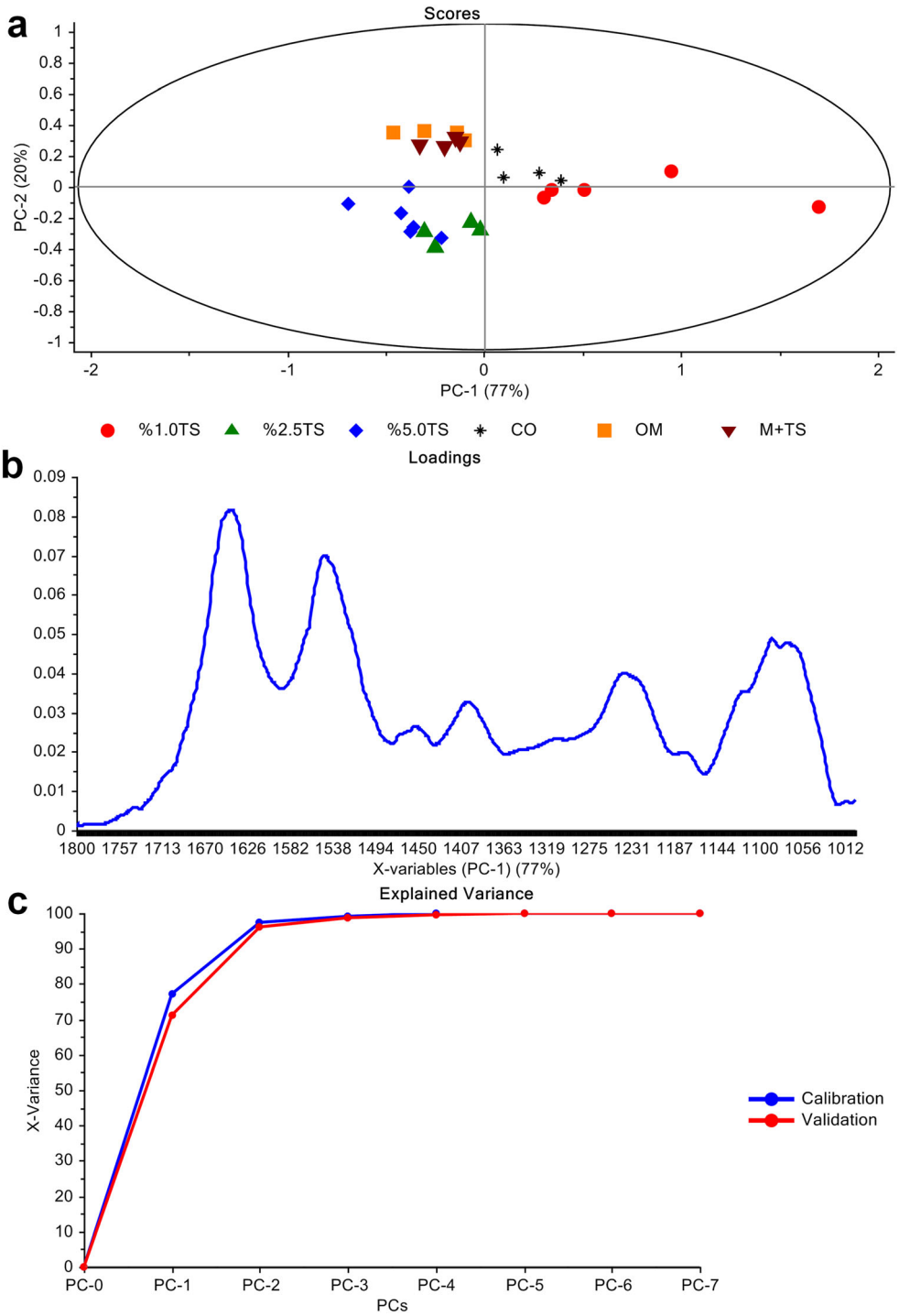


**Fig. 2** Tapioca starch modulates the cellular metabolism in *Streptococcus salivarius* M18. **a** Scores plot, **b** loadings plot, and **c** explained variance plot of PCA modeling obtained at the fingerprint (1800–1000 $cm^{-1}$) spectral region in all groups

## TS Modulates Nucleic Acid Metabolism

TS caused strong modulations in nucleic acid metabolism (Fig. 6). The nucleic acid-associated bands in *S. salivarius* M18 were resolved in second-derivative infrared spectra (Fig. 6a) and quantified in terms of their absolute intensities (Fig. 6b). The total nucleic acid concentrations were calculated considering the $PO_2$ symmetric band intensities (located at 1086 $cm^{-1}$ in second-derivative spectra), while the major conformational forms of DNA (A-, B-, and Z-forms) were quantified by resolving the second-derivative sub-bands in the $PO_2$ antisymmetric spectral region (1280–1200 $cm^{-1}$). The dose-dependent increases in the total nucleic acid concentrations and B-DNA, in line with the reductions in A- and Z-DNA, were observed. Comparable nucleic acid modulations were also detected in *S. salivarius* K12 (Fig. S6a, b).

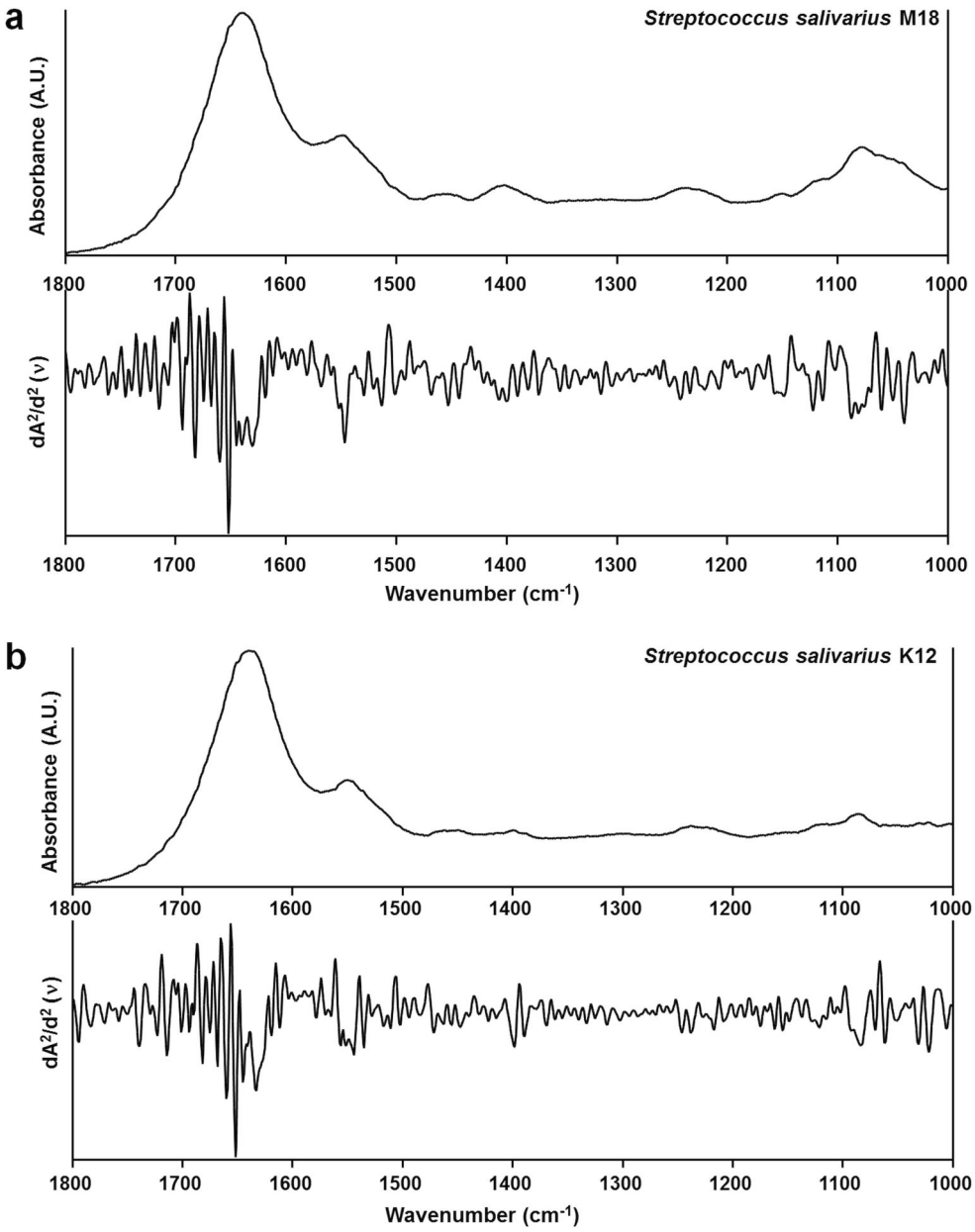


**Fig. 3** Raw and second-derivative infrared spectra obtained at the fingerprint (1800–1000 $cm^{-1}$) spectral region for **a** *Streptococcus salivarius* M18 and **b** *Streptococcus salivarius* K12

## TS Modulates Fatty Acid and Carbohydrate Metabolism

In addition to protein and nucleic acid metabolism, fatty acid and carbohydrate metabolism were also modified by TS. We investigated the fatty acid region (1445–1385 $cm^{-1}$) of infrared spectra with the same analysis approach utilized for protein and nucleic acids and quantified three different fatty acid-associated bands (Fig. 7a, b). Interestingly, TS caused a significant reduction in fatty acid concentrations in a dose-dependent manner (Fig. 7b). A similar reduction in fatty acid metabolism was also detected in *S. salivarius* K12 (Fig. S7a, b). The carbohydrate-associated bands were located at 1154 $cm^{-1}$ and nearly 1025–1031 $cm^{-1}$ positions in second-derivative spectra. The quantification of these bands revealed a dose-dependent increase in polysaccharide and glycogen concentrations in both bacteria (Fig. S8).

## Discussion

Recent clinical studies have encouraged the consumption of prebiotic functional foods and probiotics to enhance beneficial activities of probiotics. The prebiotic therapy concept has only recently been introduced in the field of oral microbiota and health care, and research is still in its infancy [33, 34]. TS affects many aspects of bacterial metabolism, and mechanistic studies dealing separately with each aspect are crucial to

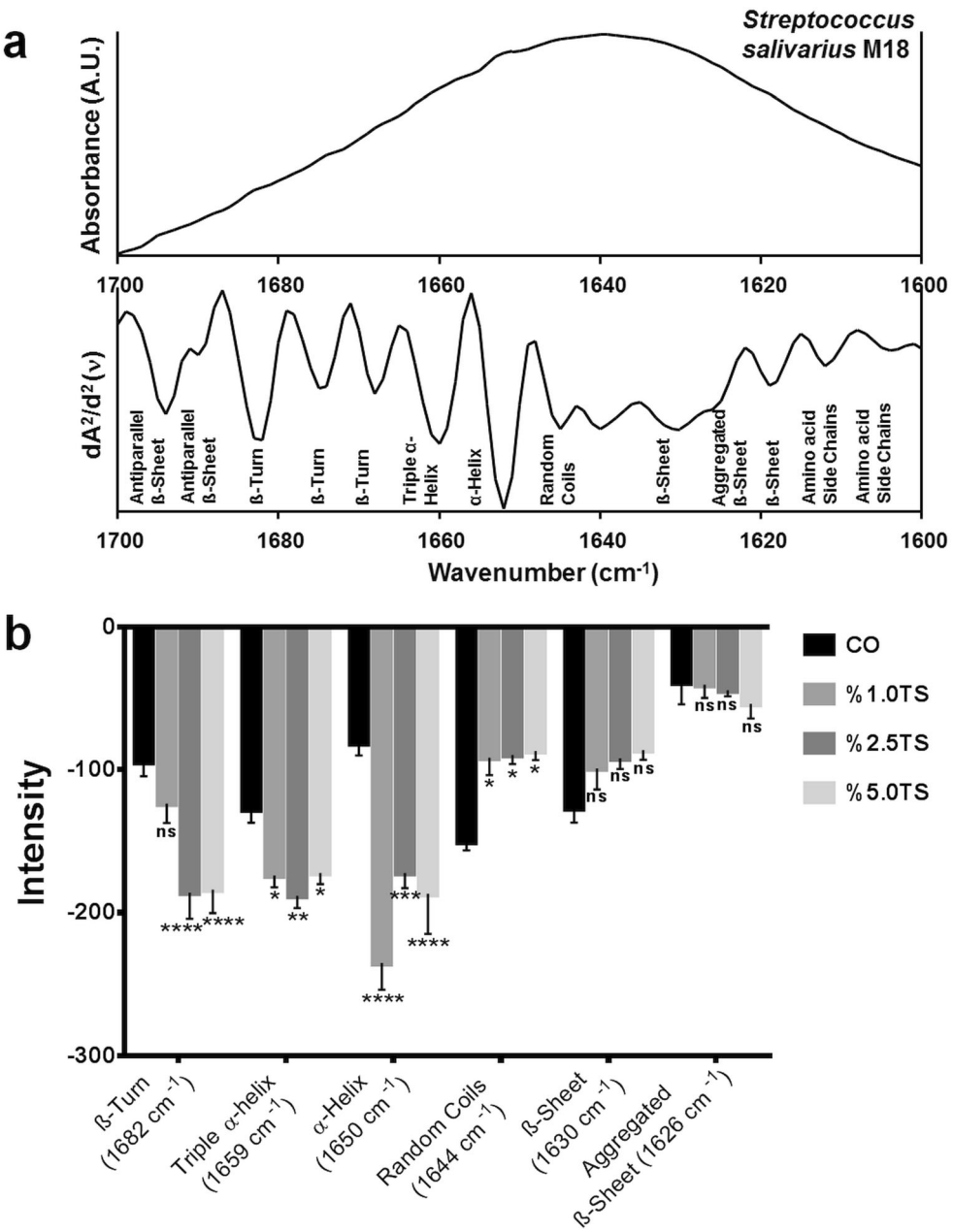


**Fig. 4** Tapioca starch modulates protein metabolism in *Streptococcus salivarius* M18. **a** Absorbance and second-derivative infrared spectra at the amide I (1700–1600 $cm^{-1}$) spectral region and **b** positions and absolute intensities of spectral bands associated with protein secondary structures. ns, non-significant; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. The error bars in reported measurements are presented as the means ± standard error of the mean

elucidate the interactions between TS and probiotic bacteria. The findings presented here can help to elucidate the molecular interactions between the prebiotics and probiotics. Generally, the interactions of probiotic *S. salivarius* M18 and *S. salivarius* K12 bacteria with prebiotic TS modulate the keystone metabolic events in these bacterial strains in a dose-dependent manner. The beneficial doses of probiotics are particularly measured based on their live cell counts: an increase in CFUs is associated with an increase in prebiotic effect [21]. Therefore, an increased number of CFUs in *S. salivarius* M18 and *S. salivarius* K12 (Table S2–S3) indicates that TS increases the survival of the probiotic bacteria. A recent study has demonstrated that native sago and tapioca starches increase the live cell counts of probiotic bacterial isolates, and the sago starch was found to have the most encouraging results [21].

The increasing doses of TS alter the typical morphology of the colonies of both *S. salivarius* strains (Fig. 1, Fig. S1). Congo red has a specific and strong binding to intact β-D-glucan polysaccharides [23]. Therefore, the accumulation of reddish carbohydrate-based structures inside the stained colonies is a phenomenon that is not observed in *L. plantarum* B-1846 (Fig. S2). The *L. plantarum* strains can ferment numerous carbohydrate-based foods [35]. The size reduction is consistent in all bacterial colonies. Bacteria demonstrate plasticity because of the adaptive pressures perfecting the shape,

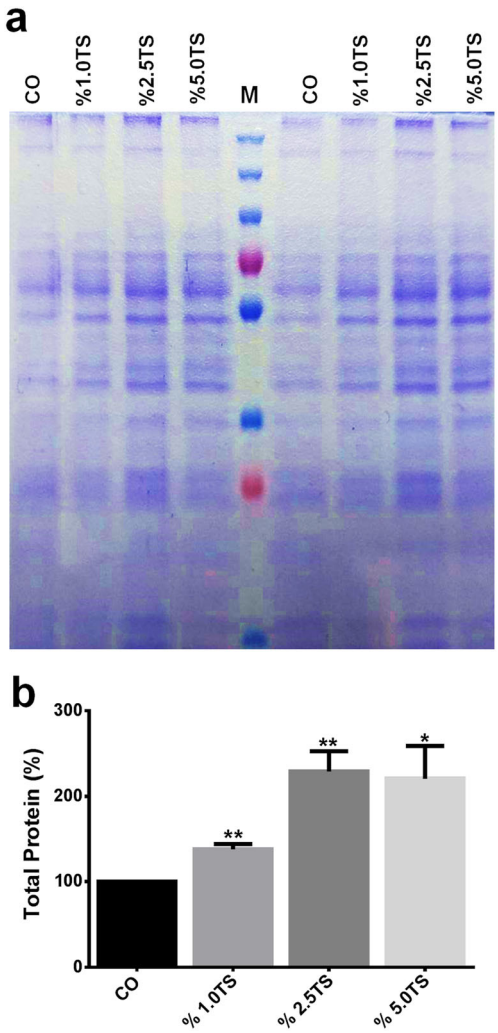


**Fig. 5** Tapioca starch modulates protein expression in *Streptococcus salivarius* M18. **a** Total protein bands profiled by SDS-PAGE and **b** total quantities of expressed proteins calculated using ImageJ software and normalized to 100%. M-Marker (Thermo Scientific™ PageRuler™ Plus Prestained 10–250 kDa Protein Ladder). $*P < 0.05$, $**P < 0.01$. The error bars in a reported measurement are presented as relative means in percent ± standard error of the relative mean in percent

which, in turn, modifies reversible cellular processes, such as intercellular communication, attachment, colonization, nutrient uptake, motility, dispersion, and adaptation to the chemical environment and physical barriers. The peptidoglycan (PG) sacculus made of polysaccharides and cross-linked peptides is a key regulator of bacterial morphology. Furthermore, the cytoskeletal factors regulate the enzymes involved in the biosynthesis machinery of PG in big protein network systems [36]. The reduced phospholipid (cardiolipin) composition of a bacterial membrane has also been linked to serious shape alterations (rod to ellipsoid), probably due to the modification of membrane organization that is ensured by fatty acid geometry [37]. The cited literature has suggested that the alteration of colony morphology is a complex phenomenon in which the diverse cellular processes remodeling colossal biomolecular systems are involved in developing an adaptive strategy for the benefit of bacteria eventually.

TS causes considerable metabolic modulations in the *S. salivarius* M18 and *S. salivarius* K12 strains (Fig. 2, Fig. S3). In the comprehensive study of particular metabolic events, the second-derivative spectra are extremely useful from which the prominent alterations in protein, polysaccharide, and fatty and nucleic acid molecules are quantitatively highlighted (Fig. 3). A wide range of cellular mechanisms associated with protein conformation might be involved in the transition of existing protein structures from random coil to regular α-helix and β-turn (Fig. 4, Fig. S4). Another possibility is that newly synthesized protein structures have higher levels of α-helix and β-sheet structures than random coils. The increased production of antimicrobial peptides (AMPs) by probiotics in the presence of prebiotics has been reported [38–40]. The AMPs provide competitive leverage to the producer bacteria in certain ecological niches because of the peptide-mediated neutralization of rival bacteria competing for available nutrients. Therefore, bacterial AMPs/bacteriocins have a crucial and operative function in defining the microbial colonization of certain habitats [41]. The sequence and structure of AMPs differ significantly, whereas the main characteristic features, such as cationic charge, large hydrophobic chain, and amphipathic character, are similar [42]. The salivaricins of the bacteriocin family are common AMPs produced profusely by *S. salivarius* to defeat pathogenic fungi, bacteria, and viruses [43]. To the best of our knowledge, the three-dimensional protein structure of salivaricins has not been reported at the time of writing this article. In the Antimicrobial Peptide Database, 60% of known AMPs are presented as unknown 3D structures. Although the AMPs with β-conformation (2.72%) and combined helix/beta packed structures (3.51%) are depicted, most of the AMPs (13.68%) are revealed as helical peptides [44, 45]. Bacteriocins are structurally flexible peptides in different microenvironments reflecting and contributing to the functional diversity [46–48]. Therefore, they play an integral, multifaceted role in microbial ecology [49].

Apart from modulations in protein conformation, TS significantly alters the total expression of cellular proteins, especially in *S. salivarius* M18 (Fig. 5, Fig. S5a). Generally, *S. salivarius* M18 and *S. salivarius* K12 depict different protein expressions. These differences can be associated with TS-induced alterations in DNA conformations directly affecting the upregulation (in *S. salivarius* M18) or downregulation (in *S. salivarius* K12) of particular genes that orchestrate the expression of proteins differently.

DNA conformation can be affected by environmental conditions, such as pH, hydration, salts, counterions, antibiotics, and metals, as well as by cellular processes, such as protein binding, RNA binding, and superhelical tension [50, 51]. The possible link between the methylation status and the

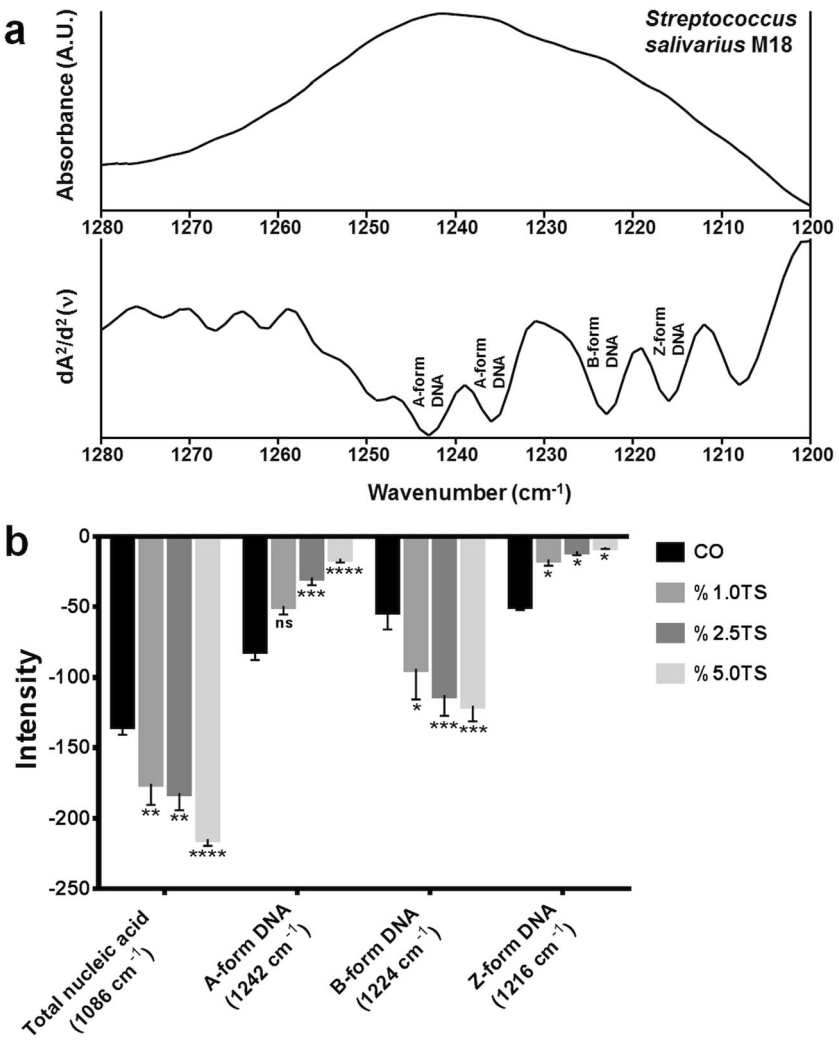


**Fig. 6** Tapioca starch modulates nucleic acid metabolism in *Streptococcus salivarius* M18. **a** Absorbance and second-derivative infrared spectra at the 1280–1200-$cm^{-1}$ spectral region and **b** positions and absolute intensities of spectral bands associated with the total nucleic acids and conformational forms of DNA. ns, non-significant; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. The error bars in the reported measurements are presented as the means ± standard error of the mean

conformational forms of DNA has also been revealed [52, 53]. The immunomodulatory effects of probiotic DNA [54] and unmethylated CpG motifs in bacterial DNA have also been shown [55]. The conformational state of DNA can be implicated in the probiotic activity. An increase in nucleic acid synthesis with the predominance of B-DNA over the A- and Z-DNA can be favorable modulations occurring in a TS-enriched environment (Fig. 6, Fig. S6).

Most antimicrobials generally target the key molecules of bacterial cell membranes, including fatty acids [56]. Diverse bacteria uniquely modify their fatty acid composition to modulate the membrane properties and minimize contact with antimicrobials [57]. In other words, the modification of fatty acid composition strictly depends on the nature, type, and concentration of indigenous fatty acids, which are unique for particular bacteria [56–58]. The reduction of fatty acids in the TS-enriched environment (Fig. 7, Fig. S7) can be associated with the inhibition of the de novo lipid biosynthesis or utilization of existing cellular lipids for distinct metabolic activities.

Extracellular polysaccharides (EPSs) produced by pathogenic bacteria are important virulence factors as they play important roles in host-pathogen interactions [59]. The EPSs of commensal gut bacteria participate in the modulation of host immune responses and help the probiotic bacteria to exhibit antimicrobial effects against challenger pathogens [60, 61] and their biofilms [62, 63]. Given the numerous beneficial functions of bioactive EPSs, probably, the EPS production in probiotic bacteria enhances its probiotic efficacy and is a favorable condition for commensal gut physiology and host metabolism [60, 61, 64–66]. Upregulated by nutritional and energy status, bacterial glycogen metabolism is associated with growth, prolonged survival, adaptation, osmoprotection, and cell wall biosynthesis [67]. The synthesis and storage of glycogen are considered vital niche factors in the host environments, promoting the probiotic properties and intestinal retention of *Lactobacillus acidophilus* and the colonization persistence of *S. mutans* [67]. In this context, the interaction with TS could create a beneficial environment for polysaccharide and glycogen synthesis, enhancing the probiotic activities

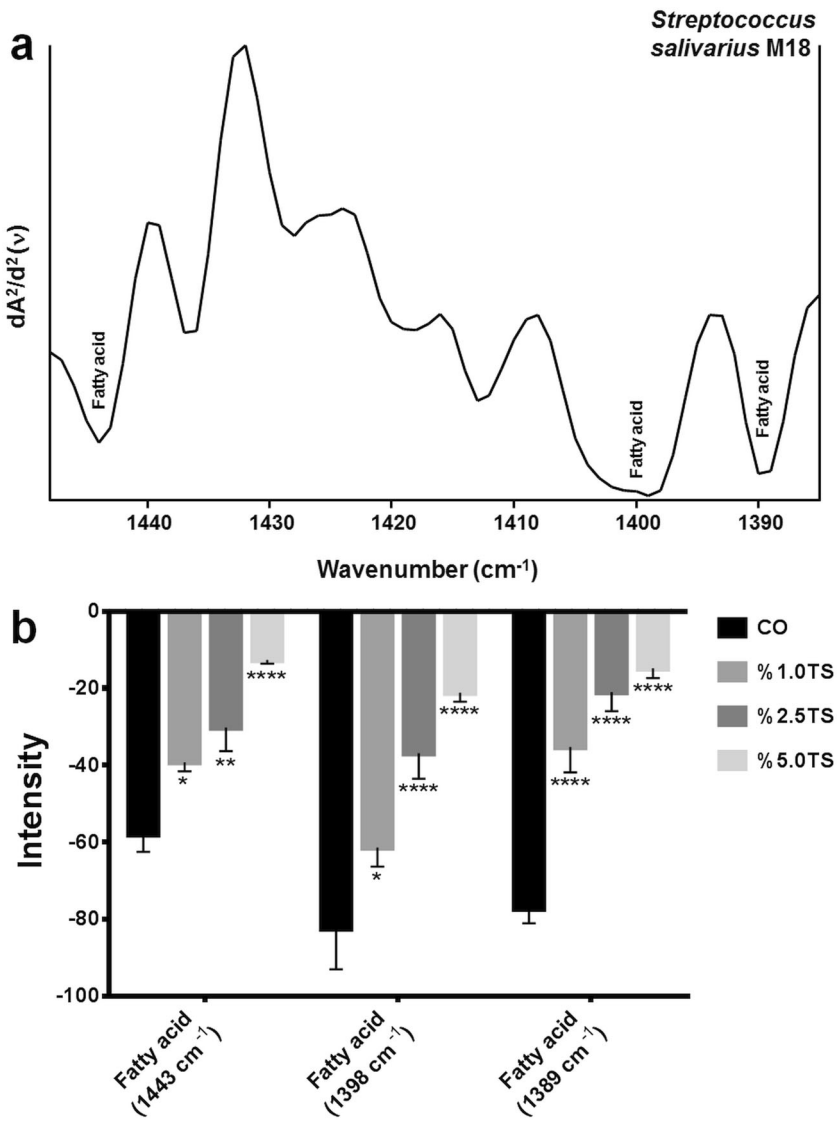


**Fig. 7** Tapioca starch modulates fatty acid metabolism in *Streptococcus salivarius* M18. **a** Absorbance and second-derivative infrared spectra at the 1445–1385-$cm^{-1}$ spectral region and **b** positions and absolute intensities of fatty acid-associated spectral bands. ns, non-significant; $^{**}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$. The error bars in reported measurements are presented as the means ± standard error of the mean

in oral probiotic bacteria (Fig. S8). The long-chain isomaltooligosaccharides synthesized from TS demonstrate the main prebiotic feature that is the stimulation of the growth of probiotic bacteria [68].

## Conclusions and Future Prospects

In summary, the interaction with TS helped the bacteria in terms of live cell counts, as the CFUs of TS-supplemented groups increased over a period of 24 h for both the strains. This increase reached up to 100× in the 5.0% TS-supplemented *S. salivarius* M18 group. In the case of *S. salivarius* K12, a similar behavior was encountered but only over 24 h. TS modulated the colony morphologies and cellular metabolism of both bacterial strains in a dose-dependent manner. The TS-associated metabolic modulations were significant in proteins, carbohydrates, and nucleic and fatty acids. Furthermore, the analysis of bacterial proteins demonstrated distinct alterations in their expression profiles.

Keeping in mind the central and crucial role of the colon in human health, one should not underestimate the importance of the oral cavity because it serves as an entrance for all kinds of transmission between us and our habitat. Gastrointestinal health strictly relies on the welfare of the oral cavity as the oral cavity can allow the bacteria in the mouth to pass to the intestine. Given that oral probiotics have the potential to produce certain beneficial metabolites, they can be considered for the protection of oral microbiota in the complementary and preventive treatment of persistent mucosal diseases. In addition, they can help prevent pathogen-associated oropharyngeal complications by regulating the microenvironment of the oral cavity. Future studies aim to investigate the interactions between human oral epithelial cells and the post-fermentative by-products of the probiotic *S. salivarius* strains produced by TS fermentation. This kind of knowledge can be

useful in the development of health-preserving real functional foods that are estimated to be the building blocks of the food industry in the near future.

## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

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